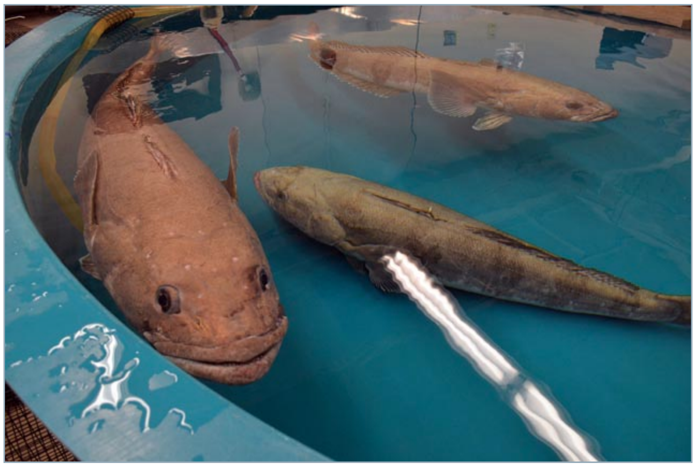

Photo Credit: Peter Rejcek

Antarctic toothfish, Dissostichus mawsoni, swim in a tank in the Crary Lab aquarium at McMurdo Station. Scientists are using them and other fish in the Southern Ocean to learn about the broad adaptations that allow them to function in freezing water.

Growing Cold

Scientists study broad adaptations of Antarctic fish to freezing waters

By Peter Rejcek, *Antarctic Sun* Editor
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The aquarium tanks in **McMurdo Station**'s research laboratory, the **Albert P. Crary Science and Engineering Center**, contained a species of Antarctic fish during the 2012-13 austral summer that has been a rare sight in recent years — *Dissostichus mawsoni*.

The Antarctic toothfish, one of the largest of the 120-plus species that make up the group of fish known collectively as **notothenioids**, has been a favored specimen for study for nearly a half-century. Like many of the notothenioids that inhabit the Southern Ocean around Antarctica, mawsoni have special adaptations that allow them to survive in the frigid waters.

Perhaps the most well-known and intensely studied trait is the ability of these fish to produce a sort of antifreeze protein that binds to ice crystals in the body to prevent them from growing. Otherwise, the fish would quickly freeze and die in seawater as cold as minus 1.8 degrees Celsius.

Photo Courtesy: Paul Cziko

Scientists Paul Cziko, left, and Konrad Meister weigh an Antarctic toothfish in the field.

The sheer size of Antarctic toothfish, which can tip the scale at nearly 150 kilograms, makes it an ideal candidate for researchers who need tissue and blood samples for various analyses, some studies delving deep into the organism's cell biology, as they attempt to learn more about how mawsoni and its cousins function in the world's coldest waters.

"You can actually get fluids from tiny little ducts and canals from the mawsoni that you can't get from the smaller fish," explained **Paul Cziko**, a graduate student at the **University of Oregon** who led the field research out of McMurdo Station this past season.

"We use the mawsoni a lot because it gives you a lot of material," noted **Christina Cheng**, a professor at the **University of Illinois at Urbana-Champaign** and principal investigator on a project to understand the genetic underpinnings that allow the mawsoni and its cold-loving cousins to exist in such an extreme environment.

"It's a precarious kind of existence," Cheng said.

The first insights into how notothenioids cope with cold conditions came back in the 1960s, largely through research by **Art DeVries**, also a professor at University of Illinois at Urbana-Champaign and a co-principal investigator on the current project with Cheng. Then a graduate student at Stanford University, DeVries discovered that antifreeze proteins circulated through the bodies of the fish.

Since then — and thanks to the **genomic** revolution that now allows scientists to reproduce the blueprint of an organism's complete set of DNA — Cheng, DeVries and their team have produced a wealth of data about the evolutionary history of the antifreeze protein trait.

The adaptation didn't emerge until relatively recently, somewhere around 20 million years ago, after Antarctica turned into an icehouse. Cheng's recent research revealed that the antifreeze protein evolved from a small bit of DNA that was attached to another gene, which was a digestive enzyme.

"That piece got duplicated and duplicated and duplicated — maybe changed a little bit — and made this really long repetitive gene that codes for protein and made this antifreeze protein," Cziko explained. "It was a completely new invention."

Photo Credit: Paul Cziko

Field team members fish at the Cape Evans wall.

"Here we actually see how the evolution of a single protein contributes to survival of a species or a whole clade of these organisms," he added.

Thanks to the antifreeze protein and other adaptations — such as the ability to maintain neutral buoyancy despite the absence of a swim bladder — the notothenioids filled many of the ecological niches left vacant by those less suited for frigid Southern Ocean.

Deoxyribonucleic acid, or DNA, is the chemical compound equivalent of both a construction and instruction manual for nearly all living things. DNA molecules are made of two twisting, paired strands, each of which is composed of four chemical units called nucleotide bases that comprise the genetic "alphabet."

The order of the bases determines the meaning of the information encoded in that part of the DNA molecule, just as the order of letters determines the meaning of a word. An organism's complete set of DNA is called its genome.

Photo Credit: Peter Rejcek

Tissue samples from an Antarctic toothfish sit in a tray of ice.

Cheng began her molecular and genomic research after working with a student who sequenced the first antifreeze protein gene. **Sequencing** involves determining the exact order of the bases in a strand of DNA.

"I found that sort of thing very interesting. I sort of taught myself molecular biology after the student left," she said.

The team has since sequenced the entire genome of the Antarctic toothfish. Now Cheng wants to look more broadly at the adaptations that mawsoni and other fish of its ilk in the Southern Ocean possess that allow them to function at nearly minus 2 degrees Celsius.

