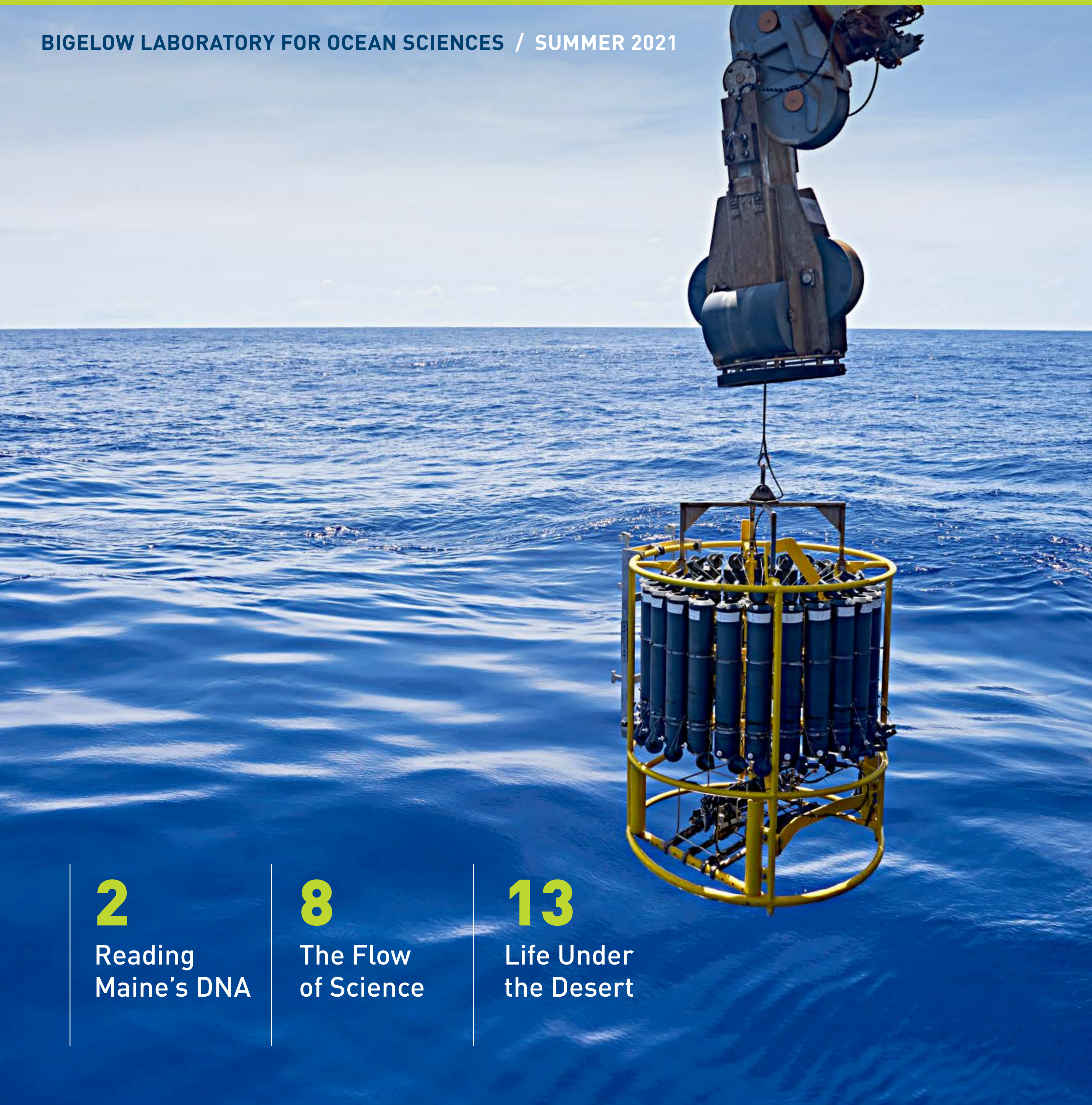
TRANSECT

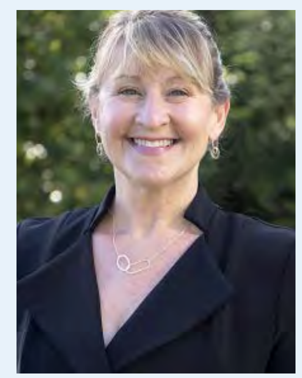
BIGELOW LABORATORY FOR OCEAN SCIENCES / SUMMER 2021



Message from the President

What a difference a year can make! As vaccination rates continue to increase, our staff is beginning to reconnect in person with each other and our supporters for the first time in more than a year. We're also seeing the first real efforts to mobilize a national response to climate change that is of a magnitude commensurate with the challenge.

The science community has been sounding the alarm on our changing climate for a very long time, launching the first science journal completely devoted to the issue in 1977. What a thrill to finally see those efforts starting to influence policy in the ways that are needed.



Our job now is to use the brain power of our diverse scientific community to understand the foundations of the problems before us, build the solutions we need at scale, and educate the next generation in the skills they will need to care for this planet.

