SEA GRANT NEWS

"Coastal Science Serving California"



Growing up to 2 meters (6 feet) in length, jumbo squid are formidable predators that hunt krill and a variety of fishes. Their normal habitat is within the tropical and subtropical waters of the Eastern Pacific. Over the last few years, however, the squid have begun moving into cooler-water areas such as Central California; see article beginning on page 4. Photo ©2003 MBARI

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First Live-Attenuated Vaccine for S. iniae in Development





(Above left) Carlo Milani, a former graduate student in the Nizet lab, injects a juvenile hybrid striped bass with *S. iniae* at Kent SeaTech's research facility in San Diego. (Right) A juvenile hybrid striped bass used in vaccine studies. Photos Jeff Locke

In preliminary trials, California Sea Grant researchers have for the first time demonstrated the feasibility of using a live-attenuated vaccine to prevent the deadly *Streptococcus iniae* infection in fish.

The success raises the possibility of being able to inoculate hybrid striped bass, tilapia, rainbow trout and other cultured species orally through feed, instead of having to inject individual fish—a prohibitively labor-intensive process for American farms.

Besides the economic benefits, live-attenuated vaccines also stimulate a more robust immune response than vaccines from killed pathogens, thus offering better protection from infection, said John Buchanan, a collaborator on the Sea Grant project.

There are currently two vaccines on the market for preventing *S. iniae* infections—AquaVac® Garvetil and Norvax® Strep Si. Both are classical vaccines based on exposing fish to killed versions of bacterial pathogens. However, neither is

approved for use in the United States, Buchanan said. In addition, AquaVac® is for use in tilapia only, and Norvax® is most effective when fish are immersed in a 60-second dip initially; subsequent booster doses can be delivered orally.

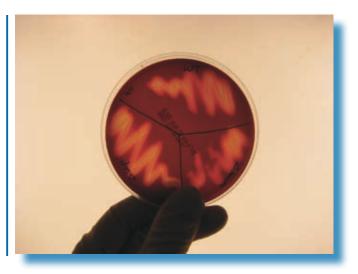
The vaccine that Buchanan and UC San Diego pediatrics professor Victor Nizet are testing, in collaboration with Kent SeaTech, is based on mutating genes of the bacterial pathogen—not on killing the pathogen outright. These mutants have weakened virulence, but they can still infect fish, eliciting a strong adaptive immune response, in which antibodies to the real pathogen are created.

In the trials so far, their vaccine has been administered through injection, which means that each fish has to be given a shot. However, as Jim Carlberg, president of Kent SeaTech, emphasized: "The beauty of live-attenuated vaccines is that you have the potential to put the vaccine in feed." The key is to be able to mutate a gene that does not wipe out the

California Sea Grant Russell A. Moll, Director Shauna Oh, Assistant Director Paul G. Olin, Extension Director Sea Grant News Marsha Gear, Editor Christina Johnson, Science Writer Steve Gabrysh, Publications This publication was supported by the National Sea Grant College Program of the U.S. Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Grant #NA04OAR4170038, project number C/P-1, through the California Sea Grant College Program. The views expressed herein do not necessarily reflect the view of any of those organizations.







(Above left) Streptococcus iniae is closely related to the bacteria that cause strep throat in children. This is how Victor Nizet, a professor of pediatrics at UC San Diego, came to study the pathogenesis of *S. iniae* in fish. (Right) The bacterial pathogen *S. iniae* growing on a blood agar plate. Photos Centers for Disease Control; Jeff Locke

"I think there is an 80 percent

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-Jim Carlberg, president Kent SeaTech

product will come out of this

research."

weakened pathogen's ability to orally infect the animal.

"A vaccine that can be put in feed would have a huge potential advantage in cost," Carlberg said.

"Oral delivery is the gold standard for aquaculture," agreed Jeff Locke, a doctoral student with Nizet, who used to work at Kent SeaTech, a large hybrid striped bass farm in Southern California.