To figure that out, she and her group need more tissue samples from the Antarctic toothfish, as well as from a non-Antarctic species of Notothenioidei, *Eleginops maclovinus*, which is as closely related to the mawsoni as possible but without the antifreeze protein or evolutionary cold adaptation.

"This whole genome study is to try to figure out what are these changes in all of the proteins that are needed for everything — from growth, development, reproduction, metabolic functions, energy production. Everything," Cheng said. "There has to be something that is different so they can work at this low temperature."

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Scientists Christina Cheng, left, and Lauren Fields prepare an Antarctic toothfish for extracting tissue samples. The large size of the mawsoni provides more samples for genetic analyses.

Photo Credit: Peter Rejcek

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Antifreeze protein only one evolutionary strategy for living in cold water

Finding the fish is half the challenge.

Until the last decade, it was relatively easy to capture mawsoni in McMurdo Sound, where DeVries and colleagues have established favorite fishing holes near the **U.S. Antarctic Program**'s main research facility at McMurdo Station. It wasn't unheard of to catch up to 500 mawsoni in a season, most of which were eventually tagged and released.

Despite nearly three weeks of fishing during the 2011-2012 season, the team, working with New Zealand colleagues at nearby Scott Base, caught just one Antarctic toothfish, a specimen barely weighing five kilograms.

"It was the smallest one we'd ever seen [from McMurdo Sound]," Cziko said.

Some researchers and conservationists believe that a commercial fishery operating in the nearby **Ross Sea** may be affecting the population of Antarctic toothfish, a long-lived species with a poorly understood ecology. The fish caught in the Ross Sea are sold commercially under the name Chilean sea bass.

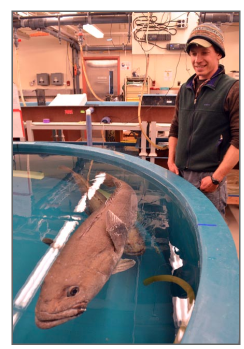

Photo Credit: Peter Rejcek

Scientist Paul Cziko observes an Antarctic toothfish in the Crary Lab aquarium room.

A change in fishing technique by the scientists this season — fishing horizontally near the seafloor instead of vertically throughout the water column — yielded about 20 specimens.

"We need to catch them so we can get their tissues and [determine] how they've changed their gene expression for adapting to the cold here," Cziko said. Genetic expression refers to which genes of an organism may be "turned on" or "turned off" under different environmental conditions.

"In the future, it may be impossible to catch [mawsoni] here. It's great to have a last hurrah here in a way," Cziko said.

Still, despite almost daily fishing trips for weeks, the catch was relatively modest, with no fish weighing more than 40 kilograms, less than half the size that DeVries and his teams would catch before the fishery started operations more than a decade ago.

"The fishery targets the big fish," Cziko noted. In addition, neutral buoyancy is an ability that comes later in life for mawsoni, so those near the seafloor are likely to be younger and smaller because they're unable to "float" up the water column.

The scientists need a range of fish sizes and ages to understand the different genetic strategies for survival at various stages through its life history. For example, fish larvae with a different species from a family of notothenioids called Bathydraconidae (Antarctic dragonfish) possess very little of the antifreeze protein. Instead, they rely on skin as a barrier to freezing.

Photo Credit: Paul Cziko

A field camp at New Harbor across McMurdo Sound where the team also works.

"The story is really complex. It seems to be quite different for each species. There seems to be different strategies for surviving this ocean at those young ages," Cheng said. "Antifreeze is no longer the whole story."

Another chapter in this story involves the ice crystals within the blood of the fish that the antifreeze protein neutralizes. They don't simply go away. Instead, they accumulate in the spleen.

"In this perennially, constantly cold water, once a fish gets an ice crystal in its body, it might be there for good," Cziko said.

Scientists aren't quite sure why sure why the ice crystals end up in the spleen, though the organ primarily filters blood. Cheng likens the presence of ice crystals to the painful condition in humans called gout, caused by uric acid crystals accumulating in the joints. However, the Antarctic fish don't seem to be adversely affected by the foreign object in their body.

Next year, Cheng and other team members will travel to the other side of the Antarctic continent to collect additional specimens from the research vessel ***Laurence M. Gould*** through June and July. Conditions are milder along the Antarctic Peninsula, though would more closely mimic those in the more southerly McMurdo Sound during the winter months.

One particularly elusive species, *Lepidonotothen squamifrons*, the grey rock cod, first found by DeVries around the Belleny Islands between Antarctica and New Zealand in the 1970s, is a notothenioid without the antifreeze protein. However, it somehow navigates its ways through layers of the water column where warmer temperatures prevail.

Photo Courtesy: Paul Cziko

Water is added to a tank containing Antarctic fish inside a hut on the McMurdo Sound sea ice.

Specimens of grey rock cod were found near **Palmer Station** in 2008, but the tissue samples were too poor to work with for sequencing its DNA. Cheng hopes to capture more of the fish for her comparative analysis with mawsoni.

"It still won't tell us why [*L. squamifrons*] can survive without the protein, but at least at the genetic level we can find out what happened to the DNA sequence that led to the absence of the protein," she explained.

The two-month voyage will also offer the researchers a greater variety of Antarctic fish species for their studies on the ice crystals found within the bodies of the organisms. The antifreeze protein is certainly a boon in one sense — but because it binds so tightly to ice, it also prevents ice crystals from melting as temperatures warm up.

"This is the neat thing about evolution … You solve one problem but create another problem," Cheng observed. "The plot thickens."

NSF-funded research in this article: Christina Cheng and Art DeVries, University of Illinois at Urbana-Champaign, ***Award No. 1142158****. Some background information on genetics for this story came from the* ***National Human Genome Research Institute****.*

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