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Sea Star

Stressed-Out Salmon

Scientists Develop Biotech Tools for Monitoring Fish Reproductive Health

By Cassandra Kamischke, Fall 2009 WSG Science Writing Fellow

As juvenile salmon migrate through Puget Sound's urban waterways and lakes, they encounter a variety of challenges. Even if they manage to avoid their natural predators, they must still face a set of ordeals that are the direct result of living in close proximity to Seattle and other major cities. Exposure to chemical contaminants, a decreased abundance of prey species and altered natural habitats are among those environmental conditions that may negatively impact the ability of young salmon to grow and reproduce.

Graham Young of the University of Washington's School of Aquatic and Fishery Sciences and Penny Swanson at the NOAA Northwest Fisheries Science Center are working with collaborators Adam Luckenbach at NOAA and Frederick Goetz at the Great Lakes Water Institute and funding from Washington Sea Grant to develop sophisticated biotechnological tools that can be used to monitor the influence of such stressful conditions on the heath of fish populations. They hope this knowledge can eventually be put toward improving fishery management practices as well as a basic understanding of fish reproduction.

To determine the effect of stressful environmental conditions on the health of fish populations, the researchers chose to examine the expression of genes involved in gametogenesis – the cellular

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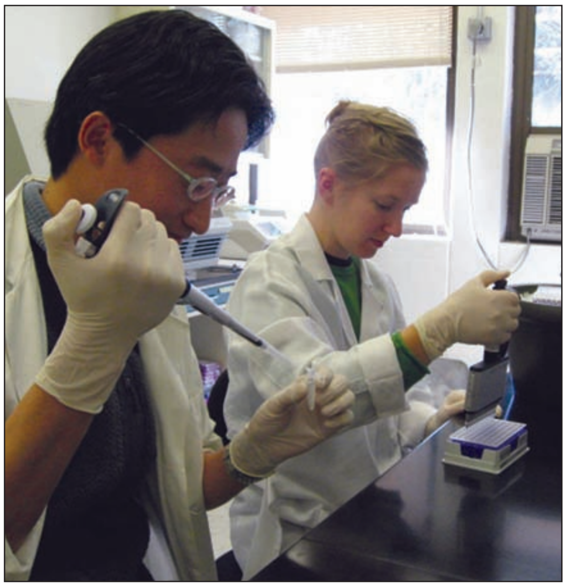
process by which an animal makes the egg or sperm cells necessary for reproduction. In molecular biology, genes are “expressed” when the cell uses the information encoded in the gene to manufacture proteins. A strand of DNA may contain thousands of genes, but not all of those genes will be expressed at any given time.



Postdoctoral fellow Yoji Yamamoto and master’s student Louisa Harding, at work in the Young/Swanson lab.

The degree to which a gene is expressed can be heavily influenced by the environmental conditions surrounding the cell. In this way, animals with similar genetic codes may exhibit very dissimilar physiological characteristics, as a result of having been exposed to different environmental conditions. The researchers targeted gametogenesis because the reproductive system is essential for sustaining populations. “Ultimately what we wanted to know is whether a certain environmental stressors put this population at risk,” says Swanson. “A population is put at risk if individuals can’t reproduce.”

The researchers chose to use salmon as an experimental model, largely because the reproductive system in salmon has been studied for a long time, so there are a number of tools already at their disposal. “Pacific salmon are rather different than most fish because they reproduce once and then they die, so they have only one chance to get it right,” points out Young. “A rainbow trout or a common goldfish – you can put them in poor environmental conditions that may disrupt reproduction but, once those conditions are removed, they can still continue to spawn. With Pacific salmon, if you disrupt them at a certain stage of development, you essentially make them sterile. If that batch of eggs fails, that’s all the eggs they have.”

Chemical contaminants known as estrogens are one type of environmental stressor that might be detected using gene expression techniques. There are a number of urban waterways containing measurable levels of estrogenic chemicals, a result of the introduction of treated sewage carrying traces of chemicals in birth control pills and other sources. One way to measure exposure to environmental estrogens is to look for a particular egg-yolk protein called vitellogenin. All fish carry the gene for vitellogenin but it is not normally expressed in male fish unless they have been exposed to estrogen. In this way, the presence of vitellogenin in male fish can be used as an indicator that estrogenic chemicals are present in the fish’s habitat. However, measuring levels of gene expression may provide a more sensitive method of detecting such chemical contaminants.

“With even short-term exposure of juvenile salmon to environmentally relevant levels of an estrogen, we saw the expression of many genes in the ovaries and testes altered, but we couldn’t yet detect the vitellogenin protein,” notes Swanson.

