San Francisco Bay, New Bedford Harbor, Massachusetts, and thousands of locations worldwide have shown the disastrous repercussions of the human-generated chemicals.
TESTING THE HEALTH OF THE BAY

BY MALLORY BICKLEY
Dr. Sarah Cohen, a marine biology professor at San Francisco State University (SF State), is one of a group of American scientists studying polychlorinated biphenyl (PCB) contamination in fish. In a collaborative study with the U.S. Environmental Protection Agency (EPA) on the East Coast, she found genetic differences in fish in polluted bays and harbors. Some have been exposed to so much toxic pollution that they have actually evolved tolerance to the poisons, thereby avoiding lethal effects on their embryonic development, day-to-day functioning, and ultimate survival.

PCBs share characteristics with tetrachlorodibenzo-p-dioxin (TCDD) or simply “dioxin,” possibly the most poisonous human-made chemical in the world. Concentrations of PCBs in some areas of the San Francisco Bay are 10 times higher than the threshold of concern for human health.

Cohen moved west after her initial East Coast research, and continues the work here, examining the genetic makeup of plants and animals in San Francisco Bay and other coastal locations. She also teaches courses in marine ecology and molecular methods in ecology, evolution, and oceanography at SF State. She works with undergraduates and graduate students, and postdoctoral associates, including Tricia Goulding, Verena Wang, Daniel Lake, Mariana Padron, Xuman Tang, Brian Ortiz, Ashley Smith, Richard Coleman, Alyssa Lai, and Vanessa Guerra. She splits her time between the SF State main campus in San Francisco and the Romberg Tiburon Center for Environmental Studies, where her lab is located. Situated on a steep hillside in Tiburon, the Center and Cohen’s office window offer a great view of the bay, its Richmond and Bay Bridges, large tankers on their way in and out of port, and myriad sailboats and ferries.

During part of her East Coast phase, Cohen worked as a research associate at Harvard University and with the EPA’s Office of Research and Development lab in Narragansett, Rhode Island (NHEERL). She looked at how contaminants affect the genetic diversity of fish in New Bedford Harbor and other east coast estuaries by comparing groups under different types of environmental stresses and examining contrasting regions of their genomes. She continued to develop a career-long approach of “using basic science to look at applied problems and using applied problems to understand basic principles in evolutionary ecology.” One such problem is studying the effect of PCB contamination on local populations as a model for understanding rapid evolution in highly variable genetic systems.

The EPA established New Bedford Harbor as a Superfund contamination site leading to numerous studies on environmental impacts and mitigation strategies to manage the extraordinarily high levels of dioxin-like compounds in this small estuary. Dr. Diane Nacci of the EPA’s National Environmental Effects Laboratory in Narragansett, Rhode Island, along with colleagues studied minnow-like killifish and characterized a remarkable tolerance in them for extraordinary levels of dioxin-like compounds.

With a National Research Council fellowship, Cohen joined the group and began genetic tracking of fish exposed to PCBs at the New Bedford Superfund site in 1999. Cohen, Nacci, and collaborators such as Joelle Tirindelli, then a Master’s degree student at RTC/SF State, have gone on to find that local fish populations show strong differences in immunogenetic potential, a measure of the population’s ability to respond to immune system challenges.

PCBs are found in bodies of water as a result of industrial wastes. Waters close to industrial sites are the most likely to become contaminated. Pointing to a map of the greater New York area, Cohen explains, “Newark site is at the bottom of Newark Airport. Jamaica Bay is at the bottom of JFK Airport. And this site right here is near a famous truck stop.” After banning the use of PCBs in 1977, Congress designated Superfund sites in some of the country’s major toxic waste areas such as New Bedford Harbor. Given these designations and the funding that went with them, environmental agencies could sponsor studies and design and undertake clean-up projects.

Although banned over 30 years ago, PCBs had a long and expansive history of use. PCBs have been used in coolants; in lubricants for cutting tools; in the power circuits of most electronics; in weed killers and pesticides; in flame retardants for wood and leather; in hydraulic fluids found in brake systems, power steering columns, and transmissions; in sealants for caulking, paints, adhesives, wood floor finishes, and paper; and in hundreds of other industrial and commercial applications. Businesses and municipalities also released PCBs into the environment while burning garbage, bleaching pulp and paper, and manufacturing certain chemicals.

