THE GREAT SQUID HUNT

When jumbo Humboldt squid disappeared from Chilean waters, it led to the demise of a world-class electrophysiology laboratory. Now the creatures are back, finds **Tony Scully**, and so are the scientists.


A chance to experiment on the axons of the Humboldt squid drew electrophysiologists to Chile.

R. F. SISSON/NATIONAL GEOGRAPHIC/GETTY

It was three years ago that electrophysiologist Francisco Bezanilla heard that the squid were back. That summer he had already travelled from the University of California, Los Angeles, to the Woods Hole Marine Laboratory, Massachusetts, in pursuit of the creatures. Like other researchers, he spent two months there each year when the Atlantic or longfin inshore squid, *Loligo pealeii,* bred in local waters. He found their giant nerve cells the best biological preparation for studying the electrical signals that cross the cell membrane. But he still struggled to isolate the weak signal generated by the flow of potassium ions.

So when Bezanilla heard what fellow Chilean Miguel Holmgren had to say, his ears pricked up. Holmgren, a neuroscientist at the National Institutes of Health (NIH) in Bethesda, Maryland, had word from family back home that the jumbo Humboldt squid, *Dosidicus gigas*, were in plentiful supply at fish markets throughout Chile. Memories from more than 40 years ago came flooding back to Bezanilla: of his old mentor, Mario Luxoro; of the bustling Laboratory of Cell Physiology in Montemar, Chile, that lured scientists from all over the world; and of long summer days spent hunched over the squid's spaghetti-like nerve axons. Bezanilla and other researchers had been forced to leave Montemar when the Humboldt squid mysteriously disappeared from local waters in 1970. Bezanilla thought that the squid's return might allow him to resurrect the old laboratory, and that the squid's giant axons — on average twice the diameter of its Atlantic cousin and loaded with many more ion pumps — might generate the stronger potassium signal he sought.

In January this year, Bezanilla and Holmgren travelled to the small seaside town of Montemar. They found the old laboratory succumbing to the ravages of time: cracks ran through the walls and a layer of grime covered every surface. "It was so sad to see my old laboratory in this state," says Bezanilla, who now works at the University of Chicago, Illinois. "The place looked awful." The two men scrubbed down the benches and unpacked their equipment. They paid local fisherman to take a trip out in search of the squid. In anticipation, they rigged up their electronics, ready to dissect the Humboldt's axons and, in doing so, continue a quest to understand a neuron's electrical properties that began over a century ago.

Nervous steps

In 1902, German physiologist Julius Bernstein was the first to measure the minute electrical charges on nerve axons. He proposed that the cell membrane somehow blocks the movement of ions and maintains a difference in voltage so that the inside of axons is negative relative to the outside. That changes when a signal propagates along the nerve. The barrier opens, negatively charged potassium ions leak out and an 'action potential' — a wave of voltage he estimated to be 70 millivolts — whips along the length of the nerve. But at that time Bernstein was unable to directly measure the amplitude of the action potential because the frog nerves he was experimenting with were too small to allow him to insert an electrode inside the cell.

Researchers took to experimenting on the cells of marine life such as the common shore crab and algae. Both were in plentiful supply and considerably easier to handle than frogs — plus working with crabs provided an excellent working lunch during long days in the lab. Electrophysiologists attached themselves to marine laboratories, of which, by the 1930s, the two most prominent were at Woods Hole and Plymouth, UK. Kenneth Cole from Columbia University, New York, for example, would spend the summer at Woods Hole

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experimenting with the large excitable cells of the sea algae *Nitella*.

It was British zoolologist John Zachary Young who first proposed experimenting on *L. pealeii*. Squid are the one of the fastest swimmers in the ocean: giant axons that run the length of the body trigger muscular pulses that propel the creature forward. Young urged Cole to take advantage of these axons, which are up to 1 millimetre in diameter, 50 times the thickness of axons found in the common shore crab and 1,000 times that of human axons. Cole found that he could thread a wire electrode right into the cell without disrupting the electrical signal propagating down the axon. Using this 'voltage clamp' technique, with electrodes both inside and outside the cell, it was possible to measure the current as ions move across the membrane during a nerve impulse.

Giant bandwagon

Others were quick to catch on. In 1938, Alan Hodgkin visited the lab in Woods Hole "because some scientists have been getting the most exciting results on the giant nerve fibres of the squid", he wrote at the time. "Cole has been getting results which make everyone else's look silly."

The Second World War interrupted work, but soon after it ended Hodgkin and his longtime collaborator Andrew Huxley sought a local supply of *L. pealeii* in Plymouth. There were a few barren years before fisherman finally delivered a major catch in 1949. In the late summer months they made discoveries using these axons that would overturn Bernstein's cellular membrane theory. Hodgkin and Huxley showed that during an action potential the voltage across the cell membrane changed by 110 millivolts (ranging from −70 to +40), not the 70 millivolts Bernstein had predicted, and that ions do not simply equilibriate across the membrane. The movement of ions is controlled by selective pores, or gates, and during an action potential the positive sodium ions rush inside the cell followed almost immediately by an outpouring of potassium ions. Hodgkin and Huxley shared the 1963 Nobel prize in Physiology or Medicine with Australian John Eccles for discovering how nerve signals travel. Hodgkin later joked that their prize should have gone to the squid.

