The Whiskey Creek Shellfish Acid Tests

Ocean acidification and its effects on Pacific oyster larvae

BY NATHAN GILLES

Alan Barton drains seawater from a large tank, filtering its oyster larvae as he goes. He will then sort the larvae by size, using custom-made screens.

The sun chips away at the marine layer on this swiftly warming May morning in the bay. On the estuary's muddy banks, clammers dressed in knee-high rubber boots dig in the dark sludge, while throughout the bay other aquatic farmers dredge for their prize: oysters.

The oysters are *Crassostrea gigas*, commonly called the Pacific oyster. These "giant oysters" measure from 3 to 15 inches long. They're huge moneymakers for global aquaculture, and they have a special relationship to this place. This is Netarts Bay, Oregon, the center of the state's oyster industry and home to the Whiskey Creek Shellfish Hatchery, one of the nation's largest producers of Pacific oyster larvae. Hatcheries such as Whiskey Creek are linchpins for industry. That's because the Pacific oyster is originally from Japan, and here on the west coast its delicate larvae grow wild in only a handful of places. For oyster growers from California to Canada to succeed, hatcheries must raise larvae. Unfortunately, as an incident at Whiskey Creek proved, the larvae are under siege.

In 2007, Pacific oyster larvae at Whiskey Creek started dying en masse. Oregon State University scientists later pinned the crime on ocean acidification. This is the term many are using to describe what's happening in the world's oceans as excessive atmospheric carbon dioxide (CO_2)—a product of human industry's hunger for fossil fuels—is dissolved in seawater. Once it's in the ocean, CO_2 forms carbonic acid, which lowers the water's pH level, making it more acidic. The consensus is that ocean acidification is just getting started. As CO_2 is continually pumped into the air, the world's oceans are expected to slide further toward the acid side of the spectrum, and that, say researchers, won't be good for animals like the Pacific oyster. That's because oysters and other mollusks make their shells from calcium carbonate, which is becoming increasingly susceptible to breaking down in our ever-more corrosive seas. This is what happened at Whiskey Creek: the seawater in which the hatchery was raising its larvae had succumbed to ocean acidification; the larvae struggled to make shells, and died. But this isn't the whole story.

Today, with Oregon Sea Grant's help, OSU researchers are continuing to investigate ocean acidification's nefarious ways. They're gaining a better understanding of oyster larvae's response to the phenomenon. They're developing better seawater monitoring techniques. And they're connecting with stakeholders in an effort to develop useful diagnostic tools for hatcheries and growers. Yet getting to this point took time. When the hatchery's larvae started dying, it was mystifying.

Acid's first inklings

The tide is very low. The moon has pulled back the saline blanket covering Netarts Bay, revealing a normally concealed landscape of sandbars, muddy flats, and Whiskey Creek's intake pipes.

"It's usually not like this," says hatchery employee Alan Barton, gesturing at the bay.

"There's usually water over all this."

Barton is standing on the estuary side of a two-lane road hugging the bay, with Whiskey Creek behind him. In front of him, a small slope declines toward the bay. Jutting from it are two intake pipes. They hang mid-air over a still-submerged bed of aquatic eelgrass. Normally, these pipes help pump between 100 and 200 gallons of water per minute into the hatchery. Not today. Today, other pipes—still underwater—do it all, pumping bay water, under the road and to the hatchery, where it's treated and then dumped into massive tanks filled with Pacific oyster and other shellfish larvae.

Barton is wearing a T-shirt bearing NASA's logo. It's an odd choice for such a down-to-earth guy, which is exactly what he purports to be.

"I don't do science," says Barton. "I work in a hatchery." Although that may be true, everyone agrees he was the first to solve the mystery.

In 2012, Barton and the hatchery gained national attention when he and several OSU researchers published a paper announcing they'd discovered that the bay water drawn from those intake pipes was killing the hatchery's Pacific oyster larvae.

Getting to that conclusion took time. At first, the only thing anyone knew was that something was horribly wrong.

