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In situ ichthyoplankton imaging system (*IS*IIS): system design and preliminary results

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Abstract

Over the last two decades, there has been an accelerating advancement of acoustic and optical plankton samplers, opening many opportunities for fine-scale studies of plankton distribution. To date, however, the imaging systems have been limited in the volume of water being sampled, thereby restricting their utility to quantifying highly abundant, small zooplankton like copepods, but not relatively rarer, larger ichthyo- and other meso-zooplankton (e.g., larval decapods, salps, pteropods, ctenophores, etc.). Here we describe an imaging system, *In situ* ichthyoplankton imaging system (*IS*IIS), that is capable of *In situ* (i.e., noninvasive) sampling of sufficiently large volumes of water at very high resolution, allowing quantitative measurement of these rare plankton, while at the same time also recording the smaller more abundant taxa. Capitalizing on state-of-the-art digital line scan cameras and high-throughput computer data transfer and storage, combined with shadow photographic lighting techniques, we have designed and built a towed system capable of imaging at 68-micron pixel resolution, yet with up to a 20-cm depth of field (with a 14-cm field of view). This system is coupled with various environmental sensors (e.g., CTD, fluorometer), enabling the evaluation of fine-scale, taxon-specific distributions in relation to environmental conditions. Field testing demonstrated high-resolution imagery of plankters, while quantitatively imaging >70 L s^{-1} continuously for a 78-min trial.

Introduction

Current technologies available for the study of ichthyoplankton and many other meso-zooplankters remain limited in comparison to the spatial-temporal resolution and data acquisition rate available for physical oceanographic measurements. Specifically, plankton measurements are made primarily by use of net collections, versus high-speed digital output possible for physical sampling. Although net technology has become quite sophisticated (e.g., MOCNESS, BIONESS, Weibe et al. 1976, Sameoto et al. 1980), enabling vertical resolution coupled with detailed physical data, net samples still require the task of being processed manually, which is a time-consuming and costly effort. Further, nets integrate organisms over the sampling distance and depth, significantly reducing sample resolution. If higher-frequency (and higher-resolution) sampling could be accomplished while at the same time allowing for much faster data analysis, there would be tremendous capacity for improved scientific inquiry. Biological oceanographers have been advancing methodologies for more rapid, higher-resolution sampling of phyto- and zooplankton via various acoustic (e.g., FishTV) and optical (e.g., OPC, LOPC, VPR, SIPPER, ZOOVIS, UVP; see Wiebe and Benfield 2003 for major review of zooplankton sampling advancements) technologies, but these techniques have typically been unsuccessful for ichthyoplankton, which although relatively larger are substantially rarer than most zooplankton.

Previous efforts to image zooplankton have resulted in high-resolution images suitable for identifying copepods and invertebrate larvae with sometimes spectacular results (e.g., Video Plankton Recorder [VPR], Davis et al. 1992, 1996), particularly with respect to resolving the fine spatial and vertical distribution of these organisms (Lough and Broughton 2007). Utilizing digital imaging systems and an off-axis illumination scheme, a parcel of nondisturbed water is imaged, providing a series of images of plankton. The critical issue for our interests (i.e., ichthyoplankton and other meso-zooplankton) is that the VPR

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(and its cousins) has been limited in the volume of water that can be sampled (e.g., the VPR is usually limited to a small rectangle to ensure sufficiently fine pixel resolution for resolving small plankters, with a very narrow depth of field—resulting in a sample volume ranging from a few milliliters to 30 mL/frame [0.9 L s^{-1} at 30 frames s^{-1}]; Davis et al. 2005). The drawback to these systems is that this volume of sampled water is too small to adequately quantify larger, but rarer plankton. Whereas copepods, and some invertebrate zooplankters, may exceed densities of 1 to 10 individuals L^{-1}, ichthyoplankton typically occur at densities of ca. 0.01 to 0.001 individuals L^{-1}. To more broadly sample ichthyo- and other meso-zooplankters, other techniques (e.g., OPC and SIPPER; Herman et al. 1992, Remsen et al. 2004, respectively) have involved imaging and/or counting plankters by size, as they pass through a narrow tube, an approach that does not enable true *in situ* observations, and which can potentially distort fragile and highly mobile plankton into nonidentifiable shapes.

