Evaluation of the In Situ Ichthyoplankton Imaging System (ISIIS): comparison with the traditional (bongo net) sampler

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Abstract—Plankton and larval fish sampling programs often are limited by a balance between sampling frequency (for precision) and costs. Advancements in sampling techniques hold the potential to add considerable efficiency and, therefore, add sampling frequency to improve precision. We compare a newly developed plankton imaging system, In Situ Ichthyoplankton Imaging System (ISIIS), with a bongo sampler, which is a traditional plankton sampling gear developed in the 1960s. Comparative sampling was conducted along 2 transects ~30–40 km long. Over 2 days, we completed 36 ISIIS tow-yo undulations and 11 bongo oblique tows, each from the surface to within 10 m of the seafloor. Overall, the 2 gears detected comparable numbers of larval fishes, representing similar taxonomic compositions, although larvae captured with the bongo were capable of being identified to lower taxonomic levels, especially larvae in the small (<5 mm), preflexion stages. Size distributions of the sampled larval fishes differed considerably between these 2 sampling methods, with the size range and mean size of larval fishes larger with ISIIS than with the bongo sampler. The high frequency and fine spatial scale of ISIIS allow it to add considerable sampling precision (i.e., more vertical sections) to plankton surveys. Improvements in the ISIIS technology (including greater depth of field and image resolution) should also increase taxonomic resolution and decrease processing time. When coupled with appropriate net sampling (for the purpose of collecting and verifying the identification of biological samples), the use of ISIIS could improve overall survey design and simultaneously provide detailed, process-oriented information for fisheries scientists and oceanographers.

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Regular surveys of early life stages of fishes provide a wealth of information for fisheries managers and fishery oceanographers. Indices of larval abundance are used quantitatively as fishery-independent measures of population abundance in stock assessments (Scott et al., 1993; Gledhill and Lyczkowski-Shultz, 2000; Simmonds, 2009). Larval fish abundance also is used qualitatively, as evidence for change in stock status (Smith and Morse, 1993; Lo et al., 2010; Richardson et al., 2010). Spawning areas and times are inferred from early-life-stage abundance and distribution, and they contribute to the definition of essential fish habitat (Brodziak, 2005; Levin and Stunz, 2005) and stock identification (Begg et al., 1999; Hare, 2005). Larval fish surveys combined with process-oriented research also help forecasting capability of year-class strength (e.g., Megrey et al., 1996; Lough and O'Brien, 2012).

Although larval fish studies make substantial contributions to the assessment of fish stocks, 3 factors currently limit their applicability. First, larval fishes are relatively rare within the plankton and estimates of variance in larval abundance can be large, limiting the power of statistical comparisons of abundance between years or locations (Cyr et al., 1992). Second, larval fishes are patchily distributed (e.g., Davis et al., 1990; Cowen et al., 1993; Pepin, 2004) but not randomly distributed; patches often are associated with fronts, thermoclines, or specific water masses (Cowen et al., 1993; Kingsford and Suthers, 1994). Most larval surveys, however, are conducted along fixed grids or as random stratified designs; significant differences in larval abundance between sampling times may simply reflect a varying intersection of sampling with dynamic larval habitat. Third, the cost of ichthyoplankton surveys is an important consideration and most programs are cost-limited in terms of ship time or the number of samples that can be processed (Tanaka, 1973; Lo et al., 2001; Simmonds, 2009).

In the United States, there are numerous federally supported ichthyoplankton programs that provide

data for fisheries management. All these efforts are limited by the 3 factors described above: rarity, patchiness, and cost. The In Situ Ichthyoplankton Imaging System (ISIIS; Cowen and Guigand, 2008) has the potential to minimize all 3 limitations, and, if successful, would provide the stock assessment toolbox with robust and timely fishery-independent measures of spawning distribution and stock size based on early-life-stage information. The overall goal of this study, therefore, was to evaluate the effectiveness of ISIIS for quantifying fish larvae and thus show the potential benefits of its integration into larval surveys, with the ultimate goal of improving stock assessments.

Specifically, we compare ISIIS with a traditional bongo sampler, which is composed of a frame supporting paired nets with mouth openings on either side of and in front of the towing wire (Posgay and Marak, 1980). The bongo has been used in ichthyoplankton programs throughout the United States since its development in the late 1960s: in the shelf ecosystem of the northeastern United States since 1971 (Richardson et al., 2010), in the Gulf of Mexico since 1982 (Lyczkowski-Shultz and Hanisko, 2007), and in the northeast Pacific Ocean since 1972 (Matarese et al., 2003). Here we present a comparison of larval fish abundance and size distribution based on results from the ISIIS and bongo sampler.

Methods

This study was conducted 54 km south of Woods Hole, Massachusetts, (Fig. 1), on 23–24 October, 2008, on the NOAA Ship *Delaware II*. The cruise immediately followed the passage of a low-pressure system, which brought strong winds to the study area; these winds diminished throughout the duration of the cruise. Sampling was completed along 2 parallel transects, which were 41.4 and 27.7 km in length and separated by ~6 km. To complete the comparison, the prototype ISIIS-1 (herein referred to as ISIIS) was towed along a transect; then the ship returned to the beginning of the transect, and net samples were made with the bongo over the same transect. Sampling along each transect encompassed both day and night periods, but no attempt was made to compare day and night differences in larval abundance or vertical distribution. Morse (1989) compared day:night catches in the region and found no significant differences for most of the taxa captured in this study. He did find some day:night bias at larger transect lengths, but, in our study, both the bongo net and ISIIS sampled during day and night, and therefore we assume this length bias was randomly distributed between the gears.