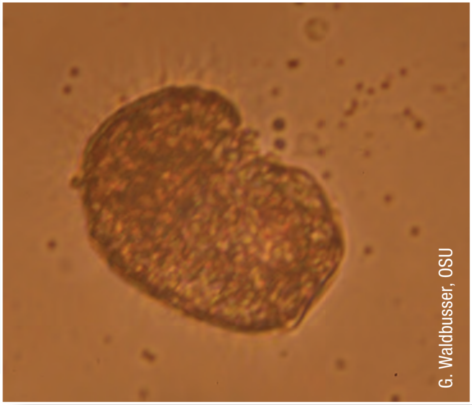
"We had three or four months when we had zero production. We'd never seen anything like it," says Mark Wiegardt.

Wiegardt owns a small oyster farm on the bay's south end. Since 1997, his wife, Sue Cudd, has owned and operated Whiskey Creek, with what he says is modest input from him. True to his words, as Wiegardt is outside, leisurely relating the tale of the hatchery's bad acid trip, Cudd bustles among the rows of enormous tanks. Using trays covered in fine netting, she's sifting tank water for nearly microscopic organisms no wider than a strand of human hair. The result of her sieving is what looks like a mess of fine mud. But it isn't mud; it's Pacific oyster larvae, millions per tray. Larvae that at two weeks of age are put on ice, placed in insulated boxes, and next-day shipped to oyster growers the world over, growers who then raise the animals to maturity before the tasty critters end up as someone's dinner.

All that's happening inside. Outside, Wiegardt is hunched over his truck bed, separating oysters for breeding and describing how the hatchery and its OSU helpers uncovered the larvae killer.

Wiegardt says the troubles began in the late summer of 2007. "To be honest, we didn't know what was causing it," he says, "but *[Vibrio] tubiashii* was present, so at first we thought it was that."

A bacteria named *Vibrio tubiashii (V. tubiashii*) was the initial culprit on the hatchery's wanted list. *V. tubiashii* preys on Pacific oysters and occasionally blooms when conditions are right. Suspecting a pathogen was at work, Wiegardt started sending samples off for testing. Sure enough, the water tested positive for *V.*



G. Waldbusser, OSU

Above: Once sorted, larvae are collected in tubes and—"like decorating a cake," says Alan Barton—are oozed out in small bundles that are then refrigerated and next-day shipped the world over. A typical bundle contains anywhere from 40 million to 150 million larvae.

Right: Pacific oyster embryo, seen under a light microscope. Formation of first shell has just begun.

Along with growing oyster larvae, the Whiskey Creek hatchery also grows their food. Above left, Sue Cudd inoculates a tank before starting a new batch of algae. Above right: Mark Wiegardt inspects one of the hatchery's 8,000-gallon, seawater-filled tanks.

tubiashii. To ward off future outbreaks, Whiskey Creek installed an enhanced system to cleanse the incoming water. This helped, but, says Wiegardt, "There were definitely still problems."

With their new defenses up and running, Whiskey Creek hired Barton as monitor. Then came another die-off, only this time the water was nearly clean of *V. tubiashii*. Something else had to be at work.

At the time, Barton was perusing Richard Feely's work. Feely, an expert in chemical oceanography at the University of Washington, had concluded ocean acidification's low pH waters were corrosive to calcium carbonate, a necessary ingredient in oyster shells. That's when Barton thought maybe the larvae slayer was ocean acidification. To test this, he sent water samples to OSU Professor Burke Hales, also an expert on ocean chemistry. After testing the water, Hales—who continues to work closely with the hatchery, and was a co-principal investigator on that 2012 paper—confirmed Barton's suspicions. "Pretty soon," says Wiegardt, "it became obvious…we had a pH problem."

Understanding how the hatchery's corrosive waters made their mayhem came soon after.

Straight to shell

"The first 24 hours following fertilization are the most important," says George Waldbusser. "That's when the larvae build their initial shells."

Waldbusser is an assistant professor at OSU's College of Earth, Ocean, and Atmospheric Sciences specializing in, among other things, bivalves' responses to acidifying waters in estuaries. He was an author with Barton on that 2012 paper. He's currently being funded by Oregon Sea Grant (OSG) and the National Science Foundation to research ocean acidification's effects on oysters and other bivalves. And he's heading up the investigation at Whiskey Creek. He also has a pretty good grasp on what it takes for Pacific oyster larvae to form their calcium carbonate shells.

"What it comes down to," says Waldbusser, "is how fast they can make their shells."

When Pacific oyster larvae are fertilized, they don't yet have shells. But they need them quickly. Without shells, the larvae can't form things like internal organs and feeding and swimming appendages—which they obviously need to survive. Waldbusser says they're also running on borrowed time. What energy the larvae still have from their lives as eggs won't last forever. The race is on; it's straight to shell, or die trying.

