Igniting research on the open seas

Fluorescence measurements are helping a microbial ecologist to investigate the undersea environment, studying microbial enzyme hydrolysis in subseafloor rocks, and how microbes respond to oil spills.

Subseafloor rocks play host to a range of novel microbial communities

The International Ocean Discovery Program (IODP) operates scientific cruises that give researchers the opportunity to explore subseafloor sediments and rocks, and the subseafloor environment. Scientists from around the world gather samples for their individual projects, performing some initial analyses on-board ship, followed by further studies back on dry land. Assistant Professor Jason Sylvan from the Department of Oceanography at Texas A&M University took part in a recent IODP drilling expedition, the first of a series of cruises to gather samples from the boundary between the ocean crust and the mantle below. Samples from these subseafloor rocks were then analyzed on board, using fluorescence-based assays to study the rates of phosphatase activities in the microbial communities encountered. Jason explained: “People have been trying to investigate the boundary between the ocean crust

and the mantle for a long time. This expedition visited an area of the Indian Ocean south east of Madagascar, where this boundary is shallower than in most other places – around 5 kilometers below the seafloor – making it easier to obtain samples."

Jason continued: "Samples are obtained by drilling into the rock beneath the ocean, collecting sequential 10 meter sections of rock which are then divided into 1.5 meter sections for analysis. As a microbiologist, I have to ensure that my samples are not exposed to contamination from the air or manual handling, and so immediately place one sample per core in a bag to keep it clean, rinsing it well with distilled water before analysis to wash off anything that might be on the outside. Working in a filter-equipped enclosure that removes particulates in the air, we break open the rock and take samples from its center, ensuring that we are only examining *in situ* communities."