Sampling gear

The imaging output from ISIIS is unique in that it provides a continual image for the entire tow duration, with a pixel resolution of ~68 µm. Such fine resolution enables detection of particles as small as a 100 µm (e.g., diatoms), although the ability to clearly resolve particles is typically in the range of 700 µm (i.e., small copepods and larvaceans) and larger sizes (e.g., larval fishes, chaetognaths, and ctenophores). One distinctive feature of ISIIS is its large depth of field (~30 cm for

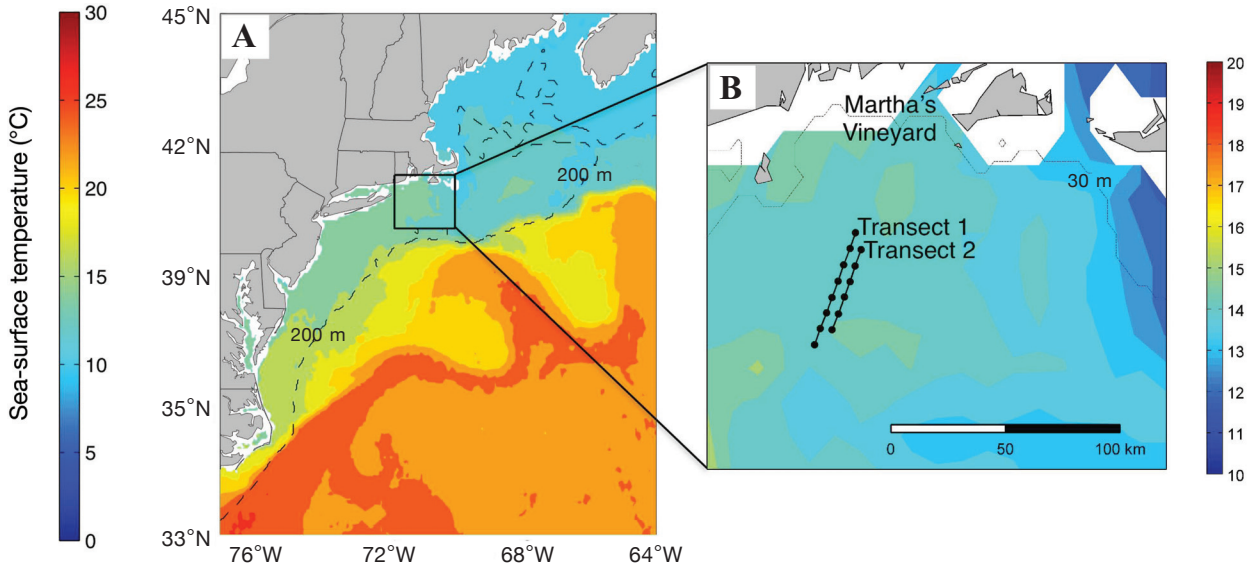

Figure 1

Eight-day average (20–27 October 2008) sea-surface temperature (SST, °C) of northeastern U.S. continental shelf from Cape Hatteras, North Carolina, to Nova Scotia, Canada. (**A**) The sampling location offshore of Martha's Vineyard, Massachusetts. (**B**) The inset shows the 2 In Situ Ichthyoplankton Imaging System (ISIIS) transects and the bongo collection locations marked by black dots along the same transects. Note the change in SST scale between the 2 panels.

mesozooplankton), which enables the concentration of even relatively rare mesoplankters, such as larval fishes and gelatinous zooplankton, to be quantified (Cowen and Guigand, 2008; McClatchie et al., 2012). Using the image analysis software that we have developed (Tsechpenakis et al., 2007, 2008), we could essentially quantify the plankton field for every centimeter of our tow, and we could match these data centimeter by centimeter with the corresponding environmental data collected by the onboard sensors (pressure [depth], temperature, salinity, and fluorometry). Consequently, ISIIS can evaluate from very fine-scale (centimeters) to submesoscale features. ISIIS sensors for this study were those for temperature (SBE 3[1] Sea-Bird Electronics, Inc., Bellevue, WA) and conductivity (SBE 4) and a fluorometer (ECO FLRT, WET Labs, Philomath, OR).

A 61-cm bongo sampler was used and fitted with 505- and 333-µm mesh nets (Posgay and Marak, 1980). A flowmeter (General Oceanics, Miami, FL) was attached in the center of each mouth opening to quantify the volume of water filtered by the net. A conductivity, temperature, depth (CTD) instrument (SeaCAT SBE 19) was attached to the tow wire above the bongo net. The CTD was used in real time to monitor the depth of the bongo net during deployment.

Sampling approach

For this study, ISIIS was towed at a speed of 2.5 m s^{-1} in a tow-yo (vertically undulating) fashion between the surface and a target depth of 10 m above the seafloor, thereby following changes in seafloor depth. The ISIIS was towed in an undulating manner by paying cable in and out from the winch, and therefore continual winch operation was required. (Since this study, a self-undulating version of ISIIS has been designed and the need for continual winch operation has been eliminated). Each undulation (surface to depth to surface) took ~10 min, resulting in a distance covered of 1.5 km, which also equates to the distance between downcasts (or upcasts). While being towed, ISIIS records environmental data (temperature, salinity, fluorescence) and imagery continually, sending the data up the fiber-optic cable for onboard recording. The continual imagery is parsed into single images of 13×13 cm at a rate of 17.3 images s^{-1}. Thus, ISIIS generates ~64,000 images h^{-1}, and for this study, an estimated total of ~478,000 images over ~7.68 h of total recording time.

Because the focus of this study was specifically larval fishes, processing of images specifically targeted larval fishes, thereby eliminating the need to capture and classify all imaged particles (e.g., copepods, larvaceans, medusae, and ctenophores). Consequently, all images were manually reviewed for larval fishes. This process is relatively rapid, although ~3 months were required to complete this task because of the large number of images. Future development of ISIIS will include automated image processing; however, the current manual processing requires viewing each image. When a larval fish was present, that portion of the image was extracted and saved to a file. All fish images were then reviewed for identification to the lowest taxonomic level possible and measured with ImageJ (National Institute of Health public domain Java-based image-analysis program available at http://rsbweb.nih.gov/ij/). Environmental data from ISIIS were interpolated across each transect with a cubic interpolation function in Matlab (vers. 7.11.0.584 [R2010b], The MathWorks, Inc., Natick, MA). The depth and environmental variables associated with each fish larva were obtained by matching time stamps from image and environmental data.