Our goal was to build on both existing knowledge (i.e., previously designed systems) and hardware to develop a very-high-resolution towed digital imaging system capable of sampling water volumes sufficient to accurately quantify larval fish *In situ*. We describe an imaging system, *In situ* ichthyoplankton imaging system (*IS*IIS), that accommodates some of the problems associated with imaging relatively rare, albeit still very small organisms *In situ* (i.e., without any indications of disturbance). This system is composed of several components: very-high-resolution imaging camera (line scan), backlighting physics, high-throughput data transfer and storage, towed vehicle, and image analysis data processing. We focus on the physical system here, and leave the image analysis component to a separate report, as it corresponds to a parallel research effort.

Materials and Procedures

Prototype overview—This camera system utilizes a combination of a light emitting diode (LED) light source, modified by plano-convex optics to create a collimated light field, which backlights a parcel of the water column and a high-resolution line scanning camera (see Figure 1). With the application of mirrors, the imaged parcel of water passes between the forward portions of two streamlined pods (underwater [UW] housings) and thereby remains minimally affected by turbulence. The resulting very-high-resolution image is of zooplankton in their natural position and orientation. When a sufficient volume of water is imaged this way, then quantification of density, organism size, and fine-scale distribution is possible.

Lighting—We use lighting that involves shadow illumination (Arnold and Nuttall-Smith 1974; Ortner et al. 1979, 1981). The focused shadowgraph technique allows for a long depth of field, not achievable with other lighting techniques such as dark field or simple backlighting. Moreover, this lighting scheme gives a very good contoured, as well as contrasted, image of small, transparent organisms such as zooplankton.

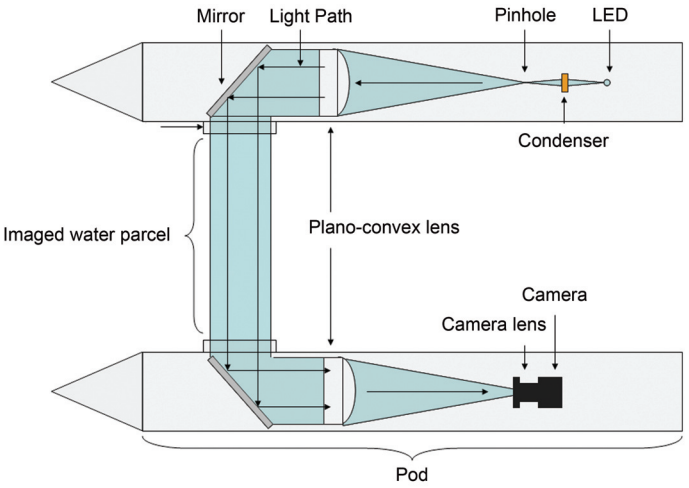

Fig. 1. Light scheme using shadowgraph technique. Light passes through plano-convex lens, thereby establishing a collimated light beam. The advantages of this approach over other lighting techniques include high depth of field (20+ cm), telecentric image (magnification level not affected by distance from object to the lens), and very sharp outlines of organisms and internal structures (facilitates automated recognition).

Because the light rays are directed toward the imaging sensor and not scattering off the actual filmed subject, the intensity of light required is extremely low compared with any other lighting technique. This avoids the use of bright light sources that may deter or otherwise compromise the behavior of organisms. Moreover, a small, compact, vibration-resistant light source such as LED can be used without the need to be overpowered or strobed. This greatly simplifies the design and, in fact, makes it even more robust.

The system is composed of a plano-convex lens (150 mm diameter and 586 mm focal length) creating a collimated beam of light through the volume of sampled water using a set of aluminum-coated mirrors (see Figure 1). Once the collimated beam of light passes through a second lens, it is refocused (Arnold and Nuttall-Smith 1974; Settles 2001) before it impinges on the camera lens (Nikkor 85 mm). This makes the system economical in terms of light intensity, since almost all the light entering the sampled volume hits the camera sensor. For our light source, we used LED technology, specifically a 5 W 455 nm (blue) wavelength LED. Because this system does not require a large amount of light, this LED is suitable for observing plankton in a relatively undisturbed environment. Moreover, this optical scheme makes the system telecentric, meaning that magnification is independent of distance from the camera to the specimen (Arnold and Nuttall-Smith 1974), thereby providing the opportunity to accurately measure size of zooplankters within the imaged volume.