L. pealeii was big, but for some it wasn't big enough. At the Massachusetts Institute of Technology in Cambridge, Francis Otto Schmitt wanted to identify the components of axoplasm, the fluid inside nerve

Humboldt squid axons can be the size of spaghetti.

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cells. It could be squeezed out of the giant squid axon as simply as toothpaste from a tube — but even then it was problematic because the squid rarely grows longer than a metre and its axon is highly branched.

In a 1955 interview with the *New York Times*, Schmitt also bemoaned the limited supply: only 300 squid per week for the annual two month breeding season. As luck would have it, multimillionaire and champion sports-fisherman Lou Marron happened to read that report. As is the wont of fishermen, he knew of a catch twice as big. He wrote to Schmitt about a species of squid he had used to bait swordfish on expeditions in the Pacific Ocean off the coast of north Chile. He offered to take him there to prove it.

Two years later, the pair set off for Chile with a small team including Schmitt's Chilean PhD student, Mario Luxoro. "I told Schmitt we had huge squid in Chile," Luxoro says, but

Schmitt had been sceptical of their existence. "He thought I was just an exaggerating South American." Still, the prospect of a bountiful supply of squid during the winter months in Massachusetts was appealing to Schmitt. And Luxoro felt vindicated when the team landed the jumbo squid now known to be the Humboldt. Named after the Humboldt ocean current that runs the length of South America, this squid grows up to two metres in length, making it one of the largest squid species in the world and twice the size of their Atlantic cousins that had been the mainstay of electrophysiologists.

The University of Chile in Santiago ran a marine-biology laboratory in Montemar and Schmitt leased a small room there before returning to Massachusetts. Luxoro remained behind, hired by Schmitt to organize the dissection, preparation and packaging of the squid axons for transport to the United States. The proteins inside degrade quickly, so the preparations of squid axoplasm were packaged into boxes with dry-ice for transport back to the United States by air. Even so, few of the packages arrived in a suitable condition for analysis. Both men were unhappy with the arrangement. The lab "was only a factory for axoplasm", recalls Luxoro, who was frustrated at spending December to February packing squid. "They were not interested in us doing pure research."

"When we had squid we would work from eight in the morning till two in the morning."
— Mario Luxoro

Birth of a lab

Luxoro felt that it was time for Chileans to do their own experiments on the valuable asset swimming nearby. With other Chilean researchers, he persuaded the chancellor of the University of Chile to buy a separate house for scientists wishing to study electrophysiology. They were given a former brothel that had a water supply in every room. In 1962, the Laboratory of Cell Physiology was born.

By now, scientists were trying to understand how different channels mediate ion transport across the axon membrane. The team grew to a dozen or so scientists and went on to compete with laboratories around the world. With little money and relatively crude equipment, the researchers could nevertheless ask sophisticated questions because they had an abundance of the best experimental material: the large surface area of the giant axons generated electrical signals strong enough to measure accurately. Luxoro and his student Eduardo Rojas, for example, were the first to provide experimental evidence that proteins within the lipid membrane, rather than the lipid

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molecules themselves, actively transport ions across the membrane[1]. It was also in Montemar that Rojas and Clay Armstrong demonstrated that movement of sodium and potassium ions during an action potential occurs via different membrane channels[2] rather than a single protein, as some had argued. The new laboratory, loosely led by Luxoro, enjoyed much independence. "It was a great time, when we had squid we would work from eight in the morning till two in the morning," he says.

Electrophysiologists flocked to Montemar during the Southern Hemisphere summer. "I loved it there," says Armstrong, a physiologist recently retired from the University of Pennsylvania, Philadelphia. "They were ingenious at setting up experiments using old parts. It was great fun to watch." During this time, a young and impressionable Bezanilla began to dissect the squid and later complete a PhD.

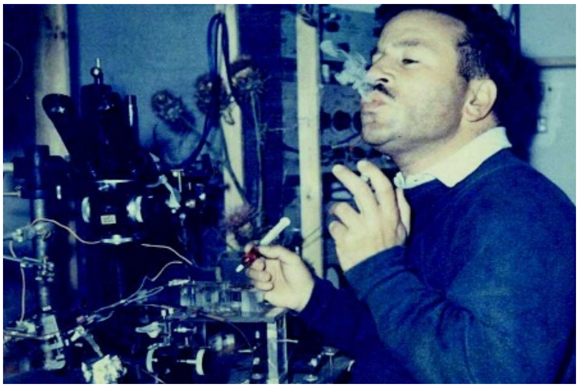
Then, in the summer of 1970, the fishermen returned empty handed day after day. Just over a decade after Schmitt and Luxoro made their expedition to Chile, the Humboldt squid migrated out of range of local fishermen, and beyond the reach of scientists in Montemar. The squid probably followed small fish, krill and other prey, which are sensitive to environmental events such as El Niño.