The researchers and their assistants, UW postdoctoral fellow Yoji Yamamoto and master’s student Louisa Harding, have also been looking at the effect of feed restriction on reproductive development in salmon. The potential for reduced prey abundance is common in urban waterways, inducing the most dramatic changes in the ovaries of the fish studied. A typical result of a reduction in food availability is the activation of pathways involved in the programmed death of the cells comprising the ovarian follicles.

“Molecular markers show indications that this ‘death’ pathway is switched on before you can see any kind of changes in the tissue,” Young says. “It’s sort of an early-warning system.”

The effects they are looking for in the fish are sub-lethal, the researchers emphasize. “It’s not classic toxicology, where you expose fish to a certain chemical and then determine how much it takes to kill 50 percent of the population,” notes Young. “These effects are quite subtle and, in fact, they may be hard to see if you just look at the fish.”

Indeed, effects that the researchers are looking for may not be apparent in a particular generation. The idea is that by determining what is happening at the genetic level when environmental stressors are introduced, it will eventually be possible to model population level effects that may occur years later. If an animal experiences reduced growth due to environmental conditions, which then leads to a reduction in the number or the quality of the eggs it produces, that animal will be less likely to produce healthy offspring. If the reproductive health is diminished in a large enough number of fish, the effects can be seen throughout the entire population.

The researchers are working to develop techniques suitable for tackling the problem of varying gene expression in local salmon species as a result of environmental conditions. This involves adapting existing methods of genomic analysis.

"You can go out and buy a DNA microarray chip, which is a way of looking at tens of thousands of genes through various, very expensive ways, and you can run a sample over it and see what genes are being increased in expression and which are being decreased in expression," explains Young. "The problem is that the sequences of DNA used to make these chips are derived from normal fish. So if you're looking for genes that are only expressed in an abnormal fish, you may miss them completely."

Rather than using a massive shotgun approach with existing salmon DNA arrays, Swanson and Young have been attempting to first determine which genes are affected when certain environmental conditions (such as a change in temperature or exposure to an endocrine-disrupting chemical) are altered. By narrowing their focus to the genes that are influenced in these disrupted pathways, they can identify the specific effects from the genes of interest.

Another aspect of the project that the researchers feel strongly about is the opportunity to train students and postdoctorate fellows in the use of these large-scale genomic approaches to understanding fish health. "We felt that it was really important to get students in fisheries much more well versed in these technologies, since this is likely what they'll be using," says Swanson. "These tools are extraordinarily powerful and could be applied to many aspects of fisheries."

Young adds, "It's not so much that we expect all our fisheries students will be using these techniques – but they may be in positions in 10 years where they're expected to be making management decisions based on these techniques. They need to be aware of what they are about as well as the strengths and limitations of these kinds of data."

Part of the challenge the researchers have faced has also been the rapidly evolving technologies used in molecular biotechnology. While working on the project for the past two years, they've seen the technology change dramatically, to the point that they may have designed an entirely different approach to the project had they started out today. That is why they are testing some of the new emerging technologies for DNA sequencing that can be used to sequence all of the genes expressed within a tissue at relatively low cost. "In two years' time, DNA microarrays may be looked on like something from the Stone Age," offers Young.

The results of this WSG-funded project could aid resource managers and owners of aquaculture facilities throughout the world to assess an array of factors affecting fish reproduction. "In Australia, for example, where the effects of climate change are now being felt, the farmed salmon have started to have reproductive problems," says Young. "The temperatures at which these fish grow are not necessarily the temperatures at which they can reproduce well. Elevated temperatures at a time when the salmon should be spawning actually inhibit the whole spawning process."

"By identifying molecular tools to assess the impact of such changing environmental conditions, fisheries that would otherwise be impaired might be more effectively restored," Young concludes.

For additional information, contact Graham Young (206.543.4291 and *grahamy@u.washington.edu*) or Penny Swanson (206.860.3282 and *penny.swanson@noaa.gov*) or see the "Research" page on the WSG Web site, *wsg.washington.edu/research/index.*html.

(Above) Penny Swanson and Graham Young; (Left) vitellogenin from coho salmon eggs contain valuable clues about the fishes' reproductive health.

Field Notes

WSG Marine Field Agent **Steve Harbell** has been appointed as a non-voting member of the Western Regional Aquaculture Center's board of directors, representing Washington State University. WRAC administers funding in aquaculture science, educational skills and facilities support within a 12-state area to provide for enhancement of viable and profitable commercial aquaculture production in the U.S. Steve is also chair of the Extension sub-committee of WRAC.