Camera—For imaging, we used an 8-bit (256 grayscale), line-scan camera (DALSA P2-22-02k40). These cameras function like fax machines, departing from traditional area-scan CCD (charge coupled device) cameras. The camera creates a picture

Table 1. Comparison of plankton imaging systems with respect to volume imaged.

System	Acronym	Sampling volume (L s^{-1})	Reference
Video plankton recorder	VPR	~2	Davis et al. 1992, 2005
Shadow image particle profiling evaluation recorder	SIPPER	10-12	Remsen et al. 2004
Zooplankton imaging system	ZOOVIS	1	Benfield et al. 2003
Underwater video profiler	UVP	12	Gorsky et al. 2000
In situ Ichthyoplankton Imaging System (ISIIS)	ISIIS	70	This report

by adding subsequent line fractions of images as the object being filmed moves perpendicular to its scanning axis. This creates a continuous image, which contrasts with sequential flash or video images in the sense that flash/video are successive images that may overlap each other or create gaps if the object being filmed (or the vehicle) is not moving in synchronization with camera's frame rate. High-speed scanning rates of the line-scan camera also allow for high-resolution images.

Image resolution in this system is driven by the camera's line sensor, its scanning rate, the field of view being imaged, and the system's towed speed. In the prototype system, we used DALSA's 2048-pixel, 36-kHz scanning rate camera with a 14-cm vertical field of view. This produced a vertical pixel resolution of 68 µm. Horizontal resolution of the system is set by the camera scanning rate and the speed of the system through the water. The goal is to match the horizontal resolution to the vertical to achieve a square pixel (to avoid distortion of the image). Sizing error due to speed variation can be corrected using the measured flow speed during the deployment, and a simple formula: horizontal scale (pixels/m) = flow speed (m/s)/36,000 (pixels/s). At 36 kHz, we set the tow speed at 2.5 m s^{-1} (i.e., 5 knots), which yielded a horizontal resolution of 68 µm. At this scale of resolution, a typical 2 by 6 mm larval fish would be imaged by ca. 2500 pixels, resulting in a very-high-resolution image (see "Results"). Moreover, this combination provided a continuous visual field that was approximately 14 cm in vertical height with a 20-cm depth of field. Thus the volume of water imaged every second was ca. 70 L (14 by 20 by 250 cm) or ~0.07 m^3. As a typical 1-m^2 plankton net filters ca. 0.75 m^3 s^{-1} (at a tow speed of ~0.75 m^3 s^{-1}), our prototype system is capable of imaging close to 10% of the volume filtered by a net, which is greater than an order of magnitude of improvement over other imaging systems (Table 1).

Towing platform—The entire system was designed to fit within a towing platform that ultimately allows undulating tows. Although there are many towed vehicle designs, there were certain system constraints (e.g., camera/light placement) that required custom design. Specifically, the vehicle holds the UW housings (light and camera) parallel and slightly forward of the vehicle to minimize turbulence within the imaged water parcel. Additionally, there was a need to minimize vibration transfer from the cable through the vehicle to the UW housings. Finally, the vehicle required a stabilizing wing (with flexibility for future undulating capabilities) that was properly balanced to aid in steady, horizontal orientation of the UW housings. Mean pitch experienced during field trials (see below) was 5.04 degrees (± 4.03 SD).

The open architecture frame is constructed of aluminum U-shaped channel bars, welded and bolted together for ease of assembly. The bars hold the battery housing and additional sensors (e.g., CTD, fluorometer, flow meter) centrally below and behind the main imaging housings (see Figure 2). The aft section is equipped with a PVC stabilizing wing and two air-filled cylinders, providing static and dynamic floatation so that the vehicle is positioned horizontally at any towing speed. The positively buoyant vehicle is weighed down with lead shot bags along the front and sides to the lower frame. The frame is designed to be towed behind a ship at a speed of 2 to 2.5 m s^{-1} with instrument depth currently determined by cable length. Tow speed (instrument through the water), and