"S. iniae is a ubiquitous disease and a fairly chronic problem," Carlberg said. "It has a huge economic impact on worldwide aquaculture."

About 26 species of fish are susceptible to *S. iniae*, which causes meningitis. Infected fish are anemic-looking, said Buchanan, who is now the chief of finfish research at Aqua

Bounty Technologies in San Diego. They can swim abnormally and have "popeye," caused by swelling in the brain.

For U.S. farms, there is no satisfactory treatment for *S. iniae*. Infected fish can be fed antibiotics, but sick fish often don't eat, Carlberg said. To treat outbreaks, which tend to occur at facilities where

warm-water fish are kept at high density in recirculating tanks, Kent SeaTech has developed its own killed vaccine for *S. iniae*. However, it too requires that fish be individually injected.

Carlberg estimates that an orally delivered, live-attenuated *S. iniae* vaccine would generate revenues of about \$10 to \$15 million annually worldwide. The savings to the global aquaculture industry could be worth ten times this amount, he said.

Nizet, Buchanan and Kent SeaTech have patented the technology that makes their live-attenuated vaccine possible and entered into discussions with pharmaceutical companies about possible licensing agreements.

Meanwhile, researchers with funding from California Sea Grant continue to study genes involved with pathogenesis. Locke, for example, has identified two new *S. iniae* genes whose mutated forms represent potential new vaccines.

Scientists are hoping to present to a pharmaceutical partner a complete portfolio of potential mutants for development of a commercial product. "There may be one gene that contributes more to virulence than all the others," Locke explained.

The U.S. Department of Agriculture has already approved two other live-attenuated vaccines for aquaculture for other diseases, so there is optimism that a live-attenuated vaccine for *S. iniae* would clear regulatory hurdles.

Buchanan said that economics is the reason pharmaceutical

companies have not pursued USDA approval for the two killed vaccines now used in Asia.

"The cost of getting the vaccine approved in the United States is more significant than the size of the market," Buchanan said. "Farms are allowed to make their own vaccines with

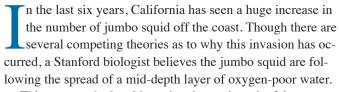
bacterial isolates from their own facilities. That is what Kent SeaTech does."

An effective oral vaccine that could treat a variety of fish species would have greater value to American farms and therefore industry would pursue USDA approval, Buchanan said. Because the USDA is considered relatively strict, its nod would grease the regulatory process in nations that otherwise might be afraid to allow a vaccine based on an infectious agent. "A killed vaccine is the norm," he said.

Kent SeaTech is optimistic about the research outcome. "I think there is an 80 percent chance that a real commercial product will come out of this research," Carlberg said.

Jumbo Squid Following Low-Oxygen Zone





This oxygen-depleted layer barely receives the faintest traces of sunlight and is home to one of the most abundant groups of fish in the sea, lanternfish—glowing, 2–4-inch fishes with big eyes, a staple of the jumbo squid diet.

There has been an expansion of the environment the squid like," said Stanford biologist William Gilly, who has funding from the California Ocean Protection Council and California Sea Grant to study the squid's influence on the California Current ecosystem. "The squid are spreading into this environment."

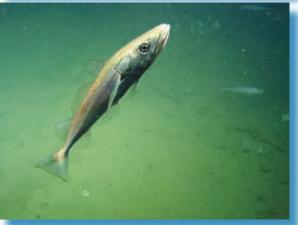
"The low-oxygen domain has grown a lot in the Gulf of California over the last 50 years," Gilly said, citing one piece of evidence for the theory.

More significantly, there is evidence that the oxygenminimum layer is shoaling, becoming shallower, in the California Current, said NOAA oceanographer Steven Bograd, who has analyzed changes in dissolved oxygen concentrations in CalCOFI (California Cooperative Oceanic Fisheries Investigations) data.

"There has definitely been a pretty significant decline in dissolved oxygen content from 1984 to 2006," Bograd said. "We see it pretty much at all depths."

