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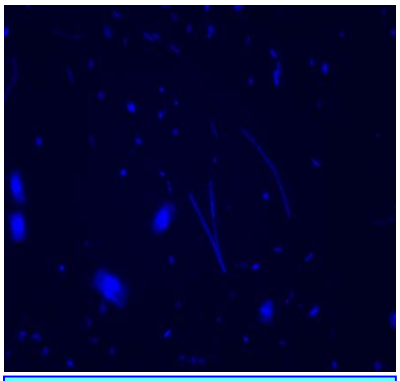
While the winter refused to surrender to spring, the winds pressed the sea ice against the peninsular coast, putting a final moratorium on boating travel. Access to our sample sites is limited to boat or sea ice travel. Since sea ice travel at Palmer gets pretty risky in the austral spring (and the ice is usually gone), we had planned on collecting samples via the Zodiac. As a final blow to our chances for boating to sampling sites, a storm with continuous 40+ knot winds pushed from the southwest all day October 20th and 21st, closing our tiny port to any but the Weddel and Southern Elephant Seals, and the long-awaited Adelie penguins. This was a great disappointment to the USAP's 308-foot ice breaker and research vessel, *R/V Nathaniel B. Palmer (NBP)*, and its crew. Only recently liberated from dense ice pack and pressure ridges (some of which rode over the railing of the ship) further south along the Palmer Peninsula, the NBP chose to conserve its fuel stores rather than push through another 30-miles of pack ice to come to Palmer Station. Ice conditions prior to this storm limited our sea-ice travel to approximately 300-meters off the shore, onto Arthur Harbor. This new ice landscape, presented to us by the same enigma that kept our fellow scientists offshore for weeks (and our Zodiac in the boathouse), freed us to venture a few kilometers onto the sea. What an excellent opportunity to collect a variety of sea ice cores!

Brandon and AK extract a core of sea ice from Arthur Harbor. Care is taken not to introduce microbes from our hands and clothing into the sample.

1.lyse- the breaking apart of a cell (usually with a specific enzyme, called a lysozyme) to release the cell's contents.
2.nucleic acid- a long chain of bases that forms a polymer, and which forms the backbone of DNA and RNA. Hence, DNA's name, deoxyribo***nucleicacid***.
3.RNA – a single-stranded copy of DNA that varies slightly from the chemical composition of the DNA. There are different types of RNA, depending upon their function.
4.ribosome- a protein synthesis machine, which translates a cell's DNA into proteins.
5.functional genes – genes that carry out cellular processes, like carbon & nitrogen fixation.
6.variable regions – areas of an organisms DNA that can differ between species and individuals.
7.conserved regions- sections of an organism's DNA that are found in other life forms. These sections may be in every life form, or unique to a certain domain, or even unique to a genus/species.
8.primer- a small section of nucleic acid designed to bind to specific regions of DNA, "open" the DNA, and copy that region.
9.DGGE- denaturing gradient gel eletrophoresis – a biotech method for separating DNA fragments of the same length, according to variation in nucleotide bases.
8.homogeneous- very consistent, similar throughout
9.fast ice- ice that forms very rapidly from fresh water on the surface of the sea; fast ice is solid, hard, and homogenous.
10.heterogeneous- varying in texture, population, or other characteristics; inconsistent
11.brash ice- fragments of floating, broken ice that may be wind-compacted and frozen together into sea ice. Brash ice varies in texture and hardness and is heterogeneous.

A Secret Community in the Sea Ice…



This slide was prepared from the bottom section of an ice core. Its proximity to the seawater, which can supply nutrients and circulate wastes, may explain the variety of bacteria in this niche. The blue dye adheres only to the DNA.

As we discussed previously (Austral Extremities, 1st Edition), we are interested in the genes marine microbes express in their natural environment, and in response to variations in environmental factors. The Archaea are of special interest to us, since they are such a large population of the microbes of the Antarctic waters. But, does the ubiquitous spring bloom of Archaea in the Antarctic seawater extend to the sea ice, as well? This question remains unanswered at this point, but the late arrival of spring has gifted us the opportunity to investigate this possibility.

We found these lovely marine algae (Coccinodiscus) in our sea ice.