Pacific oysters make their shells from calcium carbonate, the chemical make-up of other mollusk shells as well as corals, snails, and pteropods. Seawater is usually supersaturated with the stuff, but, says Waldbusser, the larvae can't wait for calcium carbonate to leisurely form their shells around them. That takes ages. So the larvae push the process along. In addition, Pacific oyster larvae can't use just any calcium carbonate. Those initial shells need aragonite, the less stable of calcium carbonate's two major forms.

"Aragonite isn't anything that's in the water," explains Waldbusser. "Aragonite is made out of calcium and carbonate and it's how those are put together that forms the shell."

To put it all together and make aragonite, oyster larvae capture carbonate, bicarbonate ions, and even CO_2 from seawater by bombarding it with protons.

George Waldbusser

They then combine these with calcium they've gathered using silk-like proteins they produce. Then the little animals chemically mix it up and, voilá, they've got aragonite. This process, says Waldbusser, is energetically expensive relative to the energy the larvae still have. If they use too much, the larvae die. That appears to be what happened during Whiskey Creek's die-offs.

Low-pH water severely curtails the available ions. (Globally, ocean acidification has resulted in a 16 percent reduction in carbonate ions, according to Feely.) Aragonite itself is also particularly vulnerable to acidification, being more soluble than calcium carbonate's other major form, calcite—which adult oysters use to continue shell building. That makes it much harder for larvae to form shells before they run out of energy. How the larvae respond to this stressful situation is the subject of Waldbusser's current OSG-backed work.

Little victims

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Graduate researchers Iria Giménez (left) and Becky Mabardy set up a lab at the Hatfield Marine Science Center for experiments on how ocean acidification affects oysters.

To mimic the bay's fluctuations, Waldbusser and his grad students have been subjecting day-old oyster larvae to varying degrees of exposure to CO_2-enriched water. They've pummeled larvae with CO_2 levels ranging from not too bad to horrible. They've examined how quickly they can gas the organisms to determine whether sudden bursts of CO_2 are worse than a slow build-up (preliminary results suggest that yes, it's worse). The whole thing is very stressful for the little guys, and that, says Waldbusser, is the point.

"We're trying to understand, over the entire larval period, how does a stressor translate into stress on the organism?" he says.

Once thoroughly assaulted, the larvae are flash-frozen in their various distressful throes; the tiny creatures' shells are then smashed—harder than it sounds, considering they're smaller than grains of sand—and dowsed with chemicals to further destroy the aragonite husks. Then they're blended in a centrifuge, and their lipids and proteins are chemically expunged. What's left is a globular mass of once-very-stressed tiny oysters, now reduced to a mere smudge of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).

"We're trying to capture a general [picture of] stress," says OSU grad student and Waldbusser team member Iria Giménez.

Giménez oversees the stress-inducing research. She says the idea underlying it is pretty simple: measure stress in a very general way by comparing the amount of RNA to DNA in the larvae.

DNA is the "blueprint of life"; the intertwined and interlocking molecules tell the body which proteins to synthesize and when. To do that, DNA needs help. Enter RNA.

RNA acts as a kind of messenger molecule, and, says Giménez, how many messengers a particular organism has at any given time tends to be a pretty good sign of just how quickly that organism is growing. And because stressed-out animals grow more slowly than normal ones, a low amount of RNA—as measured as a ratio of RNA to DNA (since the amount of DNA stays constant)—can tell you if an animal is freaking out and how badly.

"When they [oyster larvae] are under acidification stress," she says, "that growth is being depressed. They aren't growing as much, so the amount of RNA is less, so the ratio is less."

Waldbusser says that as far as he knows, this is the first time this methodology has been used to measure ocean-acidification-induced stress in Pacific oyster larvae on such short time scales. These snapshots are important, he says, because of how critical those first 48 hours are to these organisms. But it's not all make-shell-or-die for Whiskey Creek's larvae; there are some remedies.

The hatchery solution

Intake pipes pumping in bay water release a steady surge into the hatchery tanks in front of Stephanie Smith. At the OSU grad student's feet are five large bags with the words "Dense Soda Ash" written on them. This is the remedy for Whiskey Creek's upset waters.

"They put soda ash in this," says Smith, pointing at a large tank. "They then pump the soda water in until they get the state they want."

