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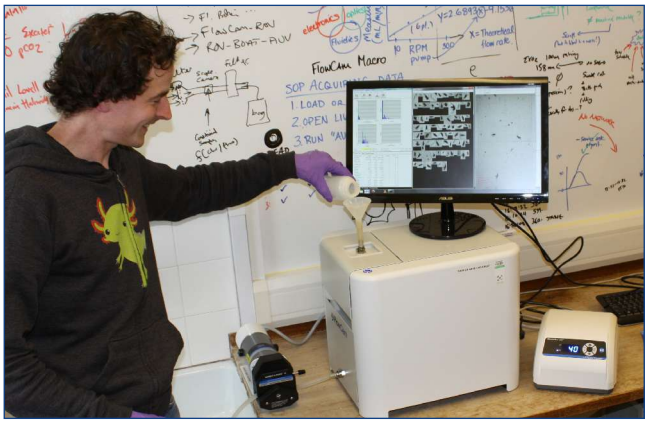
Instrumentation Advance Speeds Plankton Study

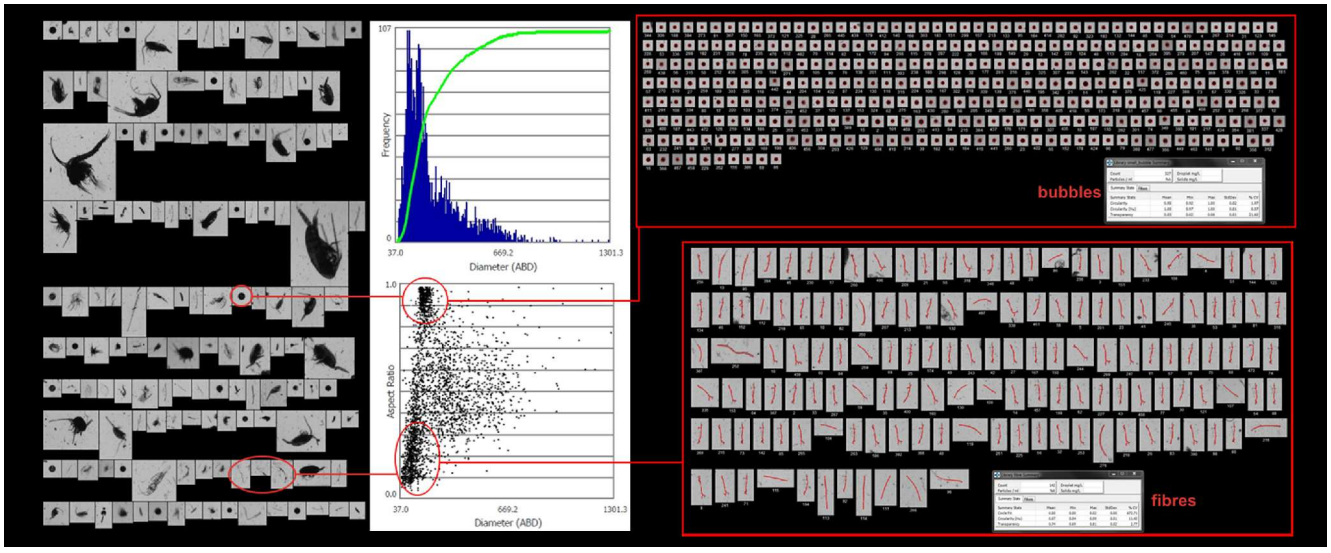
SAHFOS Climate Change Researchers Put New FlowCam Macro on Trial

By Dr. George Graham • Robert Camp

Monitoring the impact of anthropogenic climate change on the world's oceans requires accurate observations of how the Earth system changes over time. Since 1958, the Sir Alister Hardy Foundation for Ocean Science (SAHFOS) has been continually observing changes to the foundations of the marine ecosystem by gathering measurements of marine plankton and assessing its overall health, abundance, community structure and distribution across the globe. The foundation makes its data available to researchers and policy makers worldwide to help assess changes in the health of marine plankton in response to a range of physical, chemical and biological factors.