Thanks to the support of our donors, we are well positioned to do just that. During a time of great uncertainty and disruption, they maintained their focus on the ocean challenges and opportunities we're addressing together. I am immensely grateful for their steadfast generosity, which has allowed us to accelerate our work during the last year and hire multiple new senior scientists! I hope you'll join us for our virtual Café Sci series this year (page 6), during which we'll introduce you to these inspiring researchers and the unique ways they enhance our work.

These scientists will help us continue a long history of bold science at Bigelow Laboratory. We are continually advancing the leading edge of ocean discovery. In this Transect, we highlight our pioneering work in cytometry (page 8) and environmental DNA (page 2). Our scientists were instrumental in bringing the game-changing tool of flow cytometry to ocean science, which fundamentally changed the study of phytoplankton — the foundation of all life in the ocean and on the planet. The technologies to use DNA in the environment to understand and manage our coastal ecosystems are looking to be just as revolutionary.

Thank you to everyone who has helped us turn the challenges of the last year into a period of growth and transformation at Bigelow Laboratory. We're working faster and better than ever before, and we couldn't do it without you!

Deborah A. Bronk

DEBORAH BRONK, PhD

ON THE COVER

A group of Bigelow Laboratory researchers started out the year with a two-month research cruise to the southwest Pacific Ocean, led by Senior Research Scientist Barney Balch. The team was studying coccolithophores, a common algae that support ocean food webs and play a significant role in global chemical and carbon cycles. However, they are strangely scarce in the fertile waters near the equator and our researchers are trying to determine why. The pictured instrument, a CTD, was used to take measurements and collect samples as the scientists lowered it deep into the ocean.

Photo: Giuliana Viglione

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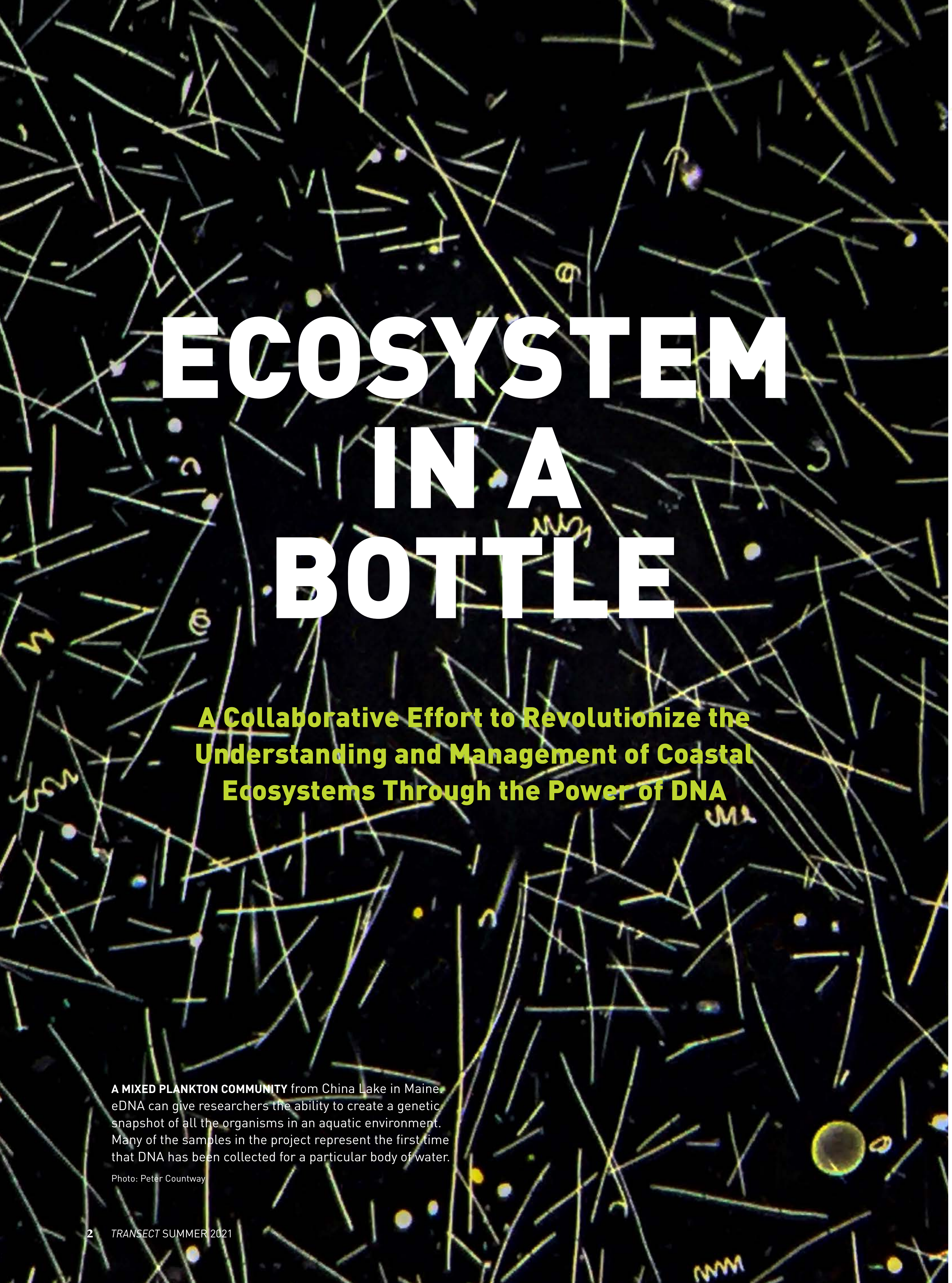
CONTENTS

***CANDIDATUS* DESULFORUDIS AUDAXVIATOR** is a microbial anomaly. It lives isolated deep underground and feeds off chemical reactions triggered by radioactivity. New research from Bigelow Laboratory recently revealed that they have been at an evolutionary standstill for millions of years. The discovery could have significant implications for biotechnology applications and scientific understanding of microbial evolution.

