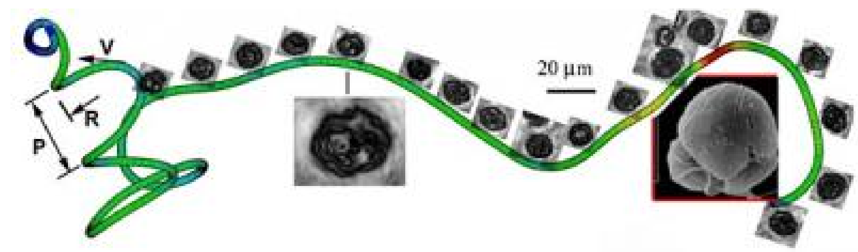
Researchers view swimming tactics of tiny aquatic predators

October 24 2007



A typical trajectory of K. veneficum along with reconstructed in-focus holographic images of the cell at selected locations. Insert: SEM images of K. Veneficum. © 2007 by the National Academy of Sciences of the USA. Insert courtesy of Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute.

By applying state-of-the-art holographic microscopy to a major marine biology challenge, researchers from two Baltimore institutions have identified the swimming and attack patterns of two tiny but deadly microbes linked to fish kills in the Chesapeake Bay and other waterways.

The study, reported in the October 22-26 online Early Edition of *Proceedings of the National Academy of Sciences*, focused on the aquatic hunting tactics of two single-celled creatures classified as dinoflagellates. These two-tailed microbes feed on even smaller prey that are attracted to the algal blooms caused by water pollution. Scientists are concerned because these dinoflagellates produce toxins that can kill large numbers of fish, but studying the predators under a conventional microscope is difficult because the tiny animals can quickly swim out of the microscope's shallow field of focus.

In the journal article, the researchers from The Johns Hopkins University and the University of Maryland Biotechnology Institute reported that they had solved this depth-of-field problem through a technique called digital holographic microscopy, which captured three-dimensional images of the troublesome microbes. The process also enabled the team to identify the tiny predatorsï¿½ distinctly different swimming and hunting tactics.

'It's like being at NASCAR with a 'magical' pair of binoculars that can keep the entire field of view in focus, so cars both near and far are equally sharp and discernible,' said Robert Belas, a professor of microbiology at UMBI's Center of Marine Biotechnology. 'Digital holographic microscopy offers dramatic increases in depth-of-field.'

'This is a breakthrough technology in quantifying dinoflagellate behavior,' said Allen R. Place, a professor of biochemistry at UMBI's Center of Marine Biotechnology. 'We can now begin to look for answers that were previously unattainable.'

Chesapeake Bay fish kills caused by dinoflagellates are considered such a critical issue that Place and his colleagues at UMBI in 2006 were awarded a $1 million National Science Foundation grant to study the biology of this problem. The same microorganisms found in the bay are believed to also pose a threat to fish elsewhere.

The research is believed to represent a milestone in the application of in-line digital holographic microscopy. This technique consists of illuminating a sample volume with a collimated laser beam and recording the interference pattern generated by light scattered from organisms with the remainder of the beam. The interference pattern 'the hologram' is magnified and recorded by a high-speed digital camera. Computational reconstruction and subsequent data analysis produces three-dimensional views of activity within a small sample of water.

'What's unique is that we were able to use this technique to study the behavior of organisms that are congregated in a dense suspension,' said Joseph Katz, who is the William F. Ward Sr. Professor in the Department of Mechanical Engineering at Johns Hopkins. 'We were able to simultaneously track thousands of these dinoflagellates over time and in three-dimensional space. And we were able to follow individual microorganisms as they swam in complex helical patterns.' Katz's group has received several grants to develop and implement digital holography as a means of tracking particles, droplets and organisms in various flows, including an NSF grant to measure behavior of micro plankton such as dinoflagellates in the ocean.

The lead author of the PNAS article was Jian Sheng, who conducted research and developed the software while earning his doctorate in mechanical engineering in Katz's lab at Johns Hopkins. Sheng currently is an assistant professor at the University of Kentucky and a visiting scientist at Johns Hopkins.

For this project, the team focused on two toxic dinoflagellates: Karlodinium veneficum and Pfiesteria piscicida, both of which feed on somewhat smaller non-poisonous microbes commonly found in algal blooms. In Katz's lab, the researchers recorded cinematic digital holograms of the two predators alone and in the presence of prey. They found that when a potential meal was nearby, the predators abandoned their random swimming and clustered around their prey. The team also discovered that Karlodinium microbes moved in both left- and right-hand helices, while the Pfiesteria swam only in right-hand helices. In addition, the researchers saw starkly different hunting tactics. The

Karlodinium appeared to slow down and wait to ï¿½ambushï¿½ its prey; the speedier Pfiesteria was a more active hunter, increasing its speed and radius of helical trajectories while pursuing its prey.

Just like lions might shift into 'stealth mode' when tracking a herd of impala on the African plains, microscopic predators apparently also need to alter their behavior in order to bring down their tiny prey, the researchers concluded. In the fluid realm of fast-swimming microbes, the scientists said, this study has shown for the first time just how the dinoflagellate predators respond to cues and alter the way in which they swim to become more formidable hunters.

Gaining a better understanding of the behavior of these microbes may lead to new ways to avert the fish kills attributed to dinoflagellate toxins.

Source: Johns Hopkins University