The bongo tows were conducted in standard fashion by following Jossi and Marak (1983). For each tow, the wire was paid-out at a rate of 50 m min^{-1} to a depth of 10 m above the seafloor, then the wire was retrieved to the surface obliquely at 20 m min^{-1}, while the ship moved at 0.75–1.0 m s^{-1}. At completion of each tow, the nets were washed down and the contents rinsed onto a 333-µm sieve. The sample was preserved in 5% buffered formalin. Samples were then sorted for larval fishes under a dissecting microscope and identified to the lowest taxonomic level following Fahay (2007). The 333-µm mesh bongo samples were used for comparisons of the bongo and ISIIS methods since this mesh size is the one that has been used for more than 20 years by the Northeast Fisheries Science Center for ichthyoplankton surveys.

To compare larval fish concentrations, each bongo tow and each ISIIS undulation were treated as replicates. There are potential statistical problems with this assumption, but to date, the decorrelation length scale in ichthyoplankton distributions in the study region has not been calculated. This assumption will be examined in future studies with ISIIS. The larval fish concentrations were transformed by the natural log, and a Shapiro test was performed to test for normality of larval fish concentrations within each gear type. Where the null hypothesis of normality was accepted, a Welch's *t*-test was used to compare larval fish concentrations between transects within gear and then between gear across both transects. Comparisons were made for total larvae, family-level larvae, and species-level larvae both within and between gears for abundance and size differences. In these tests, the nonparametric Kruskal-Wallis test was used because concentrations at the family level were zero-inflated, making transformations to a normal distribution impossible. All counts per tow (or undulation) were standardized to volume sampled (number of fish larvae per cubic meter).

All larvae collected in the bongo net were measured to the nearest 0.1 mm for notochord (preflexion) or standard length under a dissecting microscope with

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

an ocular micrometer. Larvae observed in ISIIS images were measured digitally with ImageJ software after each image was calibrated to standard pixel size. Fishes were measured for notochord or standard length (the position of the posterior end of the hypural plate was estimated if the pigmentation on a fish was too dense for the internal caudal fin structure to be visible). A subset (6 out of 409) of the fish images was discarded because orientation of the fish precluded accurate measurement. Despite our effort to remove such images from measurement, some fish sizes likely were underestimated when the observer was not able to discern the offset that may have occurred where the orientation was not exactly parallel to field of view. Lengths of all larvae were compared between the 2 gears and the 2 transects. To avoid pseudoreplication, the average length of all larvae, family-level larvae, and species-level larvae from a bongo tow or ISIIS undulation was used for comparison. Size distributions were all highly skewed, and therefore a Kruskal-Wallis test was used to compare sizes within and between gear types. Statistical analyses were performed in R software, vers. 2.14 (R Development Core Team, 2011) with the package "plyr" (Wickham, 2011) as well as visualization techniques with the package "ggplot2" (Wickham, 2009).

Results

Along 2 transects, we completed 24 and 12 ISIIS undulations and 6 and 5 bongo tows, respectively. ISIIS sampled an estimated 297 m^3 h^{-1} (or an average of 63 m^3 per tow-yo (i.e., down and up undulation), for a total sampled volume of 2281 m^3. The actual volume sampled was lower than the maximum possible because of a slight misalignment in the mirrors that occluded