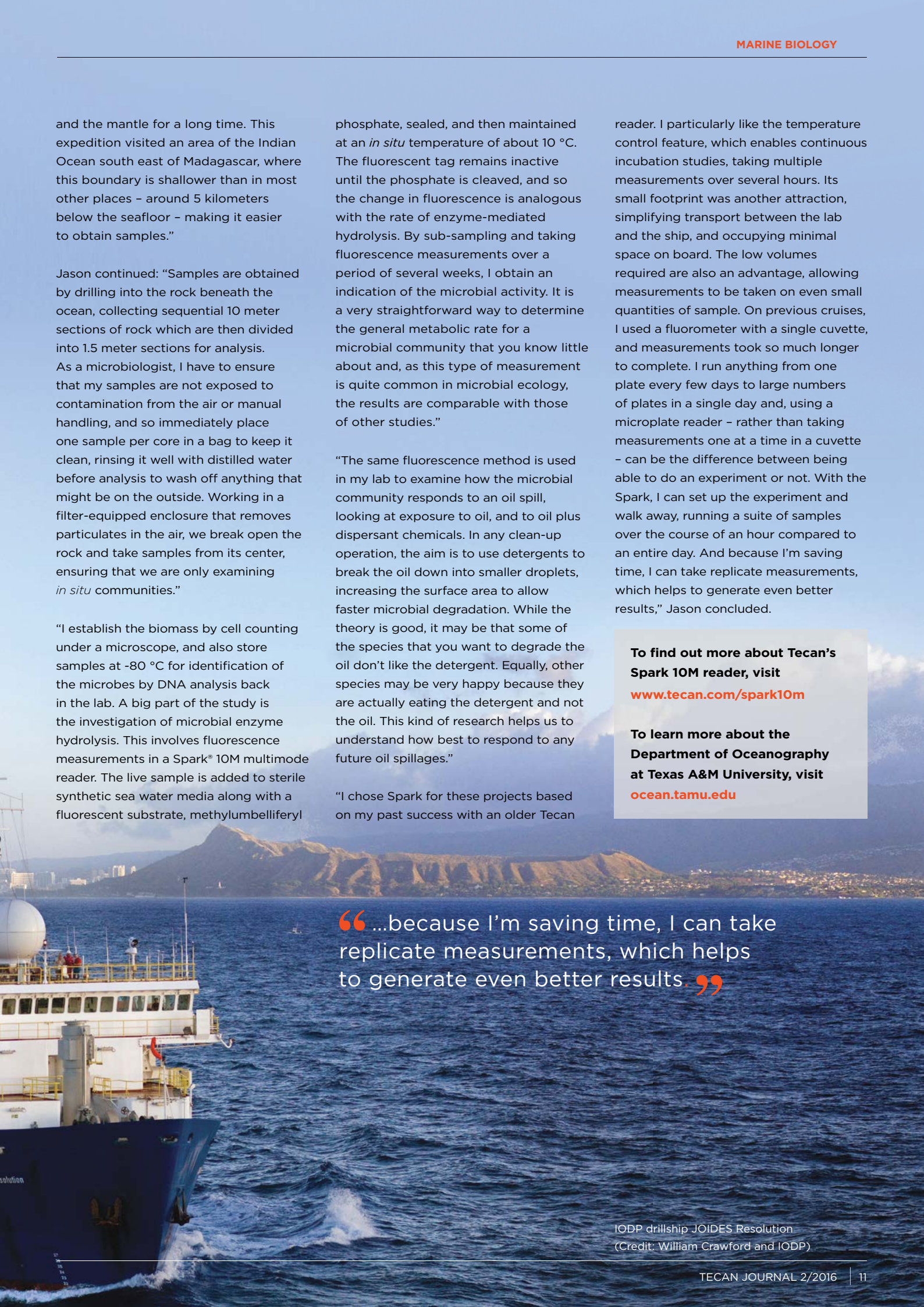
"I establish the biomass by cell counting under a microscope, and also store samples at -80 °C for identification of the microbes by DNA analysis back in the lab. A big part of the study is the investigation of microbial enzyme hydrolysis. This involves fluorescence measurements in a Spark® 10M multimode reader. The live sample is added to sterile synthetic sea water media along with a fluorescent substrate, methylumbelliferyl phosphate, sealed, and then maintained at an *in situ* temperature of about 10 °C. The fluorescent tag remains inactive until the phosphate is cleaved, and so the change in fluorescence is analogous with the rate of enzyme-mediated hydrolysis. By sub-sampling and taking fluorescence measurements over a period of several weeks, I obtain an indication of the microbial activity. It is a very straightforward way to determine the general metabolic rate for a microbial community that you know little about and, as this type of measurement is quite common in microbial ecology, the results are comparable with those of other studies."

"The same fluorescence method is used in my lab to examine how the microbial community responds to an oil spill, looking at exposure to oil, and to oil plus dispersant chemicals. In any clean-up operation, the aim is to use detergents to break the oil down into smaller droplets, increasing the surface area to allow faster microbial degradation. While the theory is good, it may be that some of the species that you want to degrade the oil don't like the detergent. Equally, other species may be very happy because they are actually eating the detergent and not the oil. This kind of research helps us to understand how best to respond to any future oil spillages."

"I chose Spark for these projects based on my past success with an older Tecan reader. I particularly like the temperature control feature, which enables continuous incubation studies, taking multiple measurements over several hours. Its small footprint was another attraction, simplifying transport between the lab and the ship, and occupying minimal space on board. The low volumes required are also an advantage, allowing measurements to be taken on even small quantities of sample. On previous cruises, I used a fluorometer with a single cuvette, and measurements took so much longer to complete. I run anything from one plate every few days to large numbers of plates in a single day and, using a microplate reader – rather than taking measurements one at a time in a cuvette – can be the difference between being able to do an experiment or not. With the Spark, I can set up the experiment and walk away, running a suite of samples over the course of an hour compared to an entire day. And because I'm saving time, I can take replicate measurements, which helps to generate even better results," Jason concluded.

To find out more about Tecan's Spark 10M reader, visit
www.tecan.com/spark10m

To learn more about the Department of Oceanography at Texas A&M University, visit
ocean.tamu.edu



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IODP drillship JOIDES Resolution
(Credit: William Crawford and IODP)