Marine plankton are especially sensitive to changes in temperature, light, acidity and nutrient availability, among other environmental variables, and respond quickly to a variety of stressors. By observing the response of plankton, scientists can document the impact of climate change on the base of the marine food chain. As the source of approximately half of the oxygen generated on Earth and responsible for removing approximately half of the carbon dioxide produced from burning fossil fuels, marine plankton play a critical role in maintaining the ratio of oxygen to carbon



(Top) A laboratory technician operates the FlowCam Macro installed at the SAHFOS laboratory in Plymouth, England. (Bottom) Observations of particles from a conventional continuous plankton recorder (CPR) sample obtained with the FlowCam Macro. Images are shown of plankton (left panel), descriptive statistics (central panel), and images grouped into classification types (right panel).

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dioxide in the atmosphere and in sustaining life of all kinds on Earth.

CPR Survey

To study marine plankton, SAHFOS operates the Continuous Plankton Recorder (CPR) Survey, recognized as the longest running, most geographically extensive marine ecological survey in the world. To advance its observational capabilities, SAHFOS is exploring the latest in autonomous technology for rapid particle counting and discrimination in order to help monitor the health of the marine environment.

In the CPR survey, SAHFOS involves a global network of more than 25 volunteer merchant vessels, cargo ships and ferries in gathering the plankton for analysis. These vessels tow the organization's proprietary CPR instrument as they sail the North Sea, North Atlantic, North Pacific and Southern Ocean around Antarctica. Operating at a depth of 5 to 10 m, the CPR filters plankton from the water and stores it on a continuously advancing roll of silk mesh. When returned to the SAHFOS laboratory in Plymouth, England, the filtering silk mesh is divided into samples representing the plankton gathered over 10-naut. mi. sections.

Since the 1950s, a dedicated team of nearly a dozen marine taxonomists have analyzed the samples using traditional light microscopy to identify and count both phytoplankton (plant) and zooplankton (animal) taxa, with almost 800 taxonomic entities routinely identified. For sample to sample consistency, this system has remained virtually unchanged since it was established. However, manually reviewing upwards of 5,500 plankton samples per year and identifying each species takes significant amounts of time. Innovation in this process to generate rapid monitoring capability and reduced cost is being explored by supplementing the existing methodology with the latest, autonomous technology.

Integrating Manual, Automated Processes

A significant international project driving this need is called AtlantOS. An undertaking of the European Union, AtlantOS was launched to achieve a transition from a loosely coordinated set of existing ocean observing activities producing fragmented, often monodisciplinary data, to a sustainable, efficient and fit-for-purpose Integrated Atlantic Ocean Observing System (IAOOS). Citing the CPR's many years of available data, AtlantOS incorporates the SAHFOS CPR data in its work packages as a key component in improving, modernizing and integrating ocean observation data. Ultimately, AtlantOS aims to deliver an advanced framework for the development of an Atlantic Ocean Observing System that goes beyond the state of the art and leaves a legacy of sustainability after the life of the project. As a member of the AtlantOS consortium, SAHFOS began exploring the feasibility of integrating new instrumentation for the rapid determination of zooplankton abundance as a complement to the manual taxonomic analysis using conventional microscopes.

Several types of instrumentation have been investigated, including systems developed specifically for the marine industry, as well as those developed for pharmaceutical or heavy industry. A range of imaging systems from flatbed scanning technology to automated, in-situ holographic imaging are being explored to measure individual micro-organisms. For example, the FlowCam particle imaging and analysis system is currently involved in a trial at SAHFOS. Originally developed at the Bigelow Laboratory for Oceanographic Sciences in Boothbay Harbor, Maine, to combine high-speed imaging, flow cytometry and microscopy in one unit, the FlowCam system can automatically detect individual particles in a sample, take a high-resolution, full-color, digital image of each one, and record more than 30 different types of measurements per particle, saving the data for later analysis. Parameters may include traditional measurements such as count, size and volume; advanced, morphological measurements such as circle fit, perimeter and roughness; spectral

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measurements addressing color and intensity; and a series of other measurements based on fluorescence. The system is capable of imaging and characterizing thousands of particles per second in real time and of differentiating particle types in a heterogeneous sample. By developing image libraries comprising individual types of particles and/or micro-organisms, the FlowCam can automatically identify and classify the particles as they are imaged. The system is in use in more than 50 countries on all seven continents by organizations such as the U.S. Naval Research Laboratory, Scripps Institution of Oceanography, the National Institute of Oceanography in Goa, India, and the Chongqing Institute of Environmental Science in Chongqing, China.