Photo: Gordon Southam



ECOSYSTEM IN A BOTTLE

A Collaborative Effort to Revolutionize the Understanding and Management of Coastal Ecosystems Through the Power of DNA

A MIXED PLANKTON COMMUNITY from China Lake in Maine. eDNA can give researchers the ability to create a genetic snapshot of all the organisms in an aquatic environment. Many of the samples in the project represent the first time that DNA has been collected for a particular body of water.

Photo: Peter Countway

LEFT Samuel Tan, one of the University of Maine graduate students working at Bigelow Laboratory on the eDNA project, collects water samples off of Chebeague Island.

RIGHT Senior Research Scientist Dave Emerson (right) and UMaine graduate student Rachel Presley prepare a sampling device to collect a sediment core in the upper reaches of the Damariscotta River. eDNA in sediment cores can provide clues about ongoing biogeochemical processes and serve as a historical record of the past.

Every ounce of water teems with affirmation of life. All aquatic plants, animals, and microbes leave genetic traces wherever they go as a natural byproduct of their existence. This environmental DNA, or eDNA, has the potential to provide scientists with a wealth of data about the organisms, how they interact, and their environment.

Scientists from Bigelow Laboratory and University of Maine are leading a $20 million National Science Foundation project to pioneer technology to harness this eDNA and make discoveries about pressing issues — from economically important and potentially harmful species to ecosystems shifting in the face of climate change.

With the help of collaborators in education, government, industry, and community groups, as well as a large cohort of UMaine graduate students, the research team aims to develop a DNA-based toolset that enables states to better monitor life in their waters.

“One of the powers of eDNA is this integrative ability to look across all organisms — literally from bacteria to whales — within a single sample,” said Dave Emerson, senior research scientist at Bigelow Laboratory and co-principal investigator on the project. “When you start putting lots of that data together, you can look at associations between different organisms and see what kinds of patterns come to light.”

For larger organisms, eDNA traces come from shed skin cells, reproductive cells, or waste products. For smaller ones, like microbes, the eDNA is gathered from the whole organism. eDNA is collected through water samples and even a small bottle of water provides the potential to find traces of a species of interest or to construct a snapshot of an ecosystem.

“We hope that eDNA will enable us to see associations we didn't understand before, because we can now look much more broadly across a community,” said Emerson, who is also leading a project team examining the use of eDNA to understand distribution patterns of marine microbes and their responses to environmental changes. “Because microbes respond most quickly, using eDNA could give us an early warning about changes that may be occurring in an ecosystem.”

Maine is being reshaped by the shifting of existing species and the introduction of new ones, some of which can be harmful. Senior Research Scientists Peter Countway and Doug Rasher are leading teams to monitor some of these changes and how they will impact species diversity and movement into new habitats.

In recent years, Maine has experienced toxic blooms of algae and cyanobacteria with increasing frequency, duration, and variety. When these toxins become concentrated, in shellfish for example, they can be passed up the food chain. Countway is leading a team using eDNA to discover where different species are located, how they

Photos: Jeremy Rich, top left; Peter Countway, top right

SENIOR RESEARCH SCIENTIST Peter Countway, left, heads across Damariscotta Lake with members of the Midcoast Conservancy to collect water samples for analysis. The Maine eDNA project engages and empowers community scientists around the state.

may impact environments, and how to identify the biogeochemical processes and microbial interactions that may lead to toxic blooms.

Information from eDNA can be used in two main ways. The first is to create a snapshot of the organisms in an ecosystem by matching up DNA sequences from a water sample to libraries of known sequences. Alternatively, eDNA can be used to find one particular organism among millions of other genetic signatures, and estimate its abundance. Countway's project is using both approaches. He first examines a broad swath of the ecosystem from microbes to fish, and identifies the potentially toxic species. He can then design a genetic assay for a particular species, much like a COVID test, that can quickly detect that organism in future water samples.

"Normally, we don't see a bloom coming, we just notice it suddenly one day when we drive past the water and it's bright green or red from a marine algal bloom," Countway said. "eDNA techniques are so sensitive that we can often detect the DNA signature from just one cell in a liter of water."

Many environmental changes are not as visible as algal blooms and can easily go unnoticed, while creating serious consequences for the ecosystem. Senior Research Scientist Doug Rasher is working with Countway on one such issue — the changing ecology of kelp forests on the seafloor.

"Maine's kelp forests provide essential habitat and refuge to a variety of marine species, including economically important fish and shellfish," Rasher said. "However, these forests are threatened by a variety of stressors, and their declining health is now recognized as a sentinel of ocean change."

As the Gulf of Maine warms, temperatures are becoming too high for some of Maine's native species, and the region is becoming more habitable for those that are historically from further south. Even compared to surveys from 2018, Rasher said ecosystems are rapidly changing, and it is important to implement these tools as soon as possible in order to keep up.