Gradual decline

It was the beginning of the end for Montemar as an international research station. Many left to fill posts elsewhere: Bezanilla was offered a postdoc at the NIH. The dictatorship of Augusto Pinochet from 1973 further deprived Chilean science of investment. "It was a horrible time," says Luxoro, who remained in Montemar, using barnacles (*Megabalanus psittacus*) to study muscle physiology. "A lot of people went away and science practically disappeared."

By the end of the 1970s, the invention of the 'patch-clamp' method by Bert Sakmann and Erwin Neher had removed much of the need for large axons. The technique involves pressing a glass tube one thousandth of a millimetre in diameter against the cell membrane, isolating a small patch from the surrounding extracellular fluid. An ultrasensitive amplifier can be used to measure the flow of ions across the membrane. In 1990, Luxoro reluctantly accepted a teaching position at the main campus in Santiago and the laboratory was abandoned.

But for some purposes, squid remains irreplaceable. David Gadsby, a membrane physiologist at Rockefeller University in New York, works alongside Bezanilla and Holmgren at Woods Hole every summer to study the action of ion channels in the North Atlantic squid. "We now have three-dimensional static images of the protein pumps and ion channels, but to understand how the conformation of these proteins change in response to varying voltages we need to use the squid," says Gadsby. Much has been learned about how a sodium–potassium pump actively transports sodium ions across the membrane to maintain the resting potential, but the movement of potassium ions is harder to measure. Bezanilla and Holmgren want to study the conformational changes in the pump as potassium ions are transported, and hoped that this would be clearer from variations in the Humboldt axon's strong potassium signal.



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Mario Luxoro in the Montemar lab, 1960 (top); Francisco Bezanilla in 2008 (below); and the lab's seaside location, 1960s (bottom).

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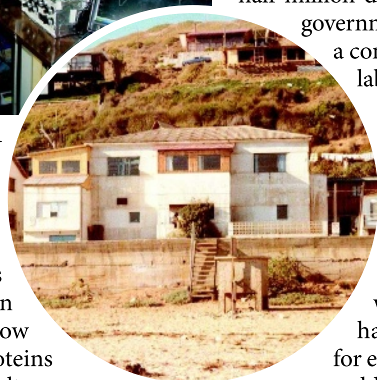

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It was for this reason, and a wish to revive his old laboratory, that Bezanilla returned early this year to Montemar with Holmgren and paid the fishermen to bring in a catch. "We normally pay US$1,700 for a delivery in Woods Hole but here we only had to pay $100," says Holmgren. Their trip and the investment were rewarded: to their great relief the first consignment of squid in nearly four decades arrived at the laboratory.

In their first experiments, the scientists tried to wash away the sodium ions in order to measure the weaker potassium ion signal alone. "We found that the signal-to-noise-ratio of the ion is improved by a factor of five or six with the Humboldt squid compared with the squid we normally use," says Holmgren. One explanation may be that the thicker membrane contains a higher concentration of ion channels and pumps per unit area.

Rebuilding the dream

After spending two weeks working at the laboratory, the scientists returned to the United States bolstered by their preliminary results — and determined to return. They are now applying for funding to go back to Montemar in December. Meanwhile, Ramón Latorre, a neuroscientist who also began his career in Montemar and is now with the University of Valparaíso, is in negotiations with the University of Chile to renovate the lab for visiting scientists. Lattore is applying for a half-million-dollar grant from the local government, which would fit out a conference room and three laboratories.

Gadsby hopes to make his first trip to Montemar later this year. Humboldt squid are now found as far north as California, but the return of the squid to the waters near Montemar has a special significance for electrophysiologists. "I've seen old photographs of the lab and I can't wait to see it for real," Gadsby says. Whether the squid will be there to greet them, though, is beyond the scientists' control. "We're dependent on the migratory patterns of the squid," Gadsby says. The researchers are well aware that the creatures could vanish as mysteriously as they appeared — taking with them the hopes for their experiments and the lab's revival.

As long as the creatures stick around, Bezanilla is hopeful that the lab will return to its glory days as an international research hub. "It was a great feeling to see the place come alive again," he says. "I hope that in the future people will realize the importance's of this laboratory, and that the Humboldt squid will once again attract researchers back to Chile's shore." ■

Tony Scully has just finished an internship in *Nature*'s Munich office.

1. Rojas, E. & Luxoro, M. *Nature* **199,** 78–79 (1963).
2. Armstrong, C. M., Bezanilla, F. & Rojas, E. *J. Gen. Physiol.* **62,** 375–391 (1973).

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