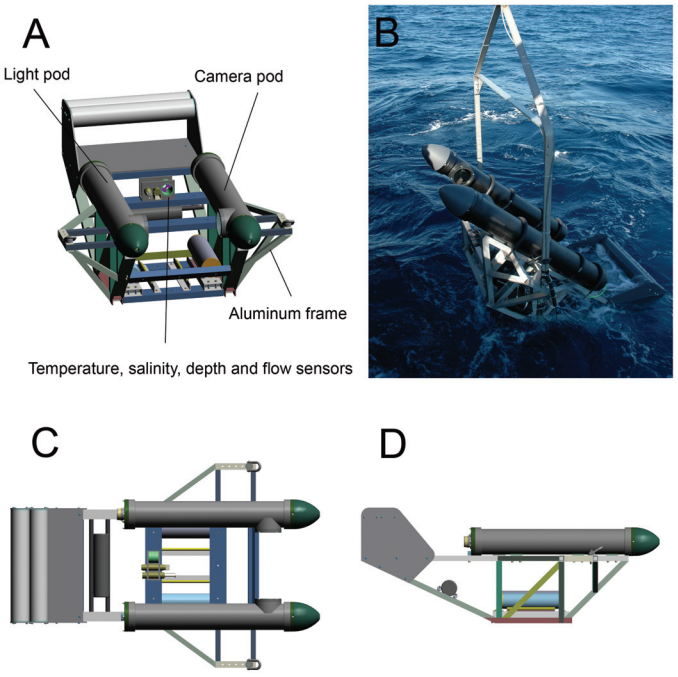

Fig. 2. Vehicle design. A. Oblique schematic of ISIIS showing main components of system. B. Photograph of ISIIS being retrieved from the water. C. Overhead view schematic of ISIIS. D. Side view schematic of ISIIS. The towed vehicle carries sensors that measure physical data such as temperature, salinity and depth, as well as imaging specimens while moving at a speed of 2.5 m s^{-1}.

depth are monitored onboard the ship in real time. A rotating stainless bridle made of flat bars allows the instrument to be towed without perturbing the water mass directly in front of the filming area. Preliminary field tows demonstrated both an ease of launch and recovery and tow stability.

The optical components of *IS*IIS are separated in two identical housings: one camera housing and one light source housing (designed and constructed by Bellamare, LLC). The pressure vessels are made of aluminum pipe (aluminum 6061-T6) precisely machined to accommodate the inside rail and lens holder system, keeping the optical instruments aligned and accessible. The forward side of each pod is fitted with a right-angle porthole covered by a watertight acrylic window. Two 45-degree-angle mirrors in front of each porthole redirect the light from the light source to the imaging sensor. The forward flat faces of the pods are covered by two PVC cone-shaped fairings, decreasing turbulence around the filming area. A third housing was added to hold a 12-volt, 105-Ah battery (Concorde Battery Co.) to power the underwater system. This housing is constructed from a large aluminum cylinder (48.26 cm long by 43.18 cm diameter) closed on each side by circular watertight aluminum plates.

Data transfer/storage—With the very-high-resolution, continuous imaging (i.e., 2048 by 36,000 pixels s^{-1}), data transfer was a critical issue. Our original plans were to save data internally on the towed system, but we decided that it was best to port the data onboard the ship to better enable eventual real-time (or near-real-time) data processing and analysis, as well as allow for greater storage capacity. Working with interface designers of line-scan cameras (Boulder Imaging, Inc.), we chose a high-throughput disc array (160 GB) for data storage that is composed of four 40-GB hard drives controlled by a high-performance computer with continuous real-time recording at up to 140 MB s^{-1}. The system configuration produces a raw data output rate of 80 MB s^{-1}, which can be compressed (at a minimal loss of image quality) by a factor of eight (MJPEG compression), thereby allowing for continual recording for 255 min (33 min uncompressed). Expanded data storage is possible for more extensive recording durations. Other means of reducing the data transfer rate include real-time image processing to eliminate areas of non-information by selecting only regions of interest (ROIs) as defined by the user.

The high data transfer rates between the towed vehicle and shipboard computer required the use of copper/fiber-optic cable. We used a shielded, three optical fibers, four copper conducting wire cable of 0.322 inch (8.18 mm) diameter (Rochester Cable, Inc.). This necessitated inclusion of an optical modem at each end of the cable and an optico-mechanical rotary joint (Princetel, Inc.) on the winch (Markey, Inc.). For the present configuration, we used two optical fibers. The electrical and optical connections between the underwater units and the oceanographic cable are made through an intermediate oil-filled (pressure-compensated) housing attached to the frame of the towed underwater vehicle.