"We're going to see a reshuffling of communities in the kelp forest ecosystem, some of which will be detrimental to the ecosystem and its associated fisheries, and some of which will create new economic opportunities," Rasher said. "In both cases, we need to be able to observe species range shifts as they're unfolding, and understand their causes."

He and his colleagues are working to use eDNA to gather information on new species that arrive to an ecosystem but are not yet abundant enough to show up with traditional sampling methods. Even if newly arrived species are still too rare to spot on a dive survey, they may leave genetic fingerprints behind.

While powerful, eDNA has limitations. For example, it can tell researchers if an organism's DNA is in the water, but it can't reveal the organism's age or if it is still alive. Part of the Maine eDNA project is to find solutions to these sorts of problems. At the same time, Rasher, a self-described "muddy boots ecologist," believes that traditional methods will long continue to provide valuable information.

"Traditional methods still do the best job at telling us what an ecosystem looks like," he said. "But when you pair them with eDNA, you can develop a more comprehensive picture of what's happening in the ecosystem, as well as what's new and may be missed by old-school methods."

Neither approach is a panacea. However, Senior Research Scientist Nichole Price thinks eDNA could be the efficient, large-scale tool that has long been needed. She is leading a team using eDNA to understand the movement and dispersal of larvae that are key to the health of commercial fisheries.

Seaweeds, scallops, mussels, and other organisms

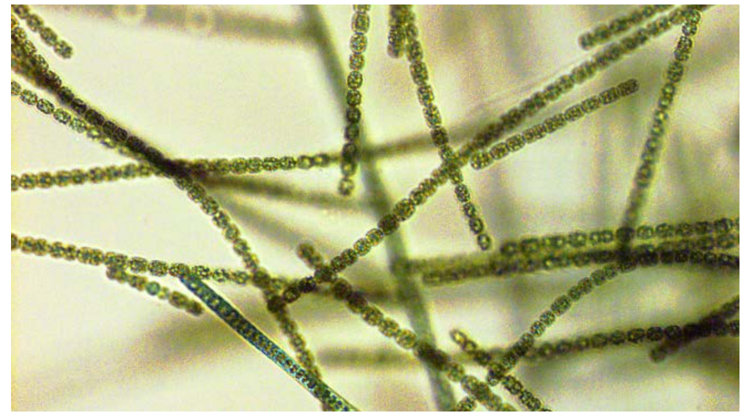
Photo: Peter Countway

'One of the powers of eDNA is this integrative ability to look across all organisms — literally from bacteria to whales — within a single sample.'

TOP Senior Research Scientist Doug Rasher and UMaine graduate students Rene Francolini and Sydney Greenlee (left to right) work at the coast, shore, and laboratory to collect eDNA and use it to create snapshots of the hidden life in aquatic environments.

BOTTOM Dara Yiu (left) and Shane Farrell, both UMaine PhD students, dive at Allen Island to collect samples and survey flora and fauna.

Photos: top left to right, Rene Francolini, Peter Countway, Jennifer Smith-Mayo; bottom, Rene Francolini



LEFT Research Associate Brittney Honisch collects water samples at a kelp farm in Casco Bay.

RIGHT Cyanobacteria floats in a sample from Damariscotta Lake, including *Dolichospermum* (rounded green cells) and *Planktothrix* (box-shaped, bluish-green cells), both of which are capable of producing toxins.

that stick to the bottom of nearshore environments begin life as microscopic plankton with limited swimming ability. They are carried around by currents until they find a suitable spot to attach and grow.

"Harvesters of these really lucrative organisms are depending on natural pulses of them to just show up when and where we expect them to," Price said. "It makes fisheries management for these kinds of species really tricky, especially in the face of climate change, which is making these settlement patterns even less predictable."

Price hopes that eDNA tools can help scientists better understand the abundance and location of these species. Traditional sampling methods have struggled with this, especially in cases where organisms are too small or similar to differentiate.

"eDNA allows us to start looking for these pulses of otherwise invisible larvae by screening the water for the DNA fingerprints of those species," she said. "We want to develop tools that can help us differentiate between the genetic signatures adult and larval stages of these organisms, which is key to ecosystem and fisheries management."

From larval development to shifting species, the true power of eDNA is in its applied opportunities. Senior Research Scientist Nick Record is working with many of the project's research teams to actualize that potential. He specializes in machine learning and big data. And, if anything constitutes big data, eDNA is it — it's not uncommon to get 20,000 individual DNA sequences from a liter of water.

Record is working with researchers to sort through this trove of information and create forecasting tools that can serve communities around Maine. They hope to identify signatures that are precursors to events like algal blooms and species shifts, similar to how meteorologists know that a drop in atmospheric pressure can signal a storm is coming. The resulting early warning systems could be beneficial to all those who work and play on the water.

"If farmers can be informed of a harmful bloom developing in a sensitive area, they could either perform their harvest a little bit early or move their shellfish out of harm's way," Countway said.

Ultimately, the project aims to develop tools that can be used by community scientists and stakeholders. Many eDNA techniques are relatively inexpensive and accessible, and the team is working with communities around New England to build their capacity for eDNA research.