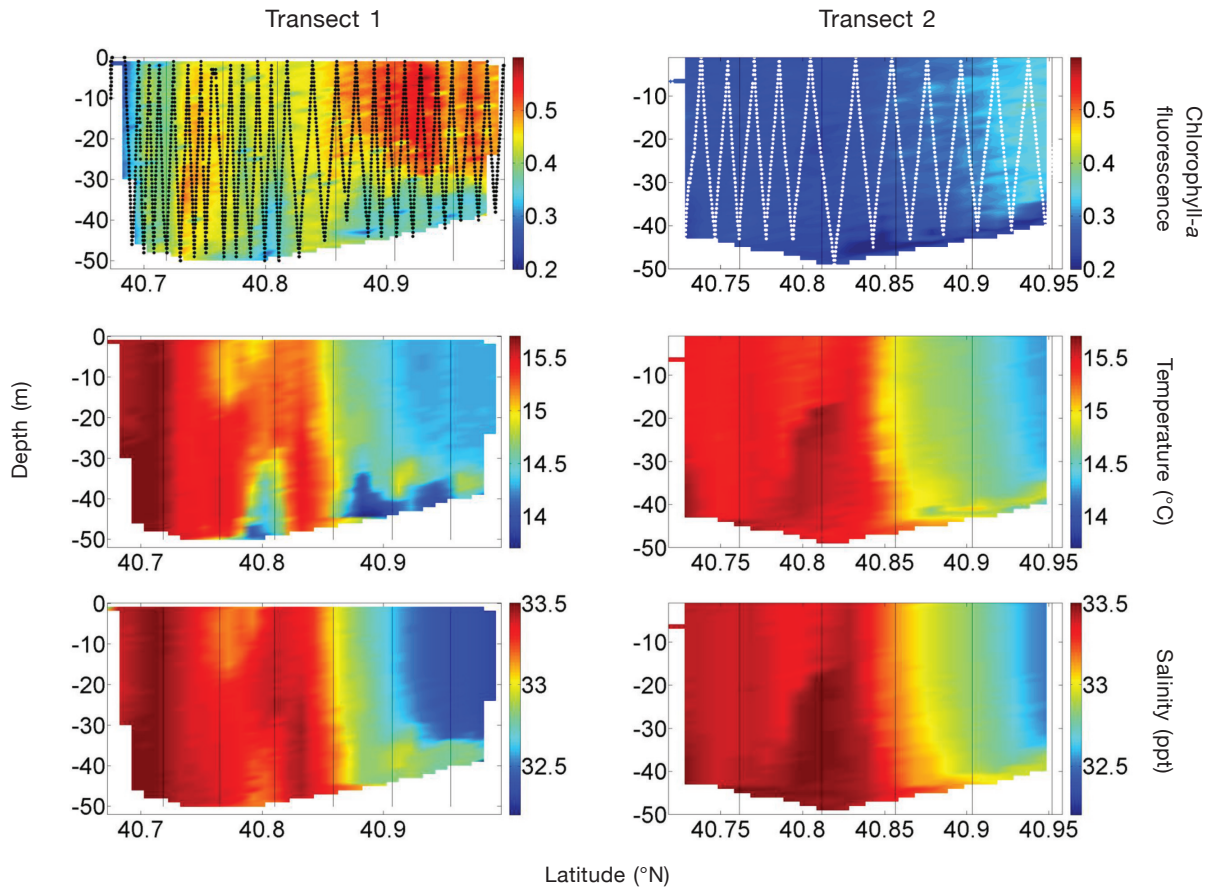

Figure 2

Fluorescence (voltage), temperature (°C), and salinity (ppt) measured from ISIIS along the western (transect 1) and eastern (transect 2) transects during 23–24 October 2008. Dotted lines in the fluorometry panels represent the undulations of the In the Situ Ichthyoplankton Imaging System (ISIIS). The vertical solid lines represent the approximate tow positions for the bongo sampler which was deployed along the length of the same transect once the ISIIS tow was completed.

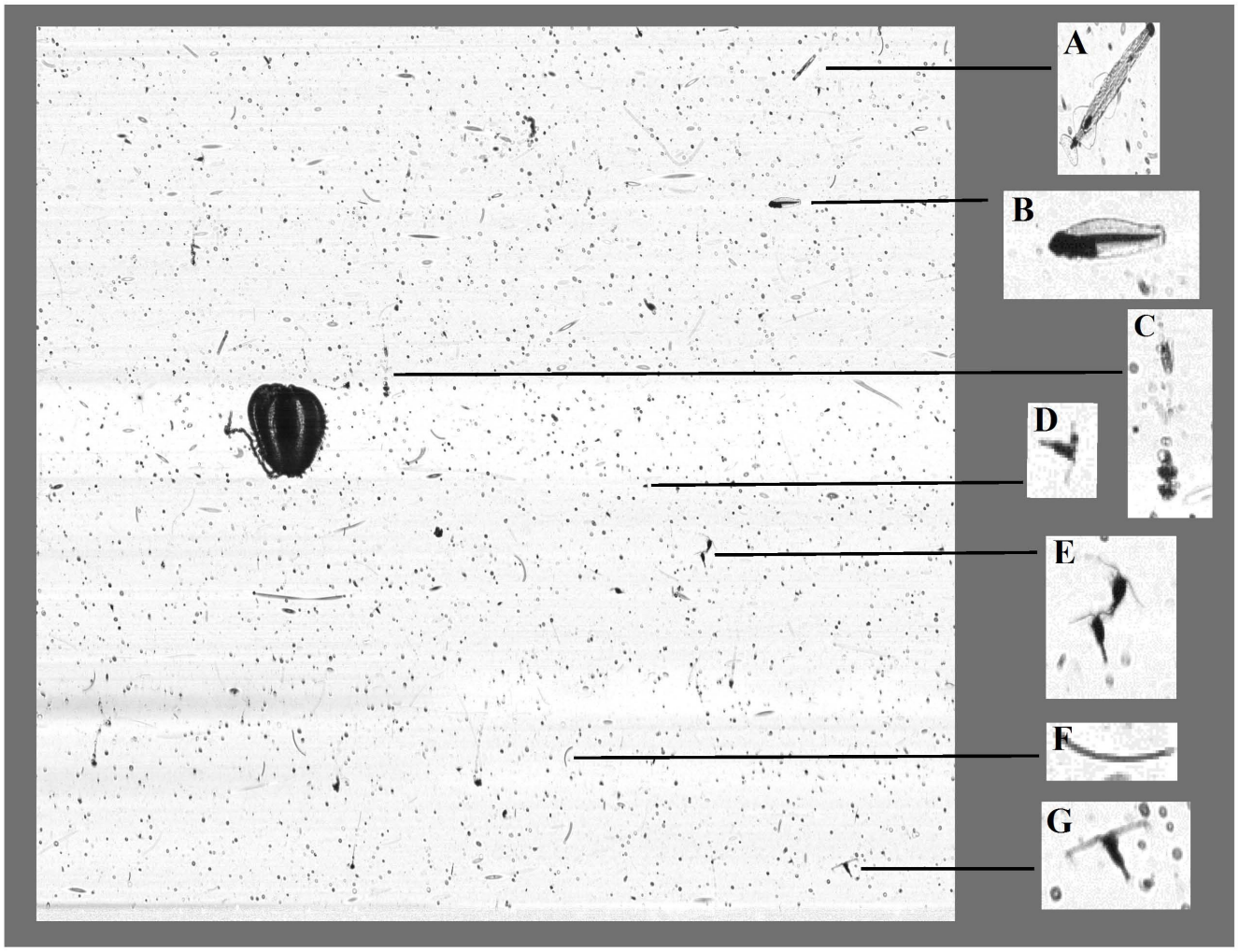

Figure 3

Example of a full-frame image collected with the In Situ Ichthyoplankton Imaging System (ISIIS). Larval fish (small [~4 mm], *Paralichthys dentatus*) and other plankters (especially copepods) are evident throughout. The small circular and elongate particles are diatoms (centric and pinnate) and diatom chains, which can be detected but are too small to clearly resolve. Also seen is a ~1.5-cm ctenophore with tentacles retracted. Several small aggregates (marine snow) are evident in the full-frame image. Overall, the full frame provides a good indication of the plankton field encountered by the observed larval fish. Surface is to the top of the image. Select plankters are shown to the right of the full frame in higher magnification (from top to bottom): (A) chaetognath (note that an improved image has been substituted for demonstration purpose only), (**B**) preflexion stage larval fish, (**C**) marine snow, (**D**) small copepod, (**E**) 2 copepods, (**F**) diatom chain (rotated to fit figure), and (**G**) copepod.

about 15% of the imaging field (i.e., the image field of view was 11 cm versus 13 cm). In comparison, the typical bongo sampled 137 m^3 per oblique tow, for a total volume sampled of 1506 m^3. The maximum depth of tows was 49 m for ISIIS tows and 52 m for the bongo tows.

