Oceanus 39(1): 12 (1996)

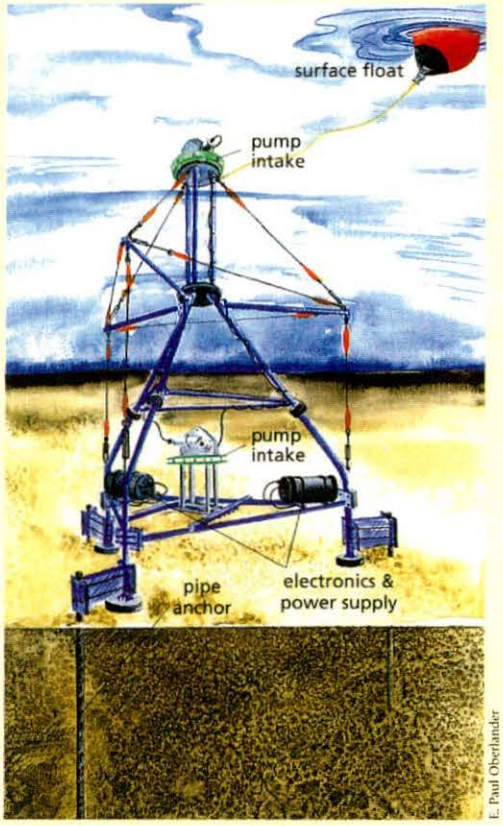
Measuring Diversity of Planktonic Larvae

Catch 'Em (and Identify 'Em) If You Can

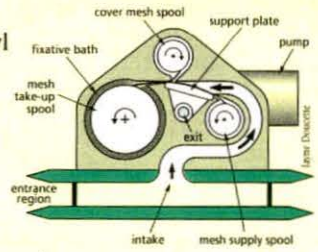
Elizabeth D. Garland
WHOI/MIT Joint Program Student, Biology Department
Cheryl Ann Butman
Associate Scientist, Applied Ocean Physics & Engineering Dept.

Imagine several million microscopic organisms living in a single glass of seawater! This is the emerging picture of planktonic ecosystems in coastal environments. The density of organisms is overwhelming, and most species have unique roles within the ecosystem. Planktonic larvae of benthic (seafloor-dwelling) invertebrates—such as worms, clams, and snails—are only temporary members of the plankton, so their importance in pelagic ecosystems is often overlooked. However, given that many larvae reside in the water column longer than the life spans of many copepods, which are considered a part of the "permanent" plankton, larvae can be important members of the planktonic food web. Larvae also colonize new habitats and replenish adult populations on the bottom. Thus, larvae can influence the diversity of both planktonic and benthic ecosystems.

Unlike the larger permanent zooplankton, many of which may be identified to species in acoustical or optical images (see *Oceanus*, Spring/Summer 1995), planktonic invertebrate larvae are so small (0.01–0.05 centimeters, 0.004–0.02 inches) and the morphological differences between them so subtle, that they must be collected directly to be identified to species. One new instrument, the Moored, Automated, Serial, Zooplankton Pump (MASZP), was designed by co-author Cheryl Ann Butman and WHOI engineer Ken Doherty to make moored, time-series collections of planktonic larvae at time and space scales comparable to data taken, for example, by moored current meters.



E. Paul Oberlander



The Moored, Automated, Serial, Zooplankton Pump tripod can be placed in water depths up to 1,500 meters to collect planktonic larvae for hours to months. The diagram shows the collection path and apparatus.

Traditional zooplankton pumps were tied to a ship's power source because they used very high intake velocities to overcome escape responses of organisms that, ironically, were escaping from flow disturbances created by the pumps! To decrease the intake velocity and thus the power needed for long-term, moored collections, the MASZP "sneaks up" on the larvae before they are able to initiate an escape response. Each discrete water sample is filtered over a portion of mesh, which is covered by another piece of mesh, and the two strips are wound together on a spool residing in a preservative bath for *in situ* storage. The sampling schedule and volume are programmed beforehand on an internal computer. The material collected is later washed from the mesh, and the larvae are sorted from the samples by hand for microscope identification—a very time-consuming and costly process.

To expedite sample processing, we are working with a biotechnology company, Hydros Inc. (Falmouth, Massachusetts), to develop species-specific "immunofluorescent" markers for larval identification. Immunological techniques for recognizing species in mixed populations require the presence of unique, diagnostic "signature proteins" within a given larval species. These proteins, called *antigens*, when injected into a vertebrate host (such as a rabbit or goat), trigger an immune response, and antibodies are released in the host to confer immunity to the antigen. The host antibodies can be isolated, purified, and tagged with fluorochromes—color-coded molecules that can be detected visually. When fluorochrome-labeled, species-specific antibodies are added to a mixed species assemblage in a MASZP sample, the antibodies recognize and bind to all individuals of that species, and they fluoresce as tiny dots in the sample dish. We hope to develop a palette of species-specific immunofluorescent probes that will paint the various species different colors in MASZP samples. A color image-analysis system will then count the number of each color-coded species from a video taped image of each sample. Thus, we are approaching the complete automation of sampling and identifying planktonic larval distributions!