"At the end of the project, I'd like to look back and say we've trained up a cohort of community scientists that has the capability to analyze a water sample taken nearby and doesn't have to wait for the gatekeepers of the technology to give them an answer," Countway said.

If communities were able to conduct monitoring on their own, the amount of data would feed enormous possibilities. Researchers hope the technology can be leveraged in ways to engage communities, expand research, and democratize science.

"There could be a thousand other people around the area contributing measurements in different places," Record said. "The data could be integrated and visualized in near real time and empower people to observe and monitor their environment in a scientifically rigorous way."

This kind of public-oriented, multidisciplinary science is at the core of the project. One of the researchers' main goals is to share and integrate the data with other monitoring efforts in Maine's coastal communities. Using eDNA to answer fundamental science questions will help validate it as a tool to gauge the health of coastal ecosystems during a time of significant environmental changes.

"One of the cool things about it is that it brings together different scientific disciplines and allows you to think about groups of organisms, as well as the way we study them, in a more common way," Emerson said. "And, to me, that's pretty powerful."

Photos: Jeremy Rich, left; Peter Countway, right

Changing the FLOW of ocean science

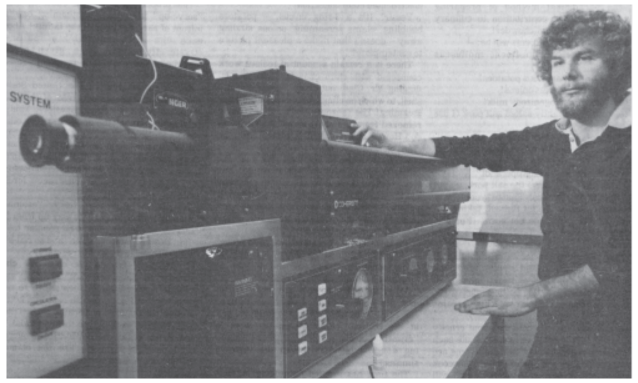
PIONEERED by researchers at Bigelow Laboratory, cytometry has revolutionized microbial oceanography. It works by creating a single-file stream of cells contained in a fluid. Each cell then passes by a laser, and sensors gather the data needed to rapidly analyze and sort them.

As Hurricane Emily bore down on Bermuda in 1987, Researcher David Phinney and Bigelow Laboratory co-founder Clarice Yentsch were there as part of a research mission that was revolutionizing microbial oceanography.

The storm rocked the island, toppled trees, and knocked out the power, bringing the team's research to a halt. When the skies cleared, the scientists emerged to survey the destruction left in the hurricane's wake. Phinney, as a good Mainer, was well-versed with a chainsaw and promptly went to work alongside colleagues and locals, clearing fallen trees to begin the recovery.

This kind of scrappy enthusiasm is the same that defined the team's endeavor to advance what would become one of Bigelow Laboratory's biggest contributions to ocean science — a technique called flow cytometry. Today, it is used around the world to study aquatic environments. The technique has been instrumental in pivotal breakthroughs, from advances in genomics technologies to the discovery of new species.

Flow cytometry is a way of quickly counting and characterizing large quantities of microscopic particles, such as cells, viruses, bacteria, and phytoplankton. It works by suspending particles in a fluid where they are forced to flow, single file, in a stream. The particles are then passed in front of laser beams where they exhibit different characteristics based on their size and fluorescence. Using this information, researchers can determine the size, shape, and basic identity of the particles, and then sort them based on these parameters.



TOP Research Associate Laura Lubelczyk loads the flow cytometer in the Center for Aquatic Cytometry as its director, Nicole Poulton, looks on.

BOTTOM David Phinney demonstrates Bigelow Laboratory's first flow cytometer for a newspaper article in 1983.

Traditional microscope techniques can be used to count and characterize cells, but flow cytometers can do it at incredible speeds — processing tens to hundreds of thousands of cells per second.

"In graduate school, I spent the whole summer in a dark room counting bacteria in samples of seawater under a microscope, and just about everything I did that summer, a flow cytometer could do in a day," said Deborah

Photo: Fritz Freudenberger, top; Tom Jones, right

TOP Participants pose at the first flow cytometry workshop for aquatic sciences hosted by Bigelow Laboratory in 1984. Clarice Yentsch is pictured fourth from left in the top row, and David Phinney is rightmost in the top row.

ABOVE Laura Lubelczyk, front, and Nicole Poulton look over data from a FlowCAM. This instrument was developed at Bigelow Laboratory in the 1990s and combines the functionality of a flow cytometer with the ability to rapidly capture images of particles.

Bronk, Bigelow Laboratory President and CEO. "It has revolutionized the study of phytoplankton and bacteria."

The technique has allowed scientists to study particles and cells in greater numbers and smaller sizes than previously possible, enabling studies that tackle harder questions. It even led to the discovery of the most abundant phytoplankton, and possibly the most abundant photosynthetic organism, on Earth: *Prochlorococcus*.

The introduction of flow cytometry to aquatic science began at Bigelow Laboratory in the early 1980s. Yentsch was speaking about the difficulties of processing cells with a microscope to a colleague who had just gotten back from a biomedical conference. They wondered if flow cytometers, which were being used to count blood and cancer cells, might offer a solution. With the help of her Bigelow Laboratory colleagues, including Phinney, she began to pursue funding to test her hypotheses.