The water column along both transects was defined by limited vertical stratification, especially in its upper 35 m (Fig. 2). A slight decrease in chlorophyll concentration below a depth of ~35 m in the inshore portion of the easterly transect was apparent and also was observed with a change in temperature and salinity; still, the differences were small. In contrast, considerable horizontal variation (south to north) was observed in hydrography along both transects with temperature lower, salinity lower, and chlorophyll fluorescence higher in the inshore (northern) portions than in the offshore (southern) portions (Fig. 2).

The productivity of the water column was evident in ISIIS imagery as a preponderance of diatoms visible throughout most images (Fig 3). Also imaged were a variety of invertebrate plankters, ranging from copepods and larvaceans to ctenophores and medusae to invertebrate larval types, such as echinoderm pluteus. Because most imagery was dominated by the smaller plankton (diatoms, copepods, and larvaceans;

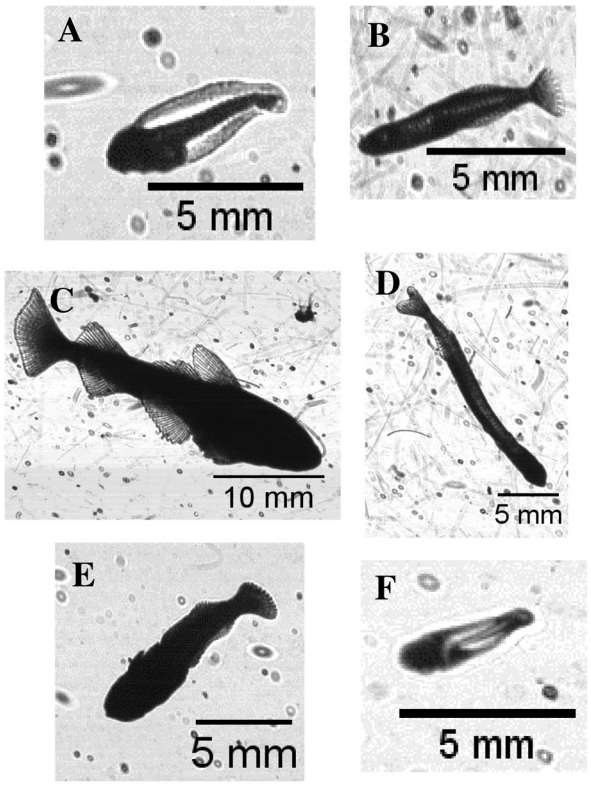

Figure 4

Examples of close-up, in situ images of different larval fish taxa imaged with the In Situ Ichthyoplankton Imaging System (ISIIS). (**A**) *Paralichthys dentatus* (4 mm); (**B**) Gobiidae (8 mm); (**C**) Gadidae (32 mm); (**D**) Clupeidae (21 mm); (**E**) *Merluccius* spp. (14 mm); (**F**) unknown (preflexion stage) (3.2 mm).

see Fig. 3), and larval fishes were relatively rare, the imagery provided a relative measure of abundance of different plankters. In most cases when fish larvae were encountered, the imagery was sufficient to discern characteristics valuable for identification at the family or genus level (e.g., shape, number and location of fins, overall body shape, fish size, and, in some cases, certain skeletal features; see Fig. 4).

The 2 sampling methods allowed us to detect comparable quantities of larval fishes. ISIIS imaged a total of 409 larvae, and the bongo tows collected a total of 359 larvae. When standardized for the volume of water actually sampled, ISIIS estimated ~0.18 fish larvae (±0.015 standard error of the mean [SE] m^{-3}), a value that was not significantly different from the estimate from the bongo tows (0.24 ±0.037 SE m^{-3}; P=0.074). Similarly, within gears, there were no differences in larval fish concentrations between transects.

The estimates of larval abundance, however, were made on the basis of the 2 gears sampling different portions of the water column. The bongo net sampled all depths equally as it was towed from depth to the surface, but ISIIS spent less time at depths >40 m than at depths near the surface (Fig. 5A). This sampling effect is evident in the difference in measured fish abundance by depth (Fig. 5B), where the apparent pattern was for a continual increase in fish abundance with depth from the surface down to 40 m and then a decrease in abundance by depth beyond 40 m. This decrease was directly coincident with the drop-off in sampling time with depth by ISIIS. When an adjusted abundance was estimated by computing depth-specific concentrations (Fig. 5C), then with the assumption of equal sampling effort per depth as with the bongo tows, an adjusted mean ISIIS fish concentration was 0.22 fish larvae m^{-3}, which is very close to the bongo estimate.

The taxonomic diversity collected by each gear also was similar; both collected larval fishes representing the same 7 families (Table 1), although bongo samples were typically identifiable to lower levels (genus and species) than those in ISIIS samples. Images of fish larvae from ISIIS were identifiable to at least the genus level for ~35% of larvae (143 out of 409). On the other hand, larvae were unidentifiable in 60 fish images and most of these unidentifiable fishes were in the early preflexion stages (~15%); in contrast, all bongo tow larvae were identified at least to the family level. Comparison of the relative proportions of taxa between the 2 sampling methods indicates that they were similar. There were a few notable exceptions: ISIIS underestimated paralichthyids and scopthalmids and estimated relatively greater proportions of phycids and ophidiids than the bongo sampler. The total number of larvae sampled was similar, but it is not known if the "unknown" category would have evened these discrepancies or added further differences among certain taxa.