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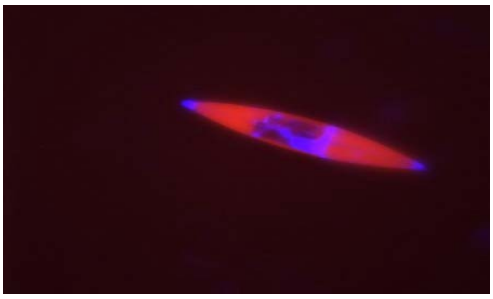
Why wouldn't Archaea flourish in the sea ice?

The sea ice is an abruptly changing environment. Fast ice can be as cold as the ambient air, and then can thaw quickly as well, exposing organisms to temperature and light extremes. Depending upon ice thickness and snow cover, sunlight can penetrate the ice to allow photosynthesis to occur. As science knows, and we witnessed in our ice cores, the sea ice is a thriving community, burgeoning with algae, flagellates, and microbes. Note the population in the DAPI slide (Page 1, bottom left), which is denser than that of the seawater slides we prepared (see 1st Edition DAPI photo). All three of the slides shown here were prepared from our sea ice samples. With the abundance of wildlife we've found in the sea ice, there must be Archaea…mustn't there?



This pennate diatom (who thrives in the sea ice) has a rigid cell wall constructed of silica, an ingredient in glass. Diatoms are also found in diatomaceous earth (and some dairy alternatives!). The blue shows the DNA, and the red shows the chlorophyll.

Although some reports suggest the presence of Archaea in the sea ice, they have yet to be confirmed. However, the sea ice community, by its very population density and variety, may preclude the presence of Archaea. Remember that Archaea are frequently found in places other organisms don't want to occupy (highly saline lakes, thermal vents, hot springs). So, we can hypothesize that Archaea may not do well when they have to compete for resources. Does the sea ice seem like a good habitat for non-competitive organisms, like Archaea?

This algae-covered ice was seen off the starboard stern of the Laurence M. Gould, on our way to Palmer. What looks to us like a colorful lattice of ice crystals and snow is a world awaiting discovery to a microbiologist.

Evidence to the contrary, the quest is still on.

One of the most difficult concepts in science is disproving by absence. Can we confirm that something does not exist, simply because we have not found it? If that were the case, we'd have quit our search for life outside the Earth many moons before this. Microbial Ecologists, like Dr. Murray, continue to search for Archaea in the exciting and dynamic environment of the sea ice. This has become part of our fieldwork here at Palmer Station. So, our first goal is to collect sea ice, and plenty of it. We use a hollow, steel hand-tool, called a SIPRE core (see top photo on page 1), which we augur down through the ice layer. The ice collects in the hollow center, and released in a core. We divide the ice cores into sections (top, middle, bottom), corresponding to the microbial communities that inhabit the ice. The surface community includes those that live in the melt pools, as well as in cracks created by tide and wave action. The middle (interior) communities live within the depth of the ice, and the bottom communities live near the ice-water interface. Each of these regions may vary in terms of exposure to light/UV radiation, exposure to the water, available nutrients, population, or other factors. Then, we melt the ice, very slowly, so we don't shock the organisms within it. We filter the organisms from the melted sea ice, perform a chemical extraction to **lyse**[1] the cells and isolate their DNA,

Dr. Murray filters the sea ice cells so we can isolate their DNA and RNA. This will help identify "who's there" in the sea ice.

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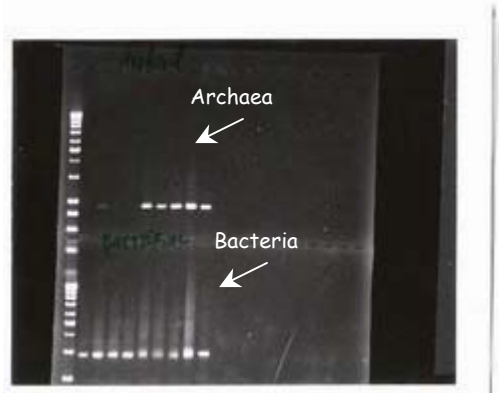
and then we use PCR (see Austral Extremities, 1st Edition) to amplify the DNA, to discover microbial diversity and their functional attributes.



A current is applied to this electrophoresis gel, which draws the negatively charged DNA across the gel. Larger DNA pieces move more slowly, and smaller DNA pieces move more quickly. The bright bands contain DNA from Archaea and Bacteria.