Others weren't as quick to see the potential, and her first attempts to secure grant support were unsuccessful. Eventually she realized that it was not because the attempts were too grand, but because they were too humble.

"We were asking for the minimum amount and needed to think bigger," she said. "We were advised that if we could demonstrate the possible usefulness of this tool in the ocean sciences, that agencies would not want to fund the Volkswagen — they would want to fund the Mercedes."

Yentsch saw enormous opportunities for flow cytometry in the study of the ocean, which teems with microscopic life that forms the foundation of global ocean health. So, she and Phinney started working in Bermuda to prove the tool's merit. The ocean around the island is home to some of the world's smallest aquatic microbes, which she thought would be a perfect demonstration of the technology's potential.

"We were trying to test it in the most difficult situation," she said. "Had we not been so enthusiastic, we would have given up. But we kept doing more experiments. We kept going and getting more and more interesting results."

By analyzing seawater samples using flow cytometry, the team completed work in a few months that might have otherwise taken a year. As the results came in, so did the funding. The National Science Foundation, Office of Naval Research, and Maine Department of Marine Resources soon stepped up to support the work, and Bigelow Laboratory became the first laboratory in the world to have a flow cytometer dedicated for use by the aquatic science community.

Clarice and her team cultivated a whole new field of oceanographic research. The process wasn't easy or immediate, but the team's enthusiasm built the groundwork for the new tool.

"Our efforts really built a sense of community," Yentsch said. "We were not just advancing our own work, but showing others how it could serve them and helping them build expertise."

Bigelow Laboratory hosted workshops and steadily fostered a collaborative group of scientists that developed the techniques and protocols for how flow cytometry would be used in ocean science. Many of them later became senior research scientists at the lab or landed prominent positions, such as Richard Spinrad, who was recently nominated by President Joe Biden to lead the National Oceanic and Atmospheric Administration.

"Through our workshops and people testing flow cytometry on their own research, it brought a tremendous amount of attention to Bigelow Laboratory," Phinney said. "Clarice really brought the community together around this tool with her enthusiasm about what it could do."

Almost 40 years after its introduction to ocean science, flow cytometry is now a staple of microbial oceanography and used at most oceanographic research institutes. It has also been instrumental to another breakthrough pioneered at Bigelow Laboratory — single-celled genomics, which allows scientists to analyze the genetic information contained in individual cells.

"Flow cytometry has completely changed our ability to quickly obtain cell estimates of what populations are out there, whether it be at the surface of the ocean, ocean depths, or deep in a mineshaft," said Senior Research Scientist Nicole Poulton, the director of Bigelow Laboratory's Center for Aquatic Cytometry. "This has enabled us to advance our understanding of the different microbes that are out there and how they are related to one another, which has revolutionized our understanding of the tree of life."

Poulton has been the director of the Center since 2014. She took over the position from Michael Sieracki, who was director after Yentsch. Along with the role, she has taken up the mantle of growing a research community around flow cytometry. She regularly runs technical workshops to share her expertise, and she was elected as a council member to the International Society for the Advancement of Cytometry in 2020, as the only representative for aquatic and environmental cytometry.

From studying phytoplankton to microbial ecology, Poulton also conducts her own research using flow cytometry. One current project aims to better understand and detect microplastics with colleagues at the University of Minnesota Duluth.

Plastic is the most prevalent type of marine debris found in the ocean and the Great Lakes, and microplastics are particularly challenging to address — and to study. Scientists do not yet know the full impact of these particles, but they make their way through food webs and into the digestive systems of aquatic animals and the people that eat them.

Much like the phytoplankton and bacteria cells that originally inspired Yentsch to begin using flow cytometry,

SENIOR RESEARCH SCIENTIST Nicole Poulton works with a flow cytometer in Bigelow Laboratory's Single Cell Genomics Center. Cytometry was instrumental to the development of single cell genomics, which was also pioneered at Bigleow Laboratory.

'Flow cytometry has completely changed our ability to quickly obtain cell estimates of what populations are out there, whether it be at the surface of the ocean, ocean depths, or deep in a mineshaft.'

studying microplastics is currently arduous and prone to errors. Poulton believes that flow cytometry can speed up and improve the process.

"We can use flow cytometry to isolate even the smallest microplastics, so we can understand what type of plastics are most likely to end up in the aquatic food chain," Poulton said. "By understanding how microplastics are distributed, we can begin to locate where they are likely going to be a problem."

Poulton is also developing techniques and instrumentation to address new challenges in ocean science. In collaboration with Senior Research Scientist Ramunas Stepanauskas, director of the Single Cell Genomics Center, she is currently working on the development of a new flow cytometer that uses both cell imaging and multiple lasers to improve the detection capabilities for isolating individual cells. This advancement may again launch a new era of groundbreaking research.

"When a new tool is developed, you realize you can go back and ask research questions again, in a different way," Yentsch said. "Suddenly, you're able to look at the world, the ocean, or single cells in a totally new way, and everyone's research leaps forward."

PROFILE

Vice chair, Bigelow Laboratory Board of Trustees

Robert Gagosian

Robert Gagosian has a passion for puzzles. Not just jigsaw puzzles, but also the ones that help us understand our planet. Those puzzles don't have a pretty picture or neat borders to guide you; they offer the pure challenge of putting all the pieces in their right places.