Smith, who also works with Waldbusser, says the bags of soda ash act like large antacids, raising the water's pH level. This process, referred to as "buffering," is part of a monitoring and treatment system that's been lovingly named the "Burkolator," after its creator, Burke Hales. Smith is one of the Burkolator's keepers. Above the roar of laboring pumps and incoming water, she explains its mechanics.

The Burkolator is a series of sensors all routed through a single computer. It calculates pH and different measures of dissolved CO_2, and it quantifies what Smith says is, without question, the most important metric for the hatchery: the aragonite saturation state. This is a measure of the "corrosivity" of the mineral, or its ability to be broken down by low-pH bay water. The state is determined by the availability of carbonate ions, where fewer ions means less aragonite and a

(Continued on page 8)

The return of *Vibrio tubiashii*

What OSG scientists are learning about the oyster-harming pathogen

In 2007, when Pacific oyster larvae began dying at the Whiskey Creek Shellfish Hatchery in Netarts Bay, Oregon, a pathogenic bacteria called *Vibrio tubiashii (V. tubiashii)* was originally blamed. Researchers later determined the culprit was in fact low-pH water (see "The Whiskey Creek Shellfish Acid Tests" in this issue). But that doesn't mean *V. tubiashii* is off the hook.

With Oregon Sea Grant's (OSG) backing, Chris Langdon, Oregon State University professor and head of the Newport Aquaculture Laboratory at the Hatfield Marine Science Center, is continuing to investigate *V. tubiashii*. He's being joined by OSU microbiologist Dr. Claudia C. Häse, an expert in bacterial pathogens; and Dr. Ralph Elston, an affiliated professor at the University of Washington and founder of a company that performs scientific testing for the aquaculture industry.

V. tubiashii presents continuing puzzles for researcher Chris Langdon.

The researchers are testing to see what effects metalloprotease, a particular protease (or enzyme) that *V. tubiashii* produces, might have on bivalve larvae. To do this, the researchers are subjecting the larvae of three different oyster species—including Pacific oysters—to a special mutant breed of *V. tubiashii* that doesn't produce the enzyme.

Langdon says that, much like *V. tubiashii*'s sudden appearance in 2007, there's been another big surprise in the knowledge that's slowly building for this bacterial pathogen. He says the current OSG research confirmed some but not all of their original suspicions.

"[Under laboratory conditions] the initial suspected toxin [metalloprotease] does not seem to be as harmful as we originally thought," says Langdon. But he says it's possible that other factors are involved in determining the toxicity of *V. tubiashii* under hatchery conditions, including the low-pH seawater linked to the Whiskey Creek larval die-offs.

"There's still a lot we don't know about this bug," he says.

lower saturation state. Smith says that when a low state is noticed, that's when you add the soda ash.

However, this doesn't always help.

The acid test

"July and August, I call that the acid test," says Wiegardt. "It's the toughest."

Wiegardt's acid test is part of a natural process called upwelling that, in Oregon, happens almost like clockwork every year in late summer. Driven by strong winds that propel ocean currents, upwelling occurs when deep ocean water is pulled into the shallow waters on Oregon's continental shelf. From there it enters Netarts Bay. This upwelled water is often high in dissolved carbon dioxide and low in pH and carbonate ions. Not even industrial-strength antacids will help.

"In the summer, this whole bay is a disaster, and buffering isn't enough," says Barton. However, upwelling might not be the only reason for this disaster.

Leaving Whiskey Creek

Netarts Bay is full of aquatic plants that could be big players in nearshore ocean acidification. That's what everyone at Whiskey Creek suspects.

"As you can see, we draw out of a puddle of eel grass," say Barton, pointing at the pipes and sea grass in front of him. Barton, Waldbusser, Giménez, Smith, and pretty much everyone else suspect that the natural respiration of ocean plants such as this eelgrass, which take in CO_2 during the day and respire it at night, is causing some weird daily fluctuations in CO_2 levels—the same fluctuations Waldbusser and Giménez are mimicking. Smith says the Burkolator has registered these oscillations. Taking what's been learned in the hatchery's controlled environment into a real-world ecosystem, she plans on further studying these ups and downs and how they're affecting Netarts Bay's wildlife. The OSU scientists also hope to get their research out of the hatchery in another way.