Off Monterey, the oxygen-minimum zone begins at about 500 meters depth and extends to about 1,000 meters. Bograd





(Above left) A pair of jumbo squid shown hunting in Monterey Bay. Jumbo squid are often attracted to the bright lights of MBARI's remotely operated vehicles (ROVs) and follow the robot submarines as they descend through the water column.

(Above right) Hake often form large schools such as those photographed by the video cameras of ROV *Tiburon*. Hake is one fishery that could be hurt by jumbo squid predation. Photos this page ©2003, 2004, 2006 MBARI

estimates that oxygen levels at 500 meters, the deepest the CalCOFI data goes, have dropped about 10–20 percent since 1984.

ROV surveys have shown that Monterey jumbo squid spend the bulk of their daytime hours at about 500 meters, the top of the oxygen-minimum layer, foraging. Similar behavior has been observed in the Gulf of California and is yet further evidence of the importance of the low-oxygen zone to these animals.

Some global warming scenarios predict a revved up microbial loop in the Eastern Pacific Ocean, in which upwelling occurs in short 1–2-day bursts, said Stanford postdoctoral researcher Lou Zeidberg, explaining the link between climate



"You have new predators on the block that are pretty awesome. They have huge energy requirements and they are starting to eat things that people care about."

—William Gilly, professor of biology, Stanford University

Jumbo squid are active predators on small fish such as the lanternfish (lower left and inset). Photo ©2006 MBARI Lanternfish are among the most abundant and diverse of all oceanic fish families. Photo NOAA *Ocean Explorer*

and the squid. Under these conditions, surface algae tend to become food for microbes at depth. Less algae is consumed by zooplankton and small baitfish, which is why global warming could lead to lower fish production off California.

Because bacteria's metabolic processes require oxygen, enhanced bacterial activity further reduces the oxygen content of water beneath the photic zone, where algae grow—in other words, the oxygen-minimum layer shoals, exactly what has been observed in CalCOFI data of the California Current.

As the habitat for lanternfish grows, presumably so does their biomass, Gilly said. It is really the swelling biomass of lanternfish that explains the squid's presence. Lanternfish comprise more than 400 species and are far more abundant than sardines or anchovies. Little is known about them, however, because they are not commercially exploited.

You can think of jumbo squid as one of the early winners of ocean climate change, Gilly said.

Others on the win list potentially include sperm whales, a jumbo squid predator whose numbers appear to have doubled off the West Coast in the last 15 years, said NOAA Fisheries biologist John Field, a collaborator on the project, who recently organized a CalCOFI symposium on jumbo squid.

In the absence of fishing pressure, some sharks could also be on the win list. In fact, Zeidberg believes that overfishing of sharks, and to a lesser extent tunas, both of which normally keep the squid's numbers in check, has probably contributed to the jumbo squid's population boom.

In the last decade, jumbo squid landings off Mexico, Central America and South America have soared from less than 200,000 metric tons to more than 700,000 metric tons. In this period, the northern boundary of the

squid's range has shifted from central Baja California to British Columbia—a staggering territorial expansion.

Some researchers speculate that California's jumbo squid, which first appeared in droves following the 1997–1998 El Niño, are the spillover from Mexico's population boom.

Gilly and colleagues, however, recently have seen a female jumbo squid off Monterey with sperm packets around her beak, proof she mated. They have also found a jumbo squid egg mass in the Gulf of California, the first ever found in nature.

Jumbo squid, which are also known as Humboldt squid, may no longer be transient visitors; they could be reproducing here, Gilly said.

One of the goals of the project is to look for squid hatchlings off Monterey. The smallest jumbo squid found to date off California has only been 10–15 centimeters long. "We want to find squid babies," to prove that the squid are completing their whole lifecycle in the region, Zeidberg said.