PCBs and the chemically related dioxins produce similar serious health effects. These include skin rashes, severe acne (chloracne), altered sex organs, various forms of cancer, suppressed immune function, learning deficits, decreased brain activity, deformities, and stunted growth and development.

In Vietnam, many thousands of people, both Vietnamese and American, were exposed to Agent Orange (AO), an herbicide that was contaminated with TCDD. The U.S. military sprayed AO during the Vietnam War to strip Vietnam’s dense vegetation so that soldiers could more easily navigate on the ground. Some Agent Orange was sprayed
Cohen and colleagues repeated these experiments using fish populations all along the Atlantic coast and focusing on one particular species of fish, the killifish.

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over the plots of subsistence farmers and quantities were stored in drums on U.S. Army bases—drums that eventually leaked into agricultural soils, ground water, and surface water bodies. As a result of both the direct spraying and the leakage, thousands of Vietnamese people became exposed to the chemicals.

Medical researchers have documented health effects in Vietnamese locals, in military veterans from the US, Vietnam, and other countries, and sometimes in children exposed directly or in utero. Agent Orange contamination has been associated with cleft palates, mental retardation, or extremities that are misshapen or missing digits. The Centers for Disease and Control and Prevention (CDC) also report that AO has been linked to diseases such as diabetes; and to cancers of the immune system (leukemia), the lymph system (lymphoma), and of the lung, trachea, larynx, bronchioles, and prostate.

Cohen’s research on PCBs led her to two Harvard professors who studied the effects of chronic PCB exposure at the time of the Vietnam War. Drs. Mathew Meselson and Robert Baughman discovered that nursing mothers who were chronically exposed to PCBs passed contamination on to their babies, since PCBs attach to the fatty amino acids found in breast milk. In 1970, those professors published an article in the journal Science, which contributed to the halt of AO spraying in Vietnam. Cohen explains that that particular story was “a fascinating example of how scientists sometimes use what they know to have political effect.”

In 2002, Cohen traveled to Hanoi, Vietnam, to attend a bilateral meeting jointly sponsored by the United States National Institute for Environmental Health and Safety and the Vietnamese government. Her purpose was to present her work with the EPA on some environmental consequences of dioxin-like compounds in American estuaries and exchange information about the environmental impact of these contaminants. The conference signified part of an important process, she recalls, wherein two governments, formerly at war, organized and began to discuss the extremely damaging shared legacy of that war. The continuing progress of this discussion and steps toward mitigation of the harm done in both countries by Agent Orange has been frustratingly slow, Cohen says, yet the process is ongoing.

An equally fascinating aspect of the PCB/dioxin story is how high concentrations of toxic chemicals similar to those found in Vietnam—with known effects on human health—could have accumulated in bays and harbors along both American coasts. The “amount of PCBs originally found in New Bedford Harbor,” says Cohen, “appears to be an order of magnitude higher than anything they found to date in Vietnam.” This could mean that people chronically exposed to contamination in Massachusetts, California, and elsewhere, could face some of the same health effects seen in Vietnamese populations.

The link between environmental exposure and human health in Vietnam holds lessons related to dietary exposure routes via American waters and their resident fish populations. PCBs bind to dietary fat, as the dioxin in AO did in humans. Fish that have been surviving high PCB levels in estuarine Superfund sites also store PCBs in their fat. Somehow, rather than dying from extreme toxin exposures, their bodies are allowing them to develop tolerance. In fact, research by Diane Nacci’s group and others has shown that the extreme toxin exposure in New Bedford Harbor appears to have driven a rapid evolutionary change in the genetic makeup of its fish.

Cohen’s research in New Bedford Harbor, aimed at testing the effects of toxins on particular genetic systems, involved sequencing the fish DNA. She did this after isolating particular genes so that she could test for mutations that might reveal evolutionary adaptation after chronic exposure to PCBs. Cohen and colleagues repeated these experiments using fish populations all along the Atlantic coast and focusing on one particular species of fish, the killifish (Fundulus heteroclitus). Earlier work by other researchers suggested that the chemical pathway involving the aryl hydrocarbon receptor (AHR) might have shut down in these fish.
In a normal fish, a PCB molecule would bind to this important dioxin receptor and initiate a biotransformation pathway that would produce highly toxic intermediate metabolites. In the killifish, although PCBs are still apparently binding to the AHR pathway, the pathway does not proceed normally to break down the toxic compounds. Instead, the fish are storing the PCBs in their fat. This apparently allows them to survive by avoiding the even greater toxicity released from the metabolic breakdown products produced during intermediate steps in the AHR pathway.