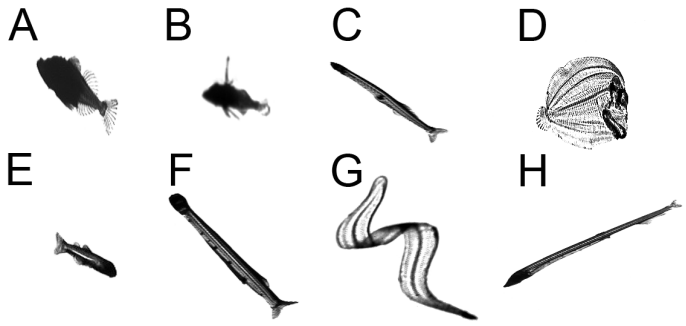

Fig. 3. In situ fish images: 0–40 m depth, Florida Current. Selected images of fish taxa captured via *IS*IIS. A. Triglidae. B. Serranidae – Epinepheline. C. Gonostomatidae D. *Bothus* sp. E. Myctophidae F. Synodontidae G. Muraenidae. H. Paralepididae – *Lestidium* sp. Note: fish sizes range from 6 to 20 mm—they are not scaled to each other in this composite image.

Assessment

Image quality under field conditions (Figures 3, 4, and 5) demonstrates several important capabilities of this system. Not only are the images of sufficiently high resolution to aid in the broad categorization of fish larvae and other mesozooplankters (e.g., larvaceans, chaetognaths), but in many cases, family or lower taxonomic identification is possible owing to the system's ability to see through transparent organisms, allowing for meristic counts and/or pigment identifiers (see Figure 3). Further, many invertebrate plankton taxa are clearly imaged, allowing for identification and observation of their natural *In situ* orientation (e.g., fine feeding structures, tentacles; see Figure 4). Even very small organisms (e.g., copepods and the cyanobacteria *Tricodesmium*) are clearly imaged (Figures 4 and 5). Finally, the system is able to discern centimeter-scaled organism aggregations and organism group orientation as seen in a single 14 by 14 cm (0.057 s) image of zoea (Figure 6). With the proposed improvements in volume and resolution (see below), the potential of this system is exciting.

Quantitatively, we processed a 78-min continuous imaging record of a short (7 km) transect taken across the core of the Florida Current wherein we undulated the *IS*IIS five times between the surface and ~40 m. Overall, we successfully imaged a total volume of 262 m^3, in which a total of 283 fish larvae were counted, which represents a mean density of 1.1 larval fish per m^3 (or 0.0011 larvae L^{-1}). When compared to 1 m^2 and 4 m^2 MOCNESS samples taken in the same location and time of year (but not simultaneously), we found that the *IS*IIS is capable of quantifying similar, if not higher, densities of fish larvae as collected by standard net methods (Figure 7A). Whether the slightly higher densities estimated by *IS*IIS represent a more efficient way of sampling or simply temporal variability in larval fish abundance is not clear. However, it does suggest that *IS*IIS is adequately sampling larval fish. Future side-by-side tests with a net and *IS*IIS will verify how well *IS*IIS samples the meso-plankton. Further, proposed improvements

