Austral Extremities

Fun with Experimental Conditions!

Altering the microbes' environment

The view from Station B towards the mountains of the Neumeyer Strait, on one of the high UV-B, low-ozone days we had at Palmer Station. Note the penguin tracks in lower left of photo.

An important part of the work we've been doing includes comparative studies of microbes at various conditions – conditions that we induce and (try to) control. Of course, these experiments are designed to guide us towards the answers to a variety of questions:

- Do microbes in the shallow (light) waters express different genes than those in deeper (darker) waters?
- How rapidly are genes expressed in response to changes in temperature, or in response to ultraviolet radiation (UV) exposure?
- If we oscillate the temperature of a microbe's environment (e.g., cold, warm, cold) how will the microbe respond?

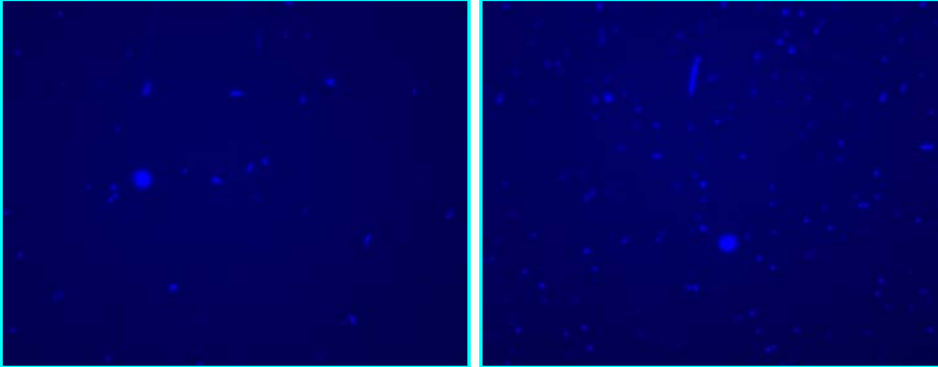
We conducted three different experiments to explore these questions.

A closer look at experimental conditions…

Experiments can be conducted under a variety of conditions. When we conduct an experiment in a test tube (e.g., allowing microbes to reproduce in a test tube), it's called "**in vitro**[1]." We can duplicate a very small part of a microbe's environment on a microscopic scale to see how the microbial community survives in a "**microcosm**[2]." Mesocosms are not quite as small as microcosms, but are much smaller than the microbe's natural environment. For our purposes, we conducted our **mesocosm**[3] experiments in about 20 to 50 liters of water. While the mesocosm allows us to look at a larger sample of the microbial community, there is still a big difference between the mesocosm and the natural environment. The experimental conditions frequently exclude many of the plant and animal organisms that normally share the microbe's natural home. Therefore, the nutrient and waste levels can differ greatly, as well as the changes in population that come from predation or containment. These are just a couple of the examples of the differences between the relatively static environment we create in the lab, versus the dynamic environment that exists in nature.

1. in vitro - an artificial environment outside the living organism
2. microcosm – a small representative system, intended to replicate a larger or natural environment
3. mesocosm - a mid-size representation of an organism's natural environment
4. heat capacity – the amount of heat (energy) required to raise 1 gram of a substance by 1° C.
5. osmotic shock – the rupture of cells (like Bacteria and Archaea) due to a sudden reduction in osmotic pressure (see next entry)
osmotic pressure – the pressure exerted across a semi-permeable membrane, due to differences in solution concentration (i.e., a "concentration gradient") on either side of the membrane.
6. freezing point – the temperature at which a liquid solidifies
7. RT-PCR – a lab method for making multiple copies of mRNA

Austral Extremities



DAPI slides comparing the microbes in a 50-liter seawater sample (left) with the microbes in about 0.5 liters of concentrated seawater (right). Note the much larger concentration of microbes in the concentrate (right) than in the un-filtered seawater (left). Is the amount of nutrients available to these two different samples the same? Are wastes accumulating at the same rate in each sample? Which group would you expect to multiply faster? To expire earlier? Why? (Answers on bottom of last page)

Sample Dilemmas

Time is critical with these experiments, because we want to know how quickly the microbes will express a gene in response to the change in conditions. The **heat capacity**[4] of water requires a lot of energy to increase its temperature by 1° C. Therefore, if we place 50 liters of -2°C seawater in a +5°C water bath, it will take quite a while before the 50-liter sample warms up. At what point during the warming phase does gene expression occur? Since we need a lot of biomass to accurately measure the results, we don't want to reduce the sample volume. We need:

- Good sample size (sufficient biomass),
- Experimental controls **and**
- Speed! We need to induce environmental changes quickly, so we can accurately determine the conditions at which genetic changes occur.