Killifish spend their lives in New Bedford Harbor, where PCBs have accumulated since the 1940s, rather than migrating in and out as do striped bass and certain other kinds of fish. Ironically, this has allowed them to survive. Because they have remained in a contaminated environment, the bodies of those that have survived have evolved different physiological and molecular mechanisms to deal with PCB pollution not seen in other fish. The killifish's survival, however, doesn't mean they are free from all toxic effects.

The repercussions of storing contaminants might be seen in several areas of their development, says Cohen. “This [AHR] pathway didn’t evolve to take care of PCBs; we invented PCBs” in the 19th century. “The pathway has been there for millennia and the pathway runs basic developmental processes. So shutting down the pathway has some big consequences.”

Cohen came to SF State in 2003. Here she has found many students who share concerns about how environmental and human stresses affect estuarine and marine species. She points her students toward applicable research and agencies that might have useful data. Sometimes, they collaborate with local and national agencies and organizations such as NOAA, the California Coastal Conservancy and Ocean Protection Council, the Smithsonian, the National Park Service, and local parks and conservation organizations that are monitoring areas of the San Francisco Bay and many other coastal environments. Students can design their own studies, collect their own samples, and carry out genotyping using the shared RTC Molecular Laboratory. Funded in large part by an award from the National Science Foundation, this lab is located at the Romberg... continued on page 30

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Dr. Sarah Cohen

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Above:
Two examples of the unusual parasites found in New Bedford Harbor killifish. Left photograph: swim bladder parasites. Right photograph: killifish heart packed with parasite cysts. Photos courtesy of Dr. Cohen.

Page 27:
Satellite view of the mouth of New Bedford Harbor as it enters Buzzards Bay, Massachusetts. The hurricane barrier at the mouth of the bay visible in this photograph restricts flow between the Harbor and the Bay.
How do organisms change genetically to adapt to environmental challenges? How does the genetic composition of populations, species, and communities change over time? Students use the RTC molecular lab, directed by Dr. Sarah Cohen, along with field and laboratory studies at the Romberg Tiburon Center, to answer questions using diverse organisms from sea stars and sea grasses to sea squirts and sea horses. The history of species, populations, and environmental change is recorded in the DNA variation that students can assess using modern molecular techniques including PCR, DNA sequencing, and DNA fragment sizing. Thanks to equipment grants from the National Science Foundation and the College of Science and Engineering, as well as donations from UC Davis, UCSF, Applied Biosystems, and private donors, students carrying out diverse studies using this joint use facility are answering ecological and evolutionary puzzles about local and distant organisms and environments. If you would like to know more, please see the website, http://rtc.sfsu.edu/in_cohen.htm.

Graduate student Verena Wang is using molecular techniques to investigate recently established populations of the invasive tunicate Botryllus schlosseri. Wang’s research compares recently discovered Alaskan populations to long-established San Francisco Bay populations to see how invasions differ with time since establishment. She is using two molecular markers: a mitochondrial gene and a recently discovered highly variable nuclear gene that has never before been used for population genetics studies. Alaska is currently relatively uninvas ed, yet under significant threat. To preserve the remarkable biodiversity of this region, it is important to examine these new invaders, early in the process, to gain information that may be used to limit further invasion.

Graduate student Tricia Goulding is researching parasites to elucidate ecological interactions related to the health of marine organisms. She studies an acanthocephalan parasite, a “spiny-headed worm,” that infects the common filter-feeding mole crabs (Emerita spp.) found on sandy beaches. Mole crabs are a critical component of sandy beach food webs, and their parasites travel between them and vertebrate hosts including diverse sea birds and otters. Genetic techniques offer a way to determine if acanthocephalans from different crab populations have genetically diverged and actually represent different species. Goulding is studying whether multiple species of acanthocephalans co-occur among California mole crab populations, and whether different species infect geographically isolated species of mole crabs.