The FlowCam is manufactured by Fluid Imaging Technologies of Scarborough, Maine. Researchers at SAHFOS had been following the company as it introduced a variety of different models, each developed for studying different types and sizes of particles. Most models were designed to excel in imaging targets ranging from 2 µm to 2 mm in size. This would be fine for studying phytoplankton, but it is too small a window to offer significant value in studying larger zooplankton.

Fluid Imaging recently introduced the FlowCam Macro, designed specifically for studying larger particles ranging from 500 µm to 5 mm in size—ideal for studying zooplankton. The FlowCam Macro was selected for SAHFOS feasibility testing, and Fluid Imaging provided training to the SAHFOS team via a series of webinars and a three-day, on-site workshop to aid in determining the ideal setup configuration for the AtlantOS project.

How the FlowCam Is Deployed

Installed at the SAHFOS laboratory, the FlowCam Macro offers a variety of customizable settings that may be adjusted based on the target group of zooplankton, concentration of particles or other questions to be investigated. These settings may be saved for easy reuse. The unit commonly operates in the fully automatic mode for maximum speed. The FlowCam has proven to consistently produce high-quality images of large zooplankton such as euphausiids, copepods and hyperiids. These are sampled from CPR routes in the North Atlantic waters from a ship transiting from the eastern United States to Iceland.

Since FlowCam measurements are based on the images, the higher quality the image, the more accurate the derived particle metrics. To use it, once the sampling parameters are set up, an operator pours a CPR sample into a funnel atop the unit and presses the start button. Then, images begin appearing on screen along with their corresponding data sets. With the FlowCam's patented companion software, called VisualSpreadsheet, the operator can acquire data at the click of a mouse and inspect individual particle images to see their measurements or filter similar particles. Alternatively, the operator may click directly on a graph or scattergram to reveal the underlying images and data. In addition, the operator may exclude particles that are not of interest to analyze those that are of interest, in real time, if desired.

Importantly, once data acquisition is complete, VisualSpreadsheet allows reanalysis to be conducted to explore the impact of software configuration changes on derived particle metrics without risk of compromising the raw data. This level of speed and convenience in data acquisition is not feasible with manual methods. At SAHFOS, laboratory technicians apply a range of software filter settings to the raw data based on particle size, shape, transparency or aspect ratio to identify and remove extraneous particles, such as fibers and air bubbles that would skew the data. This allows a clearer view of a subset zooplankton of interest. With the filtered data, expert staff taxonomists review the images, identify the types of zooplankton and develop image libraries in order to teach the FlowCam to use its pattern recognition system to identify these groups automatically when running samples in the future. The data can be exported in common spreadsheet and image file formats for sharing with colleagues worldwide.

Conclusion

The information obtained from samples run on the FlowCam is contributing to the fundamental marine observation work carried out by SAHFOS and the collection of bulk zooplankton data needed to support the AtlantOS project in answering challenging questions about the impact of climate change on marine ecosystems. Rapidly and automatically determining the abundance and biovolume of different zooplankton improves calculations of total carbon concentrations and estimates of carbon transport from the surface to the deep sea. The speed, efficiency and reliability of data acquisition are paramount, and automated systems such as the FlowCam are accelerating the pace of research into the health of fundamental components of the marine ecosystem. **ST**

Dr. George Graham is the instrumentation data scientist at SAHFOS and a seagoing physical oceanographer specializing in the measurement, characterization and monitoring of suspended particulate matter in the coastal and open ocean using novel imaging, optics and acoustics systems. He has 16 years of academic and research consultancy experience and holds an M.S. in applied marine science and a Ph.D. in physical oceanography.

Robert Camp is a plankton analyst and instrumentation technician at SAHFOS. He has 15 years' experience as an instrument technician in the fields of clinical medicine and environmental science, with a particular focus on telemetry. He is an experienced plankton analyst trained in taxonomic identification of Atlantic and Pacific Ocean species.