Size distributions of larvae differed considerably between the 2 sampling methods. ISIIS imaged a larger size range and larger mean size of fish larvae than the bongo sampler (Fig. 6, Table 2). This sampling gear pattern was evident across several individual taxa, notably the gadiform fishes, Phycidae and Gadidae, with the latter mean size from ISIIS samples being more than 3 times the mean size of this family from bongo samples (Table 2). There was also a significant difference between gear types with respect to size of Paralichthyidae, although this very small difference (0.103 mm) may not be biologically meaningful and likely was significant only because of the rank nature of the Kruskal-Wallis test. There was a significant difference in overall larval size between transects for the ISIIS samples, but there was no significant difference in overall larval size for the bongo tows between the 2 transects or for any taxonomic group between transect within gear type (Fig. 6, Table 2). Therefore, most of the differences in size were attributed to sampling gear.

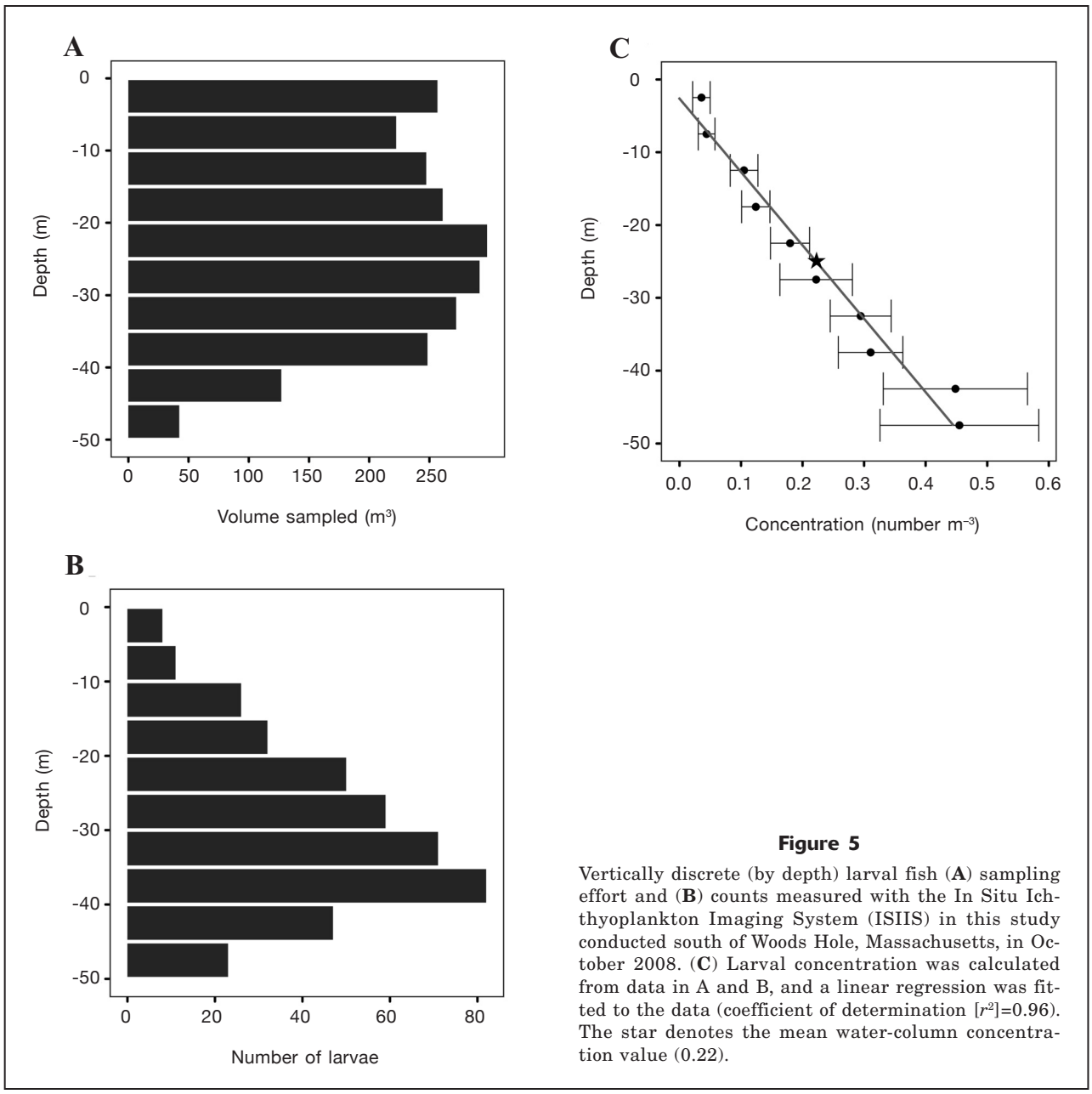

Figure 5

Vertically discrete (by depth) larval fish (**A**) sampling effort and (**B**) counts measured with the In Situ Ichthyoplankton Imaging System (ISIIS) in this study conducted south of Woods Hole, Massachusetts, in October 2008. (**C**) Larval concentration was calculated from data in A and B, and a linear regression was fitted to the data (coefficient of determination [r^2]=0.96). The star denotes the mean water-column concentration value (0.22).

Discussion

Design of larval fish surveys requires a balance of ship time, sample-processing time, and adequate sampling effort for resolution of the spatial (and temporal) variation to provide a robust measure of spatial distribution and abundance of this life-history stage. In essence, survey design is a cost-benefit issue. Greater sampling frequency will improve precision of estimates (e.g., Cyr et al., 1992), but it does so at a cost of greater ship time and laboratory sample processing. Consequently, surveys are limited, in part, by the sampling tool of choice (and its inherent limitations and benefits).