David Lake, a graduate student in the Cohen lab, is researching the population structure of flatworm parasites that have been introduced to the California coastline from the Atlantic Coast. The Parvatrema parasites use the Amethyst Gem clam as an intermediate host; this clam is native to the North American Atlantic, occurring from Nova Scotia to the Bahamas. The Amethyst Gem clam, with its broad thermal tolerance, has also become successful in San Francisco Bay and along the Pacific coast, and has displaced local populations of two species of native clams. Lake’s research examines whether this Atlantic clam brought parasites from its native range, and whether these parasites now inhabit native California clams. Invasive species threaten the stability of aquatic ecosystems, and knowing the number and extent of parasite invasions in these clams can serve as a model system for the introductions of other parasites brought by invasive species.

Xuman Tang’s Master’s thesis topic is “The identification of asexual and sexual reproduction patterns of eelgrass (Zostera marina) in the San Francisco Bay.” Eelgrass is a globally distributed marine angiosperm; it is a critical foundation species in many estuarine ecosystems, providing oxygen, food and habitat for many fish and invertebrates. Due to global climate change and disruptive human activities, eelgrass is threatened globally, including in the Bay area. Eelgrass reproduces both sexually (by flowering and germination) and asexu ally (by rhizome elongation). Tang is studying eelgrass reproductive patterns in San Francisco Bay using environmental mesocosms and fine scale genetic clone mapping. She collected seeds from three area populations, planted them in the greenhouse, and raised the plants for five months to find that blade growth and shoot reproduction rate do not differ between annual and perennial populations in the mesocosms. A fine scale clone map made by using DNA microsatellites with nine loci shows the importance of both sexual and asexual reproduction in a perennial eelgrass population, with high rates of sexual reproduction even in a perennial seagrass bed. Her work will help us understand how the surprising rates of genetic population differentiation among San Francisco Bay beds may occur.

Graduate student Ashley Smith is investigating how environmental parameters affect the reproductive biology of the six-rayed sea star along the rocky coastlines of Northern California. Ashley is measuring how hydrodynamics shape the reproductive output of the brooding sea-star. Specifically, she is using a wave dynamometer to measure local wave forces in both high- and low-energy habitats. Despite the crashing waves and rapid water currents in the rocky intertidal zone, brooding Leptasterias hold their young beneath their bodies. In this position, the majority of the stars’ tube feet are used to protect her brood. With just a small fraction of tube feet remaining attached to the substrate, and strong lateral forces from water movement, brooders have a great risk of brood loss. This data, coupled with active monitoring of both brood and embryo loss in varying hydrodynamic habitats, will give us a better understanding of the costs and benefits associated with this important reproductive strategy.

Richard Coleman, a graduating senior in Dr. Sarah Cohen’s lab, has been researching the ecological genetics of six-rayed sea stars in the genus Leptasterias. His research addresses pressing questions about how habitat diversity may promote species diversity in nearshore habitats. Although there have been numerous systematic studies on Leptasterias, few have focused on those living in Central California and thus their taxonomic status remains unresolved. Coleman has characterized, in detail, both genetic and morphological traits of 150 individuals from diverse microhabitats using PCR and sequencing to identify different species. Preliminary analysis suggests a novel clade, possibly at the species level, as well as intriguing differential distributions of genotypes between different habitat types, independent of geographic distance.

Mariana Padron, a graduate student from Venezuela working jointly with the Cohen lab at RTC and Dr. Healy Hamilton’s lab at the California Academy of Sciences, is examining geographic patterns of genetic connectivity in Caribbean and Atlantic seahorses. She is interested in the application of molecular genetics in prioritizing areas for conservation. Seahorses are economically valuable fish species that are listed as threatened. Previous studies have suggested that each one of the Caribbean seahorse species, Hippocampus erectus and H. reidi, may actually be a species complex, because of high levels of morphological variability and broad geographic distributions. She is using several types of molecular markers to help discriminate between closely related and cryptic species. This information will contribute to the management of exploited seahorse populations as required under the Convention on International Trade of Endangered Species (CITES).
Undergraduate and graduate student researchers at RTC carry out field studies, laboratory experiments, and genetic analysis to answer questions about environmental change and human impacts on local and distant habitats. Their research provides answers to pressing applied questions on human impacts and also addresses basic questions in evolution and ecology that give broader answers about biological processes. A focus of the Cohen lab is in developing a greater understanding of processes that act upon biological diversity in its broadest sense, from the molecular to ecosystem levels.