Back in the lab is where things get exciting. The incredible advances made in biotechnology provide us with a myriad of tools to decipher the genetic material we isolate from the sea ice cells. We will be looking at two types of genetic material, DNA and RNA. If you recall, DNA consists of two long strands of **nucleic acid**[2], wound into a double helix. These strands of DNA are made up of nucleotides, the individual bases that make up the DNA "backbone." The DNA for an organism, known as its genome (gDNA), is made up of many genes. For example, a bacterium that has about 2 million bases in its gDNA includes about 2,000 genes that may ultimately be expressed. When a gene is expressed, its DNA is transcribed, or copied, into a new molecule called **RNA**[3]. We are studying ribosomal RNA (rRNA) and messenger RNA (mRNA). rRNA gets folded into a **ribosome**[4] (see photo→). Ribosomes consist of a small sub-unit (SSU) and a large sub-unit (LSU), which come together to make proteins. mRNA is a molecule derived from DNA that contains information to make a particular protein. When a gene is expressed, the two sub-units of the ribosome, made up rRNA, come together to translate the mRNA. What this means is that we have two places that we are looking for information. First, we'll analyze the ribosomal DNA from our sea ice cells, to see "who's there." Then, we'll analyze its mRNA, to see which genes are being expressed.

Scientists at the University of California - Santa Cruz used x-ray crystallography to generate the first detailed picture of a ribosome, showing the LSU (gray) and the SSU (purple). Reported by Cate, et al., in Science, 24 September 1999.

There are many variables that determine whether or not an mRNA is translated and a gene is expressed. Environmental condition, for example, can play a key role in gene expression. For our project, we're looking for **functional genes**[5] in the microbes of the ocean and sea ice, that are turned "on" (expressed) or "off" (not expressed), depending upon changes that occur in the natural environment, or that we induce in the lab.

Where terrestrial (glacier) ice meets the sea... We collected many of our sea ice samples from here in Arthur Harbor.

Every living organism's DNA has regions of its DNA that are **variable**[6], and regions that are **conserved**[7]. That is, some areas of the DNA backbone are very different from creature to creature, while other areas of the DNA backbone are the same, whether the DNA belongs to a microbe, an oak tree, or a human being. As you might suspect, if every single life form shares certain conserved regions of DNA, couldn't we use these regions to identify the relatedness between organisms? This is indeed true. In fact, the sections of DNA that encode for rRNA (i.e., the rRNA genes that form the ribosome) have highly conserved in all known organisms, and are a powerful tool for studying diversity. If we were to find another life form elsewhere in the universe, analyzing its DNA (if it *had* DNA) for known conserved regions (like the rRNA genes) would be a good place to look for its relatedness to organisms we've identified on Earth. For now, however, we

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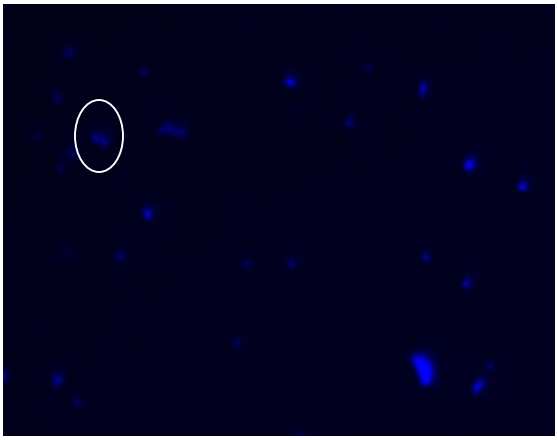
are looking for Archaea in sea ice, so we will look for conserved regions known to exist in Archaea - namely, the Archaeal rRNA genes.



The doublet shown inside the circle may be Archaea.

We select **primers**[8] to bind to conserved regions of the cell's DNA. If we select a primer that will target a conserved region that we know exists in Archaea, and the primer binds to the DNA we've isolated from our sea ice cells, then we know we have Archaea in our sea ice samples. We can repeat this process with primers specific to other marine microbes, to see who else is living in the sea ice.