Results indicate that data collected with this prototype version of ISIIS are comparable to data collected with a bongo sampler. Measurements of larval concentrations were similar, although identifications of larvae fish were possible with ISIIS only at a coarser level of taxonomic resolution compared to that with the bongo sampler. In waters with relatively low species diversity of ichthyoplankton, like the shelf of the northeastern United States, the taxonomic resolution possible with ISIIS is adequate for conducting an array of studies, particularly when data are verified with net samples. However, in species-rich waters, the taxonomic resolution possible with ISIIS may limit the applications of

Table 1

(**Upper**): Comparison of taxonomic resolution between bongo and In Situ Ichthyoplankton Imaging System (ISIIS) samples collected south of Woods Hole, Massachusetts, in October 2008 as part of this study. Data are presented as "total," which is the combined lowest level of identification across all taxa; "family," which is a comparison just at the family level (where all taxa are subsumed into relevant family taxa), and "species," where only identifications to species level are presented. (**Lower**): Summary comparison between the bongo sampler and ISIIS gears for number and proportion of identifications at family, genus, and species levels, as well as number and proportion of unknowns.

Taxa	Identification level					
	Total (lowest)		Family level		Species level	
	Bongo	ISIIS	Bongo	ISIIS	Bongo	ISIIS
Clupeidae	1	3	2	3		
Brevoortia tyrannus	1	0			1	0
Gadidae	3	13	3	13		
Merlucciidae	0	0	48	44		
Merluccius bilinearis	48	44			48	44
Phycidae	0	83	48	104		
Urophycis spp.	48	21			48	21
Ophidiidae	0	29	7	34		
Lepophidium profundorum	7	5			7	5
Gobiidae	3	6	3	6		
Paralichthyidae	1	62	217	135		
Citharichthys arctifrons	14	0			14	0
Etropus spp.	10	8			10	8
Paralichthys oblongus	4	0			4	0
Paralichthys dentatus	188	65			188	65
Scopthalmidae	31	10	31	10		
Unknown	0	60	0	60		
Total larvae	359	409				

	Numbers		Proportion	
	Bongo	ISIIS	Bongo	ISIIS
Family	39	206	0.11	0.50
Genus	58	29	0.16	0.07
Species	262	114	0.73	0.28
Unknown	0	60	0.00	0.15
Total	359	409	1	1

the technology. The version of ISIIS used in this study was an early prototype (Cowen and Guigand, 2008); considerable advancements have been made in the image sharpness and depth of field since the field work reported here, and these changes should improve identification of individual fishes, especially of smaller taxa.

Larval lengths were different for ISIIS and the bongo sampler. The bongo sampler collected smaller larvae, indicating limitations with our ISIIS image-processing procedures for recording larval fishes <5 mm (and obvious diagnostic morphological features on small larvae). On the other hand, ISIIS imaged larger larvae, indicating that avoidance of the ISIIS by larger larvae was reduced. With the potential of an increase in image resolution to advance identification of smaller larvae (e.g. the improved image of a chaetognath in Fig. 3, upper right), the overall size range sampled by ISIIS could be a significant improvement over the range of the bongo sampler that has been used by the NEFSC for the past 30-plus years. If there is an effort to merge abundance time series between the bongo and ISIIS, careful calibration studies would be required to account for variances, including length-based, diel, and regional differences in detectability. These types of calibration studies also are necessary to combine data across different mesh sizes of the bongo sampler (see Johnson and Morse, 1994; Richardson et al., 2010).

Our results indicate that ISIIS could be a valuable addition to the survey sampling toolbox because it suc-

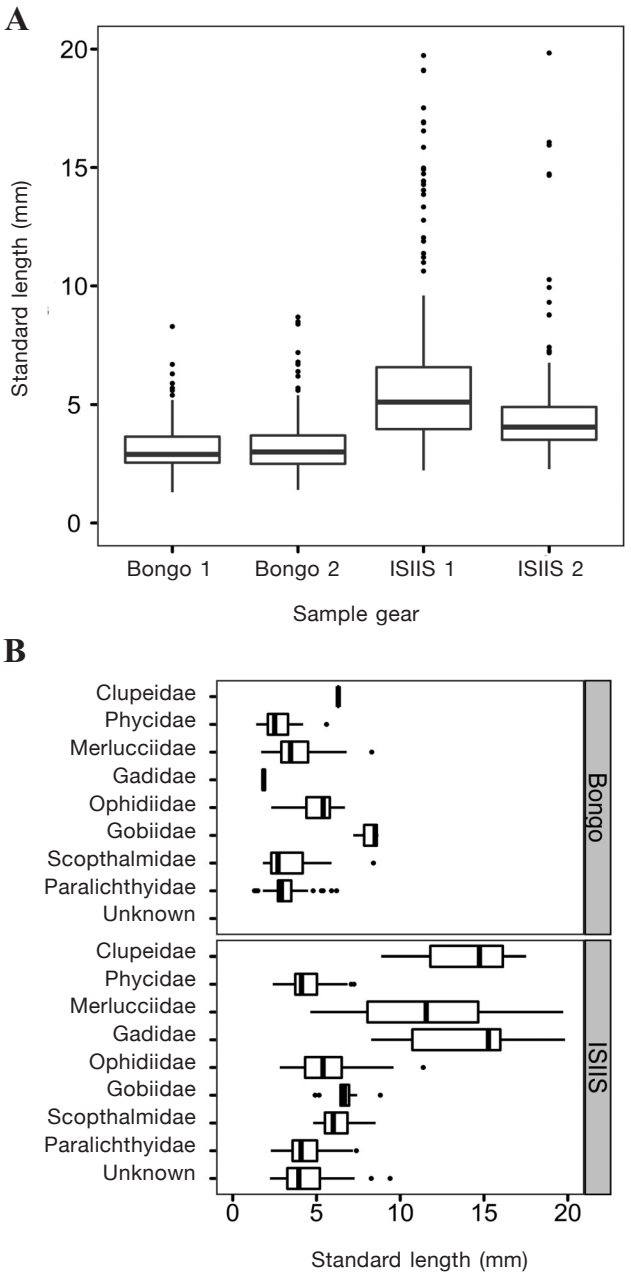

Figure 6

(**A**) Summary statistics (box plot) of larval size distribution by sampling gear and transect (1 and 2). The vertical bars of the box plot represent the range, the box represents the 1st (lower) and 3rd (upper) quartile, and the central (horizontal) line is the median of the distribution of observations. Perceived outliers are denoted as separate points beyond the range. Sampling was conducted with the In Situ Ichthyoplankton Imaging System (ISIIS) and a bongo sampler south of Woods Hole, Massachusetts, in October 2008. (**B**) Taxon-specific comparison of fish lengths between bongo sampler (top) and ISIIS (bottom). Note: the box plots are rotated 90°relative to A; however, basic features are the same as in A.