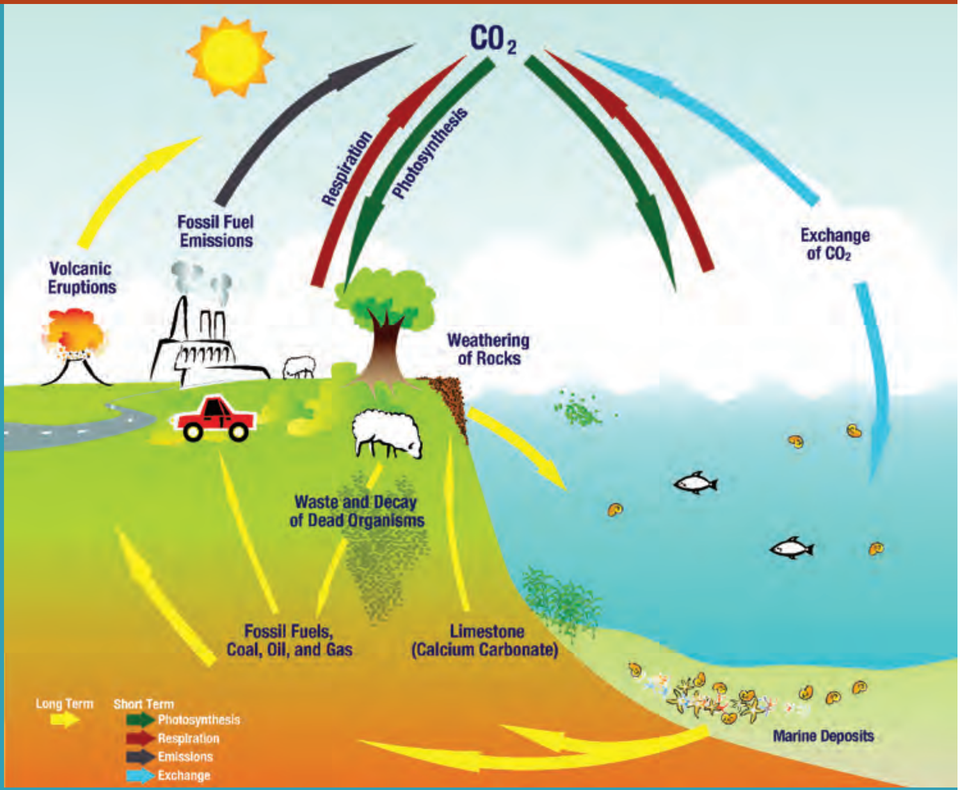
As of this writing, OSU grad student Becky Mabardy, with OSG support, just finished conducting a survey of shellfish industry stakeholders that she says has revealed some interesting results.

"They feel like the science is sort of locked up in the academic community and it's not made accessible," says Mabardy.

To change this, Mabardy is investigating what would be the best medium in which to convey OSU's research, and—be it on an app or a website—what information from the myriad collected will be most useful to people in the shellfish industry. She says she suspects the most valuable data will be about local conditions, which—as Whiskey Creek's long, strange trip illustrates—can deviate wildly from global averages.

That also seems to be where the science is going. As Giménez puts it, "All those [local] things really matter. The oysters don't care about mean global changes; they care about what's happening right here and right now."

WHAT CAUSES OCEAN ACIDIFICATION?



Carbon is continually exchanged between the atmosphere, ocean, biosphere, and land on a variety of timescales. In the short term, CO_2 is exchanged continuously among plants, trees, animals, and the air through respiration and photosynthesis, and between the ocean and the atmosphere through gas exchange. Other parts of the carbon cycle, such as the weathering of rocks and the formation of fossil fuels, are much slower processes occurring over many centuries. For example, most of the world's oil reserves were formed when the remains of plants and animals were buried in sediment at the bottom of shallow seas hundreds of millions of years ago, and then exposed to heat and pressure over many millions of years. A small amount of this carbon is released naturally back into the atmosphere each year by volcanoes, completing the long-term carbon cycle. Human activities, especially the digging up and burning of coal, oil, and natural gas for energy, are disrupting the natural carbon cycle by releasing large amounts of 'fossil' carbon over a relatively short time period.

Source: National Research Council. 2010. Ocean Acidification: A National Strategy to Meet the Challenges of a Changing Ocean. p. 3. Washington, D.C.: The National Academies Press.