A World of Small Discoveries: Zooplankton Barcoding

by Rob Jennings

It is early enough in the morning that, even if you looked off the deck of the ship as it rolls gently in the warm waters of the Sargasso Sea, you could not tell where the sun will rise. Despite the hour, I am part of a team of fellow scientists and crew members assembled at the stern of the ship, ready with buckets of cold sea water as the winch draws meter after meter of steel conducting wire up from the depths. Our excitement is palpable as a huge, 10-meter-square frame of metal—the front end of the gear that collects our sample—appears at the sea surface, the long mesh nets trailing only half-visible below. The frame and nets comprise a MOCNESS (Multiple Opening/Closing Net and Environmental Systems Sampling), and this particular MOCNESS has been down thousands of meters in the Sargasso Sea—to just above the ocean floor—collecting tiny invertebrate organisms, many of which are rarely seen alive and intact.

At the tapered far end of each net is a sort of small plastic bucket, called a cod end, where all of the organisms captured by the net are collected. It is these cod ends that we are anxious to remove and take with us, bathing the organisms inside with the cold seawater in which they are used to being immersed. Once the net system is safely stowed on deck and the cod ends chilled in their separate buckets, the real work begins.

The cod ends are full of zooplankton, the small invertebrates that spend their entire lives suspended in the waters of the world’s oceans, and they are the reason we are spending 30 days here in the Sargasso, the region roughly east of Florida and north of the Caribbean. Our scientific team is part of the Census of Marine Zooplankton (CMarZ), which in turn is part of the Census of Maine Life (CoML), an international consortium of scientists tasked with finding and enumerating all of the species of zooplankton in the world, from the North Pole to Antarctica and from the surface to the deepest ocean waters. Once we get our precious samples safely into the scientific labs on the ship, we will preserve half of the zooplankton for future research, and immediately begin sorting through the other half, trying to see as much “real live zooplankton” as possible before they begin to die and degrade.

Our work for the Census may seem a bit shocking, or maybe even unneeded. Haven’t we been exploring the oceans for long enough now that there are no new surprises? On the contrary—most people would be shocked to realize how little we know about what is out here in the oceans, particularly away from coastal regions, and beneath the easily-sampled surface waters.

Some 7000 different species of zooplankton are known—spanning a huge amount of diversity, from the beautiful and delicate gelatinous ctenophores (“comb jellies”), which beat their long rows of comb-like paddles to propel themselves around, to the shrimp-like euphausiids called krill, which are the main food source for many of the world’s whale populations. But at least another 2000 species are estimated to be “currently unknown”, waiting to be discovered in remote parts of the ocean by cruises such as ours. Part of the mission of CMarZ is to build excitement at this frontier-like world of discovery. Most of us are accustomed to knowing the organisms around us on land—the familiar grass, trees, birds, squirrels, etc. But imagine driving to work or school in the morning, and not recognizing three out of...
An individual arrowworm, belonging to the biological genus *Eukrohnia*, is one of the myriad zooplankton found during an April 2006 CMARZ cruise aboard the NOAA vessel *Ron Brown*.

*Atlanta peroni,* a pteropod (or “wing-head” mollusk, must be an artist’s dream. This animal swims, coiled shell and all, using the long wings near its head as oars.

every ten organisms you saw! Sometimes your reaction would be, “I’ve seen squirrels, but never a squirrel with fur *that* color!” But other times, you would only be able to say, “I don’t even know what the closest kind of animal is to… whatever *that* is over there!” And now imagine if, *every time* you drove to work, the same thing happened.

The goals of CMARZ and the larger Census are even more crucial in light of the current state of human affairs. We are just now seeing the implications of decades of pumping carbon dioxide into the atmosphere. Fishing technology has allowed us to reach more and more remote habitats, and our harvesting of these marine communities has far-reaching effects on which species thrive and which suffer. The makeup of the oceans could be changing (albeit slowly) right beneath our hull as we sail through these waters… and with so many undiscovered species in the ocean, we want to finish our census quickly. We have to know how much is out here, in order to fully realize what is at stake—how much could disappear from the oceans.