cessfully has estimated larval fish concentration, and, in an environment of relatively low diversity, as in this study, resolved the taxonomic composition of the larval ichthyofauna. Under such conditions, the rapid sampling speed of ISIIS could be used to increase spatial and temporal resolution of ichthyoplankton patchiness, without the need for additional ship days. For example, rapid undulation of ISIIS resulted in 24 vertical forays through the water column being repeated every 1.7 km along the 41.4-km transect in just 4.6 h. In comparison, 6 bongo tows were completed along the same transect in ~6 h for a spatial resolution of 6.9 km. Therefore, ISIIS can provide 3–4 times the spatial resolution of a bongo sampler over a comparable (or shorter) time frame. Other benefits of ISIIS include its ability to resolve very fine-scale patchiness because its sampling rate is both continuous and rapid. Consequently, depending on how it is towed, ISIIS can be used to assess detailed vertical distributional data, a feat that is not possible with a bongo sampler, or even with opening and closing net systems, without very extensive (and expensive) sampling efforts. Further, simultaneous sampling by other environmental sensors provides detailed concurrent image and physical data. Information about nearest-neighbor scaling and fish larval distribution in relation to their predators and prey, as well as environmental conditions, would be possible because of the fine-scale, in situ information available in the ISIIS imagery. Such sampling with ISIIS would allow targeted, process-oriented studies, even while general survey designs are being employed.

Still, the results of this study indicate several specific functional aspects that need to be considered or addressed for ISIIS to be a highly effective sampling tool for survey and process-oriented studies. First, ISIIS detected fewer smaller larvae than did the bongo sampler. Further, the small larvae detected with ISIIS were largely classified as unknown. These results indicate that the image resolution of ISIIS should be improved to increase the detectability and identification of small larvae, although preflexion larvae will likely always be problematic because of their limited morphological distinctiveness. An increase in detectability will require an increase in the depth of field such that particles that pass between the viewing ports are *all* in focus, thereby eliminating regions of out-of-focus particles that potentially can obscure the remaining image. The current version of ISIIS (ISIIS-2) has been successful at extending the depth of field from ~30 cm to the full 50-cm space between viewing ports, adding to the volume sampled and the overall clarity of imagery (Cowen and Guigand, unpubl. data).

The second issue is the need for rapid, accurate image processing. The large number of images produced makes computer-aided image analysis a requirement for large-scale application of this instrument. We were able to use manual assessment of the images taken in the current study (by focusing only on fish larvae), but further analysis of these data or more extensive surveys

Table 2

Kruskal-Wallis test for comparison of size difference (in mm) by transect and sampling gear for all fishes combined, as well as for the 3 most dominant fish families, from this study where 2 gear types were used: bongo sampler and the In Situ Ichthyoplankton Imaging System (ISIIS), to sample fish larvae south of Woods Hole, Massachusetts, in October 2008. (**Upper**): comparison within gear between transects. (**Lower**): comparison between gears. Asterisks (*) denote significant differences.

	Bongo Transect 1	Bongo Transect 2	*P*	ISIIS Transect 1	ISIIS Transect 2	*P*
Mean size—all larvae	3.514	4.397	0.275	7.223	4.959	0.001*
Paralichthyidae mean size	3.809	5.044	0.547	4.672	4.122	0.061
Phycidae mean size	2.335	2.980	0.221	4.617	4.295	0.199
Merlucciidae mean size	3.998	3.550	0.783	13.701	12.037	0.496

	Bongo	ISIIS	*P*
Mean size—all larvae	3.858	6.468	1.67E-12*
Paralichthyidae mean size	4.385	4.488	0.0001*
Phycidae mean size	2.622	4.486	5.41E-05*
Merlucciidae mean size	3.795	13.398	3.806E-06*

with ISIIS will require automated computer analysis. Several different options may be available for addressing some of these needs (e.g., Davis et al., 2004; Hu and Davis, 2005; Luo et al., 2005;; Culverhouse et al., 2006; Benfield et al., 2007; Zhao et al., 2010), although these alternatives have not been tested with repetitive processing of millions of images. Consequently, we are currently developing and testing algorithms suitable for segmenting and classifying individual organisms from full image files. These algorithms must be capable of processing data at high speeds (or with multiprocessor computers) and must be able to handle large data sets (e.g., Tsechpenakis et al., 2008). With such analysis capabilities, the typical time between research cruise and ultimate data analysis could be reduced greatly.

Conclusion

Although ISIIS can be a powerful tool for resolving fine to mesoscale patchiness in both vertical and horizontal distributions of plankton, it is limited by the fact that it is a nondestructive sampler (i.e., it does not collect specimens). ISIIS will not replace nets for all studies. There is still a strong need for sample collection, whether for identification verification (for larvae or eggs) or for more specific studies, such as projects on food habits, growth, and genetics, that require specimens. In addition, many nets, including bongo nets, can be used by a greater variety of vessels and in a wider range of weather conditions than the ISIIS instrument package. When these different tools are combined, however, ISIIS could be used to establish the vertical and spatial setting of fish larvae. This information could be used to identify locations for targeted net samples. This melding of samplers also would lead to more efficient requirements for ship time and processing time (i.e., less time spent with nets and on processing the survey samples from areas where the targeted specimens are rare or absent). Therefore, ISIIS (and the technology it represents) is a valuable addition to both process-oriented studies and routine surveys. This technology can contribute both to the understanding of the relation between larval fishes and their biological and physical oceanographic habitat and to the quantification of larval fish abundance and distribution for use in stock and ecosystem assessments.

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