**Top left:** samples are prepared for genetic analysis using techniques including DNA and RNA extraction, PCR, cloning, sequencing, and fragment sizing, in the RTC gene lab.

**Second from top left:** Population level analysis requires collection, curation, and analysis of hundreds of samples to make comparisons of variation within compared to among populations. Samples become an invaluable resource archive for analyzing temporal as well as spatial patterns of variation in response to changing environments. Properly archived samples can be used for morphological and genetic analysis decades to 100s of years after collection providing the essential baseline for studies of change. The San Francisco State University Invertebrate Biodiversity Collections include irreplaceable samples collected by Ed Ricketts of Pacific Grove, the famed invertebrate naturalist featured in John Steinbeck's novels.

**Third from top left:** the Pacific mole crab, Emerita analoga, is just one of the many abundant species researched using the above mentioned methods, and featured in the sidebar on page 29.

**Bottom left:** former graduate student, Joelle Tirindelli, analyzing Atlantic killifish immunogenetic changes in the RTC gene lab.

**Top right:** Master’s student Xuman Tang, in collaboration with Dr. Zheng-Hui Ho’s lab, creates protein alignments from data in the NIH database to search for new genes to aid understanding of population health in marine flowering plants.

**Second from top right:** graduate student David Lake examines native and invasive clams for parasites that complete their complex life cycle in vertebrate hosts.

**Bottom right:** undergraduate Richard Coleman, now a graduate student at the University of Hawaii, began his field work at SF State in the Marine Ecology class learning census methods.

Photos top left and right, second from top left and right provided by the SF State Science Graphics Center. All other photos courtesy of Dr. Cohen.

Tiburon Center. At this facility, students work with Cohen on diverse species from seagrasses to sea stars, sea squirts (also known as tunicates), copepods, various types of parasites, and fish. All the projects are aimed at understanding the evolutionary ecology of estuarine and marine species in environments with varying exposure and responses to human impacts.

Regarding contaminants, working on the West Coast has presented Cohen and her students some serious challenges. The estuaries on the East Coast, for example, are smaller than San Francisco Bay and are distinctly separated from each other, making it easier to find cleaner reference sites among contaminated ones. The industrial histories of the two coasts are also very different. Eastern waters have been subjected to a number of different pollutants during 400 years of fishing and 100 years of industrialization. Development and commercial fishing are newer in San Francisco Bay, but because the Delta system controls water flows into the Bay, it is particularly vulnerable to contamination and sedimentation.

Moreover, estuarine organisms can be larvae in one location and
to another recent article from the National Resources Defense Council (NRDC) called “Contamination of Fish from the Bay Poses Health Threat to Those Who Catch and Eat Them,” consuming fish from the Bay is unsafe due to high levels of PCBs in the water, sediments, and fish themselves. Due to these health risks along with declining productivity, little seafood from the Bay is currently sold on the retail market. Herring is the last commercial fishery in the Bay, Cohen says, and “may not be viable for much longer.”

The San Francisco Estuary Institute (SFEI) is a non-profit organization focused on studying levels of toxic chemicals in the Bay. They have noted that sites near industrial areas along the shoreline and in local watersheds are the most likely to be contaminated, as in New Bedford Harbor. In a 1997 study, for example, they report that 50 percent of San Francisco Bay fish sampled by SFEI workers were contaminated with levels of PCBs and mercury 10 times greater than the threshold for human concern.

Thousands of people still fish in San Francisco Bay, however, and eat not only the fillets, but also more contaminated parts like fish organs. Save the Bay, a nonprofit organization dedicated to cleaning and preserving the Bay, conducted several surveys and found that 70 percent of people fishing and eating from the Bay were not aware of the health risks.

The world is a living laboratory for the effects of human-generated contaminants. Each ocean-going tanker, each refinery, each car driving over an elevated bridge, each instance of chemical warfare, and each operating incinerator helps to contaminate the world’s waters. Sarah Cohen’s work, along with her collaborators and students, serves as a reminder that such problems are not just on other coasts and other countries; they can be in our own backyard.