Another major goal of the project is to estimate the squid's biomass in the California Current, which stretches along the entire U.S. West Coast, and to document the squid's feeding behavior—to provide managers with ballpark numbers of the squid's impact on species of management concern such as hake, market squid and rockfishes.

"We have no direct evidence the squid is impacting fisheries," Field said. But, it is reasonable to assume they could—or are. Adult jumbo squid off California can weigh 30 kilos. The animals reach this super-size in about a year.

"You have new predators on the block that are pretty awesome, Gilly said. They have huge energy requirements and they are starting to eat things that people care about."

Finding the Cause of "Soft" Caviar

alifornia Sea Grant is funding research to identify the cause of an undesirable farmed caviar trait known as soft-egg syndrome, which affects caviar texure and is blighting up to 20 percent of farmed white sturgeon eggs. The hunch is that the farmed white sturgeon's diet is to blame.

We have to sell soft caviar as a lower-grade product, maybe as a C-grade," said the project's leader Kenji Murata, a biochemist at the Department of Animal Science at the University of California at Davis. Murata will be collaborating with UC Cooperative Extension and the white sturgeon caviar producer Stolt Sea Farm in Sacramento.

At issue is whether sturgeon feed has changed in the last few years.

Because feed recipes are proprietary, the balance of fishmeal to vegetable protein could have changed



slightly, or the quality of fishmeal could be lower or deficient in some micronutrient due to changes in wild fisheries, and the farm would not know, said Stolt's manager Peter Struffennegger, citing an example of what could be going awry nutritionally.

"We know it is not our processing techniques," he said, "because the eggs are soft going into the tin. We have photos of eggs cracking. You add salt and the salt reacts with the egg, and the egg becomes even softer."

He also does not think the soft eggs are due to stress caused by handling and transport, though both will be studied.

Given the hunch about diet, the Sea Grant project will focus on how diet affects egg burst force and other more technical egg qualities, such as the incidence of ovarian follicular atresia (the degeneration of the egg follicule) at harvest. Water temperature is another key environmental factor that will be explored.

"There are a lot of smoking guns," Struffennegger said. "It is about finding out what direction the bullet is coming from."

The Biggest Flaw of Fisheries Policy?

In her recently published 500-plus page doctoral thesis, Sea Grant Trainee Carmel Finley defends the theory that the policy of Maximum Sustainable Yield (MSY) and its implicit assumption that all stocks have surplus production has been the single, biggest mistake of American fisheries management.

Finley, a former journalist, argues that MSY was not about good science. Instead, she said, it was adopted in 1949 to further U.S. foreign policy objectives and territorial claims, in particular the nation's "open seas, open skies" doctrine.

"Between 1949 and 1955, the United States maneuvered to have MSY declared the goal of international fisheries management," she said. The international treaty eventually adopted in 1955 established the rights of foreign fleets to fish off any coast. Any nation wanting to exclude foreign boats had to prove the target stocks were overfished. Fisheries science was turned into a game.

"The practical effect of MSY was that wealthy nations were able to fish unhindered off the coasts of poorer nations, taking as much fish as possible, until these countries began expanding their Exclusive Economic Zones during the 1970s," she wrote.

Finley, whose husband is a commercial salmon fisher in Oregon, points to the collapse of the West Coast groundfish fisheries in 1996 as a contemporary example of how science remains subservient to politics in establishing fisheries quotas.

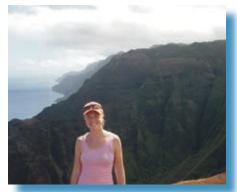
"Why wasn't the evolving scientific picture (on rockfish longevity) built into the management plan? Scientists knew the harvests were too high."

Finley's thesis, "The Tragedy of Enclosure: Fish, Fisheries Science and U.S. Foreign Policy, 1920–1960," is available to the public through the University of California library system.



Photo Rick Starr, Sea Grant Extension Program

Sea Grant State Fellowship Winners Announced



We are pleased to announce this year's recipients of California Sea Grant State Fellowships. These recent graduates, two of whom hold master's degrees in marine conservation and the third a law degree, will each receive a 12-month stipend to learn about and contribute to California marine management and policy.