We can also use **DGGE**[9] to identify, in a general sense, who's in the sea ice. DGGE uses a gradient (e.g., temperature, nucleotide sequence, etc.) to separate the sea ice DNA into information we can interpret. We use the PCR reaction to target and amplify the rRNA genes of all bacteria or all Archaea in our sea ice samples. For example, there may be 40 different species of bacteria in our sea ice samples. These different microbes will have species-specific differences in their rRNA gene sequences (different species = different sequences). The DGGE will differentiate these different sequences into a separate band for each ***different*** (think "variable regions") DNA segment. So, if we inject a soup of an unknown number of species onto a DGGE, and we see 11 bands, we know we have 11 different types of DNA (and potentially 11 different species). Thus, we use *conserved regions* of DNA that flank the *variable regions* to see organisms' diversity.

Sea ice, depending on when it was formed can be very **homogenous**[8] (**fast ice**[9]) or **heterogeneous**[10] (**brash**[11], multiple freeze-thaw cycles, etc.). These ice cores are very heterogeneous and break into sections quite easily. The sections indicate distinct layers of ice within the core.

What difference does it make if there are Archaea in Antarctic sea ice?

If we find Archaea in the sea ice, it will confirm growing evidence that Archaea, while perhaps originally adapted to extreme environments, also inhabit more moderate places. Have these ancient life forms adapted to the milder conditions of today's Earth?

Let's think about some other implications. We know that scientists have found Archaea in some sea ice, but not in other sea ice. If we look at very localized sea ice samples, and confirm the presence or absence of Archaea, how they are stratified within the sea ice column, and who their neighbors are, we may be able to draw a better picture of who the Archaea thrive with and where they prefer to live. Who else is there? What nutrients are missing or present in these places? On a much grander – and far more hypothetical – scale, what are the implications of their appearance or disappearance? What if we find Antarctic Archaea, known psychrophiles, in an area we've never found them before? Can they be an indicator species for climatological variation? Can these tiny, ancient life forms, residents on this Earth since the days when life was confined to a biological soup, help us learn about life in the future, as well as life in the past? The answers to these questions are beyond the scope of our work here, but worth our consideration.

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So where do we go from here?

The Ice Pirates arrive at Torgeson Island…

When Dr. Murray and Brandon return to the Desert Research Institute (DRI) in Reno, NV (their home lab), much of the real nitty-gritty work awaits them. They will determine whether or not the DNA from our sea ice cells contains genes that are catalogued in a clone library of known genes. (While we can look at slides of the cell's DNA, we cannot confirm who's present without molecular biology techniques, like PCR and DGGE, that require the tools at DRI and other facilities in the states.) They will perform a variety of molecular analyses (DNA microarray hybridizations) that focus on the development of environmental genes that are expressed by the marine microbes here in Arthur Harbor. And maybe they will find proof that Archaea live in the sea ice samples… Since Archaea have been found in some habitats, and not in others, the real mystery seems to lie in understanding their *ecology* and their specific distribution within the environment.

Outside the Science World

Finally released from our one sampling station 300-meters from the shoreline, we took the opportunity that the expanded sea ice provided to venture towards Torgeson and Humble Islands. These islands serve as summer mating grounds to a swelling population of Adelie penguins. Joyously gliding across the snow on their bellies, they ventured out to greet us as we wound our SIPRE core down through the frozen cap over the harbor. Questing further for future seawater sampling grounds, we found ourselves in the raging silence of Loudwater Cove, whose power to calve deafening columns of glacier ice into the ocean was evident despite its state of frozen stillness. Tucked away from the rest of the harbor by Elephant Alley, haul-out extraordinaire for the Southern Elephant Seals during thaw, the rocky shores of Loudwater Cove had been colonized by a league of giant petrels (colloquially known as "jeeps," or GPs), preparing to birth their young into the new summer. It was a day that woke us from the shivering winds of the past four weeks, and opened our hearts to the immense beauty of the Antarctic.

Elephant Alley – the spectacular gateway to Loudwater Cove.

We are surreptitiously preparing Halloween costumes, in eager anticipation of the Laurence M. Gould's arrival (a $2 bet on a guess at its **actual** arrival goes towards the farewell party) and a big bash for the incoming Long Term Ecological Research (LTER) science group. Dave Hoffman, **the** man in the know when it comes to the ozone hole, will be our guest lecturer, whenever the ship arrives, to educate us on the work his atmospheric research group has done in that arena. Big science! A lecture none of us will miss…

We miss you all and are eager to share the results of our brief season here with you, once we arrive home. Please visit Dr. Murray's web site, **http://www.dri.edu/DEES/Faculty/Murray.html,** if you missed our 1st Edition and to view our final newsletter (3rd Edition) in a couple of weeks!