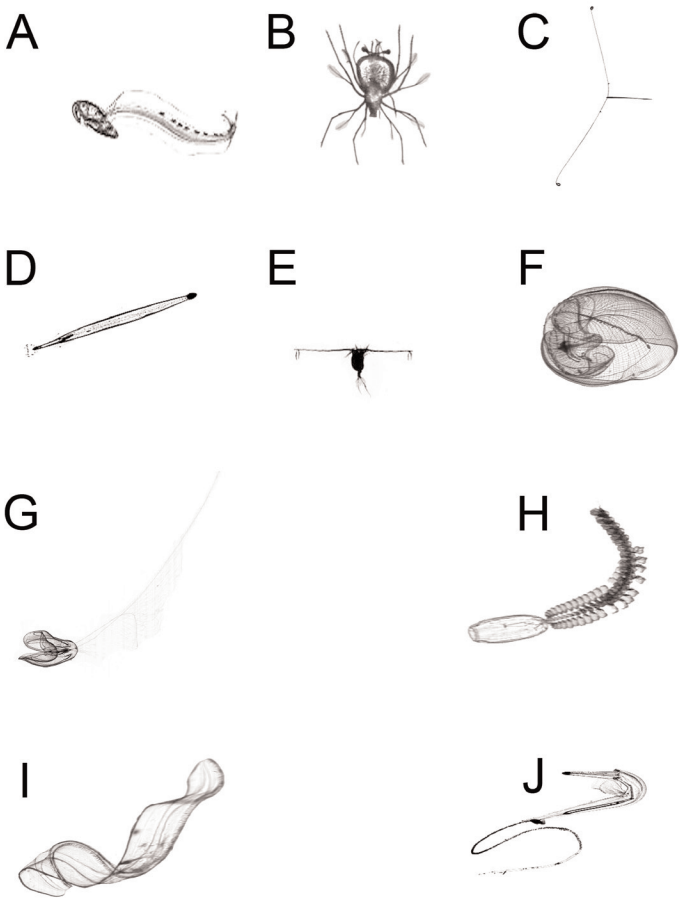

Fig. 4. In situ invertebrate zooplankters. 0–40 m depth, Florida current. Selected images of invertebrate plankton captured via *ISIIS*. Organisms are not scaled to each other in this composite image; sizes range from a few millimeters to several centimeters. A. Larvacean (*Oikopleura sp.*). B. Scyllarid lobster larva. C. Unidentified larval crustacean (?). D. Chaetognath. E. Copepod with eggs. F. Ctenophore. G. Ctenophore with feeding tentacles extended. H. Aggregate phase Thaliacean salp with reproductive buds. I. Ctenophore (*Velamen sp.*). J. Pterotracheid heteropod.

in the volume imaged and image resolution (see below) should greatly improve this system's capacity for fine-scale resolution of ichthyoplankton distribution and environmental interactions.

Beyond simple density estimates, continuous sampling allows parsing of data into very fine time/depth stanzas. Such data can then be plotted to examine fine-scale distributions in association with environmental measurements such as temperature, salinity, density, Chl *a*, and dissolved oxygen. For

Fig. 5. In situ cyanobacteria. Close-up of image of the cyanobacteria *Tricodesmium*. Although very small (ca. 2–4 mm), its unique shape renders this organism easily discerned by the system.

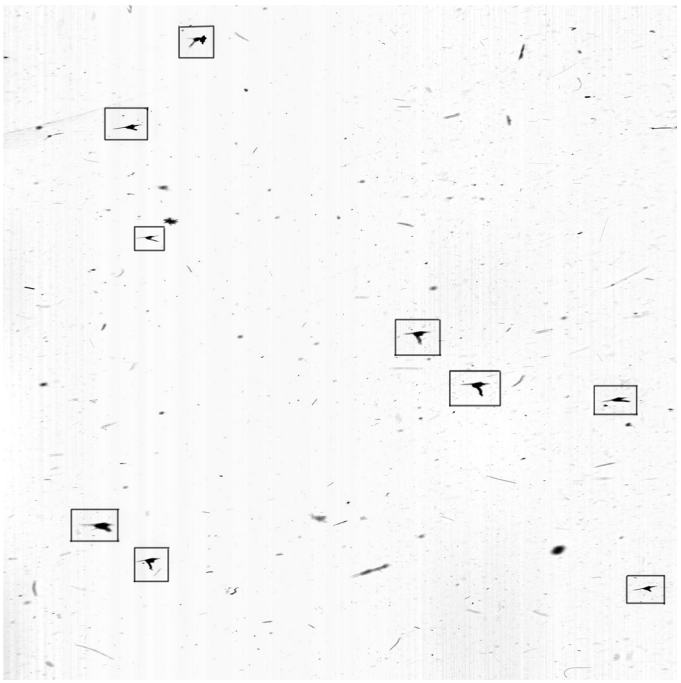

Fig. 6. Raw image of zooplankton aggregation. Single 14 by 14 cm frame showing aggregation of similarly oriented crab zoea larvae.

our particular example, the water column in the upper 40 m of the core of the Florida Current is well mixed, and therefore few fine-scale vertical features were observed. However, when the distribution of larvae with respect to their nearest neighbor was compared with random expectations, we saw evidence of aggregation at very small scales (i.e., 2–4 m; Figure 7B). Although preliminary, this analysis demonstrates some of the potential results possible with more extensive sampling and in more vertically/horizontally stratified environments.