And Bob is not one to shy away from a challenge. In addition to his role as vice chair of the Bigelow Laboratory Board of Trustees, he is the senior advisor to the president of the Desert Research Institute in Nevada, after previously serving as president. He also previously served as president and director of the Woods Hole Oceanographic Institution, and as president of the Consortium for Ocean Leadership.

Bob's love of scientific puzzles started in high school, when a teacher took him and other students to MIT for 10 weeks to learn about the new and exciting discovery of DNA.

"And that's a jigsaw puzzle, right? The way DNA molecules are constructed, that's exactly what it is," he said. "So I just loved it. I love the process."

After college, Bob had a 22-year career as a marine geochemist and published about 90 research studies. Over time, he found he got more joy out of watching his students' careers grow than his own. He transitioned to department chair and began helping young faculty members succeed in their teaching and research. It was from there that he went on to helping foster organizations through his leadership roles.

"Taking different pieces and fitting them together has been a theme of my whole career," he said. "I just get sheer joy out of watching people succeed. I spend a lot of time telling people when I think they're doing a good job. I don't think we do that enough."

Bob said that board service is one way he gives back for all the help people have given him throughout his career. His approach is focused on supporting organizations' people, encouraging them to think carefully about how they spend their time, and helping them avoid short-term distractions and focus on the future.

He remembers sitting next to Bigelow Laboratory co-founder Charlie Yentsch in a meeting at Woods Hole Oceanographic Institution where Yentsch talked about his vision to move to Maine and start a new research facility. Over the years, he maintained close connections to the Laboratory.

Bob prefers to work with private, nonprofit institutions like Bigelow Laboratory because of their ethos and potential. He enjoys working with organizations that prioritize mission over profit, and he appreciates the resourcefulness of researchers who fight to make a difference. His experience has also shown him that private research institutes can quickly pivot and adapt to meet innovative opportunities and pressing environmental needs.

"Bigelow Laboratory has a critical niche with marine microbiology, which is such an important area for understanding the future of this planet," he said. "Climate change, carbon sequestration, and aquaculture: it all starts with microorganisms."

While the ocean holds more questions than answers at the moment, he thinks its future looks bright. Because of technological advancements, such as genomics and artificial intelligence, he believes we are on the cusp of fantastic discoveries in microbial ocean science.

"A place like Bigelow Laboratory can make a huge leap in our understanding because of its focused approach and these new technologies," he said. "I think we're going to be able to find out so much more about the ocean in the next 25 years than we have in the past 300."

Much like microbes have a global impact in spite of their modest size, Bob thinks Bigelow Laboratory has a significant place in the puzzle to understand our planet.

"Bigelow Laboratory is really focusing on the most important problems for the future of the planet," he said. "That's why I see it playing a huge role, even when compared to much larger institutions. It's a gem. It really is."

'Climate change, carbon sequestration, and aquaculture: it all starts with microorganisms.'

FIELDWORK

Notes from the Field

Photo: Detra Page

MOJAVE DESERT, Nevada

BY DR. JULIA MCGONIGLE, Postdoctoral Scientist and NASA Postdoctoral Program Fellow

Water is a rare and precious commodity in the Mojave Desert, the site of my recent field excursion. There, on the ancestral lands of the Nuwuvi, or Southern Paiute, water was conceptualized as a sentient being that required human interaction to remain healthy. Springs and seeps were associated with particular families within the larger tribe and passed down alongside oral traditions.

Our field site is near a series of such springs in the protected Ash Meadows Wildlife Refuge, home to rare and threatened species of desert pupfish. Being a microbiologist, I was out in the Amargosa Valley seeking a far smaller, and likely more common, form of life living in the subsurface below the crystal blue-colored springs.

The research well we sampled water from is located just outside of Death Valley. This half-mile deep well was drilled on behalf of Inyo County, California, in 2005 to provide hydrologic data on the lower carbonate aquifer it taps into. Water from the well comes out at 135 degrees and can originate from sources around 60 miles away at Yucca Mountain.

The well represents a rare window into an ecosystem deep below the surface. Life in such extreme environments is poorly understood, yet it's estimated these microorganisms represent around 13 percent of all biomass on Earth. We hope our work will provide insights on life in the deep biosphere. The multi-state collaborative team includes several senior research scientists from Bigelow Laboratory: Ramunas Stepanauskas, Beth Orcutt, David Emerson, and Nicole Poulton. The field team was led by Duane Moser of the Desert Research Institute in Nevada.

Fieldwork in the desert is not easy. You deal with overbearing sunshine, temperatures around 90 degrees, and the threat of stepping on a rattlesnake. For this project, logistics involved deploying a deep fluid pump with sterile methods, connecting a water-tight pipe with sterile sampling ports, and creating a mobile lab space. In addition, all of the stagnant water that had been sitting in the well for roughly 13 years needed to be flushed out before my experiments could begin.

Day one involved hourly sampling for chemistry and biology as the pump replaced this stagnant water with water from deeper in the aquifer. We hypothesized that the number of subsurface microorganisms, such as the sulfate-reducing *Desulfotomaculum* species, would increase throughout pumping.