Sample Solutions

- We can concentrate the microbes from a 50-liter sample down to a more workable volume (e.g. 1.5-liters). But do the microbes in the concentrated sample behave exactly like those in the raw seawater sample? Are the concentrations of nutrients, waste products, and water the same? What about **osmotic shock**[5]? These questions remain to be answered.
- We can use warmer water baths, to induce a rapid temperature change. Immersing our 50-liter -2° C seawater sample into a 15° C water bath will get our sample up to 5° C much faster. Caution: we need to be sure we don't overheat the sample!

We chose to run both types of samples (raw seawater and seawater with concentrated bacterioplankton cells) simultaneously, under equal conditions, for some of our experiments. This allowed us to take advantage of the benefits of both approaches, and get some insight into the possible shortcomings of each method.

Austral Extremities

What is Thermal Stress?

The marine Bacteria and Archaea that we are interested in live in Antarctic seawater at a temperature around –1.8° Celsius (C). The salts in seawater lower its **freezing point**[6], permitting the seawater to remain liquid below 0°C. Since these organisms are adapted to a colder environment, which is relatively stable year-round, what happens if we expose them to warmer water? Exposing an organism to warmer or colder temperatures than its normal environment creates thermal stress. Research by others has indicated that the highly studied bacterium, *Escerichia coli* (*E. coli*), expresses specific genes in response to temperature changes in a very short time, perhaps 1 to 2 hours after the exposure. How long would it take for the Bacteria and Archaea of Arthur Harbor to respond to thermal changes in their environment? How dramatic would the temperature change have to be to induce a response?

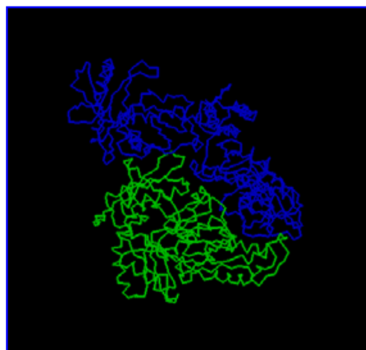
The never-ending chore of hauling 50-liter seawater bottles (2 to a sled) from the field to the lab induced significant thermal stress in the Murray Field Team members!

In our first thermal stress experiment, we exposed our two sample types (50-liters of seawater and 1.5-liters of concentrated seawater) to the following conditions:

- **either** 4-hours of incubation at approximately -1.8°C, **or**,
- 4-hours of incubation at approximately +5°C.

In another experiment, we exposed three concentrated seawater samples to the thermal conditions shown below:

Sample No.	Initial Temperature	1st Incubation	2nd Incubation
Sample 1	-1.8° C	+5° C	N/A
Sample 2	-1.8° C	+10° C	N/A
Sample 3	-1.8° C	+5° C	-1° C
Control	-1.8° C	-1° C	-1° C



Reverse transcriptase has two sub-units. The larger SU is shown in blue.
Photo: http://www.bmb.psu.edu/597a

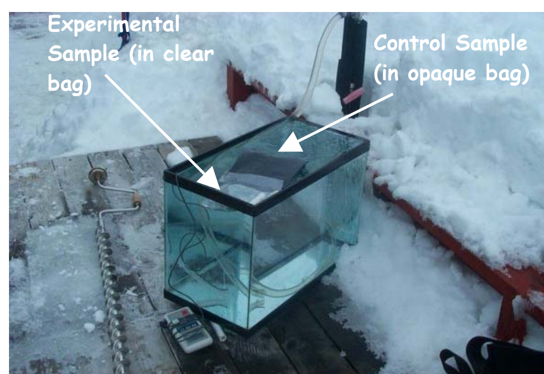
The conditions described above allowed us to look not only at what genes were expressed in response to temperature increase, but also how quickly the microbe recovered when returned to the temperature nearer its native climate.

The samples incubated at –1.8°C are used as a method control, to see what genes are expressed in the background samples over time, after they've been removed from their natural

environment. Back at the Desert Research institute (DRI), Dr. Murray and Brandon will use a special type of polymerase chain reaction, called **RT-PCR**[7], to search each sample for the different mRNA (see Austral Extremities, 2nd Edition) that will be present depending upon which genes are expressed. RT-PCR uses reverse transcriptase (RT), an enzyme that can synthesize DNA from an RNA template. Remember that DNA usually transcribes RNA, not the other way around. Hence the name of the enzyme is "*reverse* transcriptase."