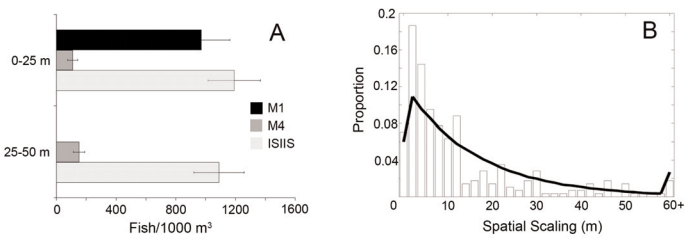

Fig. 7. Quantitative comparisons. A. Comparison (mean and SD) of larval fish sample densities from 1 m^2 (150 µm mesh size) and 4 m^2 (800 µm mesh size) MOCNESS nets versus the prototype *ISIIS*. Samples were compared over two depth bins, 0–25 m and 25–40 m. Note that samples were taken in same area and time of year, but different years, so some variation may be interannual variation. 1 m^2 MOCNESS samples were not yet analyzed for 25–50 m depth bin. B. Estimation of the degree of aggregation of larval fish at different spatial scales. The red line is expected proportion of fish if randomly distributed. Blue bars are observed data. Greater than expected co-occurrence of larvae at 2- to 4-m scales was observed.

Discussion

The name of this system suggests that its use is focused on larval fish (ichthyoplankton), but its utility goes far beyond fish. Any meso-zooplankton (from as small as a few millimeters to >10 cm) can be clearly imaged by this system. Further, given the large volume being sampled and the continuous format, organisms can be quantified over fine scales (centimeters to meters), thereby opening up opportunities to examine process-oriented questions about various organism–feature interactions such as thin layers as an aggregation/food source for zooplankton, role of convergences in concentrating organisms and the relative association of zooplankters to their fine structure, and transport by internal waves/bores (e.g., Kingsford and Suthers 1994; Pineda 1999; Holliday et al. 2003; Lough and Broughton 2007). These represent a variety of interdisciplinary studies that could be well suited to linking the high-frequency/high-resolution sampling possible with *IS*IIS with that of other environmental sensors (e.g., CTD, ADCP). Similarly, when used in a survey mode, this system may be a cost-effective means of obtaining fisheries-independent data on spawning stock biomass via the measurement of egg abundances over relatively large spatial regions, as well as providing more detailed knowledge about what environmental features are critical for spawning to occur. Finally, the fact that this system images water parcels with minimal disturbance of organisms will allow a variety of behavioral/orientation studies to be conducted relevant to feeding, floating, diurnal rhythms, vertical migration, etc. Thus, previously very difficult-to-study zooplankton behavior may be more accessible to a large group of zooplankton scientists.

Future efforts will entail improvements to image resolution and depth of field to maximize the volume sampled. Technological advancements in line-scan cameras, coupled with improvement in LED design and light outputs, will enable us to improve the resolution of our imaging. Simultaneous advances in computing and data transfer and storage will also be required. Improvements in the depth of field will be addressed by further minimizing the size of the light source. Although we are currently using a relatively small source (LED diameter ca. 2–4 mm), reducing this diameter further can have a significant impact on the depth of field (Settles 2001; G.R. Settles, personal communication). One straightforward means of doing this is to put a light condenser in the light pathway to concentrate the light source through a 1-mm pinhole before passage through the plano-convex lens (see Figure 1). Using this approach, we plan for a twofold increase in the sample volume per unit time (i.e., going from 70 L s^{-1} to up to ~140 L s^{-1}).

We believe that our prototype *IS*IIS has performed well and holds considerable promise as an operational system. We are now in the position to address specific design improvements to significantly increase the volume sampled and resolution of the system. Further, as the system becomes operational, the obvious need for automated image analysis (e.g., Culverhouse et al. 1996; Tang et al. 1998; Hu and Davis 2005; Luo et al. 2004, 2005; Benfield et al. 2007) must be addressed. With the resulting increase in the volume imaged with concurrent very high image resolution, this system significantly expands the scope of rapid, quantitative plankton sampling available for biological oceanographers.

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