On day two, after pumping continuously overnight, I was able to set up incubation experiments. This involved adding small amounts of various chemical energy sources to bottles of subsurface water and letting it sit overnight. The experiments test if acetate, carbon monoxide, and hydrogen can stimulate microbial activity, or if most cells exist as non-active spores.

Previous work using DNA sequencing revealed spore-forming microbes, like *Desulfotomaculum*, are common in the fluids. DNA can tell us a lot about microbial communities, but lacks information on what those microbes are doing: a dormant cell's genome looks the same as an active cell's genome.

Cell activity in our experiments is determined through a novel technique, using a cellular dye, developed within Bigelow Laboratory's Single Cell Genomic Center. Obtaining these samples involves working with your hands immersed into a plastic bubble filled with a constant stream of nitrogen gas to keep oxygen out of the subsurface fluids.

The work is difficult in the middle of the desert, but worth accomplishing. These samples will help achieve a goal of this National Science Foundation-funded project: to connect genomic information to microbial activity and bring us closer to understanding life below the surface.

GIVING OUR SINCERE THANKS

Bigelow Laboratory is an independent, nonprofit institute. Our impact is only possible with the help of our community of supporters. Their generosity fuels our discoveries, powers our solutions, and enables us to inspire the next generation of ocean leaders.

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Photo: Fritz Freudenberger

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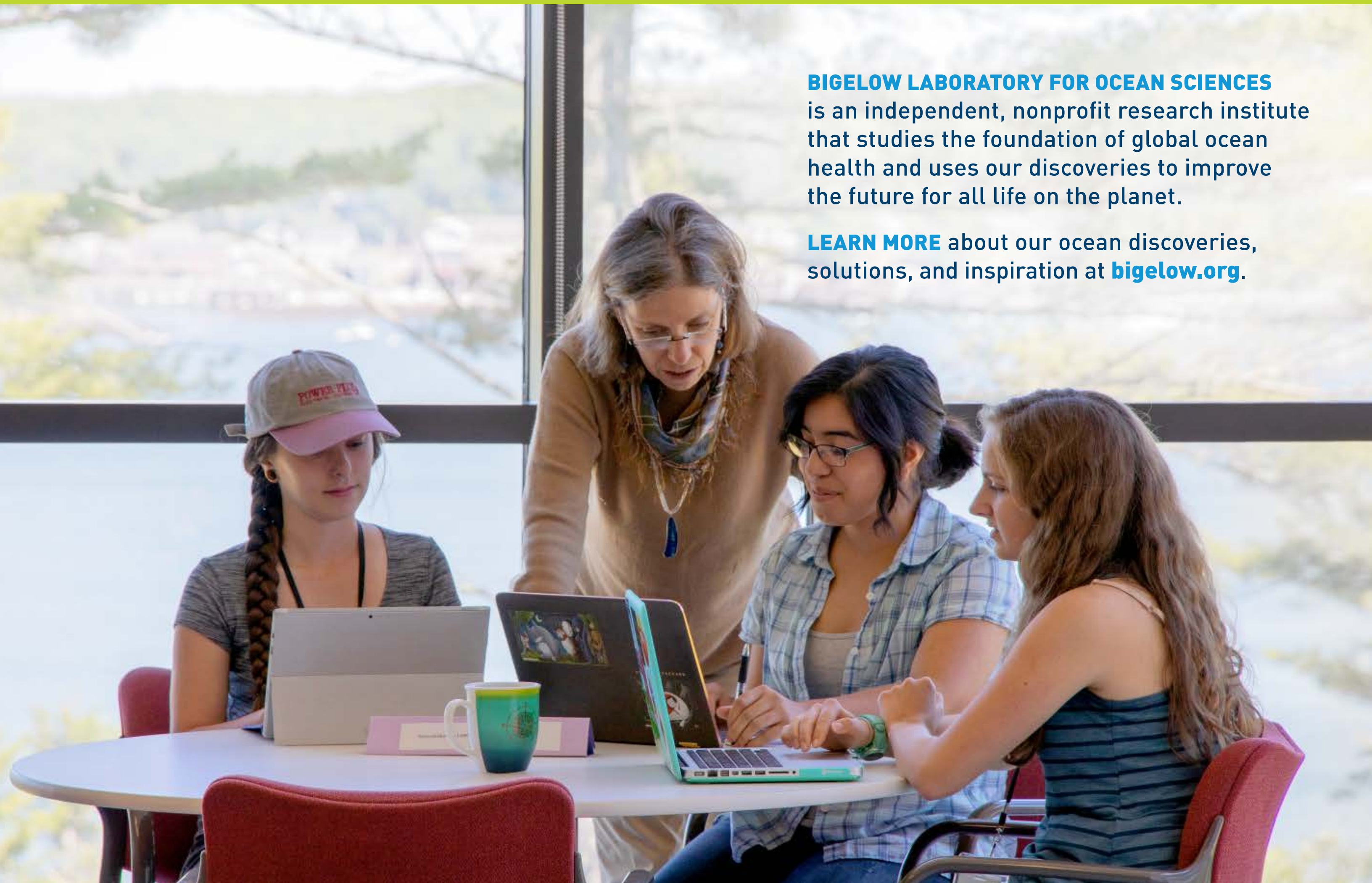

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