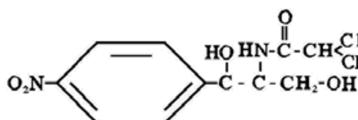
The Influence of Ultraviolet Radiation

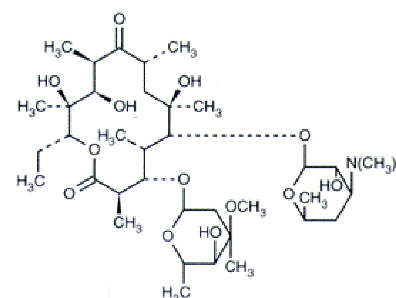
Palmer Station's location in Antarctica gives us a unique opportunity to look at the effects of UV radiation, since the presence of a "hole" in the ozone around the South Pole and surrounding regions is periodically present. The ozone hole permits higher levels of UV radiation to penetrate the area under the hole. Thus, more UV-B radiation penetrates the lower atmosphere and contacts the Earth in this area than elsewhere on our planet. You may recall that UV-B, as opposed to UV-A, is the more hazardous form of UV radiation. The nature of the atmosphere here results in increased UV-B penetration during the austral spring, which is when most organisms are breeding and rearing their young. For this reason, other science groups are looking at the influence of the increased UV-B exposure to marine life forms, especially those that live in shallow waters, where the UV-B exposure is expected to be high.



Reproducing UV exposure conditions. Notice that the control sample is in a dark container, with no exposure to the UV-B, whereas the experimental sample is in a clear container – allowing maximal UV-B exposure.

How does the increased presence of UV-B affect gene expression in marine microbes? Might we see its effects in shallow and deep-water residents? For now, we have decided to look at organisms from the shallow water, and how they respond genetically to UV radiation at Palmer Station. One sample of concentrated seawater was placed in a clear container and another in an opaque container. Both were put in an ambient water bath outdoors and exposed to the UV radiation from the sun. As with most of our work, the results from the experiment are unknown until the lab work can be completed back at DRI.





The chemical structures of chloramphenicol (above), and erythromycin (right), two antibiotics we used in our experiments.

Using Antibiotics as a Genetic Tool

Sometimes we can use the action of specific antibiotics to control our

Austral Extremities

experimental environment. For example, erythromycin and chloramphenicol both inhibit protein synthesis at the large sub-unit of the microbe's ribosome (see Austral Extremities, 2nd Edition). These antibiotics are effective against Bacteria, but not Archaea. If we expose our samples to antibiotics, might we be able to eliminate some of the Bacteria from our sample, while retaining the Archaea? This is a method of sample enrichment – "killing" the microbes we don't want (Bacteria), while preserving those we do (Archaea).

The antibiotics were added to concentrated seawater samples incubated in the dark, to mimic their natural environs. We treated one sample with erythromycin and ammonia. Ammonia is a source of nitrogen, which is a nutrient (food) for microbes. Why would we give food to a sample that we're trying to kill off with a bactericide? (See answer at bottom of last page). We exposed a second treatment with chloramphenicol, and no "food." Dr. Murray and Brandon will explore the results of all of these fascinating experiments, and hopefully gain valuable insight into the lives of the Archaea and those other ubiquitous Antarctic microbes, the Bacteria.

Departure – how time flies!

In little over six weeks, our time had come to an end. The overwhelming task of compiling all of our data and samples into a moveable feast back to the U.S. kept us all distracted (well, almost) from the fact that the Laurence M. Gould had been unable to pick us up from Palmer Station as scheduled, due to ice pack. This threw through a small wrench into our machine – and Brandon nearly built wings to escape out of discarded Halloween costumes! But it allowed us unexpected sightseeing opportunities and a farewell sunset on Torgeson Island. We bade our newfound friends and priceless co-workers "adieu," bowed a solemn goodbye to Palmer from the stern of the Gould, and entered the reflected heavens of the Neumeyer Strait (November 8, 2001). The last page says it all – thanks for joining us this season!

Just as there is a diverse community of terrestrial and sea animals that we can see with the naked eye, there is a dynamic community in the microscopic world. Each and every element plays an important, if unknown, role.

Austral Extremities

A Farewell Gift from Antarctica…

"Sample Dilemma"Answer (Page 2): The DAPI slide of unfiltered seawater on the left has fewer organisms competing for resources like food and oxygen. The slide on the right (concentrate) is "overpopulated" with organisms. Therefore, it has less fluid to dilute waste products, and fewer nutrients available for each organism. Therefore, we can expect a higher success rate for the organisms in the raw seawater sample.

"Antibiotic Tools" Answer: (Page 5) We expect erythromycin to "kill" off the bacteria, while we expect the "food" (nitrogen) to help increase the population of Archaea. In this way, we "selected" our organisms of choice – Archaea.