It is both of these things—the sense of discovery, of seeing such beautiful organisms freshly plucked from their normal environments, but also the size and importance of the task before us—that pulls us to the middle of the ocean. “Oh, just find *everything* out there and tell me where it is!” can seem like an overwhelming task. And that is why CMARZ has turned to some innovative approaches. We are not only traveling the oceans in search of zooplankton; we are *barcoding* them.

Wait… does this mean that you’ll begin seeing jellyfish at the beach with a series of black-and-white bars tattooed on them? No, no, no… you can’t see our barcodes with your eyes… and they are a natural part of each species of zooplankton already! Our barcodes are short pieces of DNA from each organism’s own genome… a small part of one particular gene with some very useful properties. The DNA sequence of this gene—the series of A’s, C’s, G’s, and T’s, make a different pattern for each different kind of zooplankton. What does this mean? Well, just like the barcodes on the boxes of food at the grocery store, the different DNA barcodes help us identify *this* kind of krill from...
Rob Jennings, logging data for sequencing DNA from zooplankton aboard ship.

that kind of krill. So on this ship, our team is made up of taxonomists—scientists who can look carefully at our zooplankton under the microscope and tell exactly what species it is, and molecular biologists—scientists who can take a small piece of the organism and determine its DNA barcode. CMarZ combines these two kinds of knowledge, the details of its body morphology and the details of its DNA, to understand who exactly these zooplankton are. And not only does that help us better understand the species we've known about for decades… it helps us discover more of those 2,000 “we know you're out there!” new species even faster.

So what happens to these beautiful, colorful, charismatic organisms after we bring them up from the depths and take a good close look? For the first time in zooplankton history, instead of bringing the organisms back to the DNA lab on land, we have brought the DNA lab out to sea. The company who makes our DNA sequencing machine thought we were crazy—and even laughed at me when I suggested it!—but we knew we could make it work… and it has been working! Having a DNA lab right on the ship presented some new challenges, because none of the equipment was designed to rock back and forth, or be surrounded by wet, salty air. But we knew it would help us do our work even faster. When those zooplankton come up into the bright light, the warm air and water, and the lower pressure at the surface, most of them are not happy. Even when we put them back in cold seawater under our microscopes, we have limited time before they begin to really suffer, and need to be preserved in ethanol so we won't lose them entirely. But even preserved, their DNA slowly degrades, breaking down to the point where we can't get our barcode. That is why we decided to bring the whole process out to sea. With all the knowledge and equipment right there, we don't have to worry about old, preserved organisms that have been sitting on some dusty shelf, losing their DNA and turning into a pile of goo. We have everything at our fingertips, and the results are clearly better—more intact organisms, higher quality DNA, and less uncertainty about who's who.

The synergy that results, when two pieces come together and produce more than either one could alone, has opened up some interesting new doors. We think that some of those “elusive 2,000” have turned up right under our microscopes. For example, scientists have known of the organism Atlanta inclinata for many years. It is a kind of marine snail, called a pteropod, that swims around—coiled shell and all!—using the long wings near its head as oars (“pteropod” means “winged head”). But our scientific team noticed in the fresh collection that some of these snails had transparent appendages while others had gold-colored appendages. So what, right? Some people have blue eyes and some have green. Well, when we analyzed the DNA barcode for the clear and the golden type, they were very different. We might have been calling two different species Atlanta inclinata when they're not the same things at all. And after sitting around in alcohol for months as specimens usually do before an expert can take a good look at them, who knows if the striking color difference would still be detectable? So out here on the rolling waves, we may be breaking new “ocean”, but the opportunities are potentially a gold mine.

About the Author:

Robert Jennings is a post-doctoral fellow working with Ann Bucklin (UConn Marine Sciences), and the Census of Marine Life’s marine zooplankton unit. Their goal is to assess the biodiversity of animal plankton throughout the world ocean. Rob’s work focuses on the genetics and evolution of invertebrates in the sea.