The winners are:

Cina C. Loarie, a graduate of the Center for Marine Biodiversity and Conservation at UC San Diego (2007), now working at the California Ocean Protection Council. The council is implementing Gov. Arnold Schwarzenegger's Ocean Action Plan. She is currently helping write staff recommendations that will fund a series of expositions of tall ships in 2008.



(Left to right) Cina Loarie, Hugo Selbie and Carina Uraiqat. Photos Cristina Salvador, Lisa Lurie and Melissa Coates

Hugo Selbie, also a graduate of the Center for Marine Biodiversity and Conservation at UC San Diego (2007), is spending his fellowship at NOAA's Monterey Bay National Marine Sanctuary. Selbie is interested in interviewing local commercial fishermen about what they catch, where and what they observe while at sea. He is especially interested in local shark and ray fisheries.

Carina Aisha Uraiqat, a graduate of UC Davis School of Law (2007), is at the California Ocean Science Trust. Uraiqat will use her legal expertise to interpret marine related laws.

The California Sea Grant State Fellowship program was conceived as



an adaptation of the highly successful national John A. Knauss Fellowship program, which places graduates with advanced degrees in marine science or policy in legislative and executive offices in Washington, D.C. The State Fellowship program does the same with "hosts" in state offices, state agencies or other programs with direct relevance to state marine resource management. The goal of both fellowships is to inject a higher level of scientific sophistication into the crafting and execution of marine-related policies.

For more information about the California Sea Grant State Fellowship program and how to apply, visit www. csgc.ucsd.edu.

Knauss Fellow Lands Position at D.C. Budget Office



2007 Knauss Fellow Stuart Levenbach. Photo Lisa Levenbach

Former Knauss Fellow Stuart Levenbach has been hired at the Office of Management and Budget in Washington, D.C., as one of only two full-time employees whose sole responsibilities are to evaluate NOAA's budgetary requests.

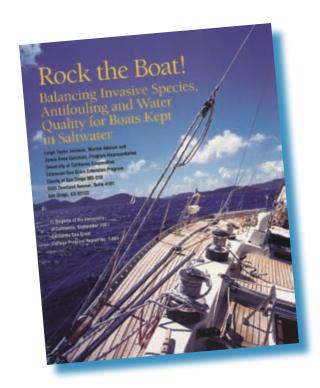
"I am essentially the president's expert on the 'wet side' of NOAA," Levenbach said. NOAA Fisheries, the National Ocean Service and Sea Grant are among these "wet" programs.

Levenbach, whose official title is Program Examiner, earned a doctorate in marine ecology from UC Santa Barbara in 2007. As a Knauss Fellow, he worked with Republican staff on the Ballast Water Management Act and the Coral Reef Conservation Amendments Act, among others.

"Where the rubber meets the road on policy is where you spend money," said Levenbach, explaining his interest in preparing the president's budget. "OMB is a great place to get a macroperspective on policy."

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New California Sea Grant Publication



Rock the Boat!

Because of the fouling organisms that can grow on boat hulls, boats can spread unwanted organisms into new locations. At the same time, the use of toxic hull coatings to kill these organisms can leach undesired amounts of copper into waterways.

How can one control the spread of invasive species while maintaining water quality? Why should one care about invasive species anyway? A new report published by California Sea Grant Extension Program can assist boat owners, marine businesses, harbor managers and resource managers, among others, in weighing their options.

The glossy four-color publication contains pictures of common fouling organisms and discusses why their spread is problematic and proposes steps boaters can take to control them. You may obtain a hard copy of the brochure free of charge by contacting California Sea Grant Marine Advisor Leigh T. Johnson by phone at 858-694-2852 or emailing her at ltjohnson@ucdavis.edu. A PDF of the brochure may also be downloaded for free from the Publications page of http://seagrant.ucdavis.edu.