Marine Habitat Specialist **Jim Brennan** has been appointed a member of the proposal review panel for the NOAA Climate Program Office's Sectoral Applications Research Program (SARP) for 2010. SARP is an interdisciplinary program that supports research on the effects of and potential responses to climate variability and change in coastal, water resources, health and other vital social and economic sectors.

Alex Berezow** is the new WSG Science Writing Fellow for the Winter Quarter 2010. He was preceded by fall 2009 Fellow **Cassandra Kamischke**, a UW graduate and Institute of Environmental Health employee, and by summer 2009 Fellow **Christine Coltellaro**, a UW Environmental Studies major and former curatorial assistant at the Burke Museum of Natural History. WSG Science Writing Fellows assist in the development of communications products pertaining to issues in marine-related research, education and outreach. For information on spring 2010 WSG Science Writing Fellowships, visit the fellowship Web site (*wsg.washington.edu/education/fellowships/science_writing.html*) or contact UW science-writing instructor **Deborah Illman**, *illman@u.washington.edu.*

Smith Island Study Scratches the Surface of Ambient Undersea Sou

By Whitney Neugebauer, WSG Communications Intern

In the summer of 2007, University of Washington researcher Peter Dahl deployed several networks of hydrophones on the seafloor of northern Puget Sound. His intent? To measure the levels of ambient human-produced sound that marine mammals, and in particular the state's killer whales are exposed to.

Southern resident killer whales are protected under the federal Endangered Species Act. Three major threats to these endangered marine mammals have been identified: reduced stocks of salmon (the southern residents' preferred prey), toxins in the water and increased noise in the marine environment. Because killer whales use a complex sonar system to communicate and locate prey, it is thought that a noisy environment could add stress to an already struggling population of animals.

The public's interest in this iconic species, the largest and, perhaps, most impressive member of the dolphin family, sparked Dahl to investigate background noise in Puget Sound. Over the past century, the sounds of recreational boating, commercial shipping and shoreline construction have been steadily increasing, directly contributing to the ambient noise levels in the Sound.

Washington Sea Grant funding enabled Dahl, a principal engineer at the UW's Applied Physics Laboratory and associate professor in the Department of Mechanical Engineering, to measure ambient noise in Puget Sound during a four-day pilot study. Dahl selected his study location, a marine sanctuary north of Smith Island that is also habitat for southern resident killer whales. The site afforded Dahl access to a pair of nearby monitoring stations dedicated to tracking commercial vessel traffic and local weather conditions. It was not directly in established shipping

Congratulations to **Delisse Ortiz** (Washington State University Vancouver), **Danielle Rioux** (UW), **Ian Smith** (Western Washington University) and **Joseph Zelasney** (UW), newly accepted Dean John A. Knauss Marine Policy Fellows. The four fellows will spend a year in Washington DC — Ortiz with the National Marine Fisheries Service (NMFS) Office of Sustainable Fisheries; Rioux with the Office of the NOAA Administrator; Smith with NMFS' Seafood Inspection Program and Zelasney with the U.S. Department of Transportation's Committee on the Marine Transportation System. Also, cheers for **Chelsie Papiez** (Evergreen State College). As the recipient of the 2009 NOAA Coastal Services Center's Coastal Management Fellowship, she is now working for Maryland's Chesapeake and Coastal Program to target tools and techniques for sea level rise adaptation and response. A final hurrah to UW PhD candidates **Jocelyn Lin** and **James Thorson**, recipients of the 2009 Sea Grant/ NOAA Fisheries Fellowship in Population Dynamics.

Water Resource Educator Sue Blake is now one of 12 Local ECO Network coordinators for the Puget Sound Partnership. Her duties will be carried out in Whatcom County, through the existing Whatcom Watershed Information Network (WWIN). WWIN is a network of local environmental educators working together to support and improve watershed education, stewardship, information exchange and public involvement efforts in Whatcom County. Current projects include planning for an upcoming water festival, program development/ evaluation training and a speakers series. To join the network, contact Blake at 360.676.6736 or *sgblake@ wsu.edu*.

Editor, David G. Gordon; Designer, Robyn Ricks; Web Editor, Marcus Duke; Communications Manager, Dan Williams. Photos: Cover, courtesy Joseph Anderson; Page 2, courtesy Penny Swanson; Page 4, Ron Mielke; Page 5, courtesy Peter Dahl; Page 6, courtesy Jessica Silver and Michael Grilliot; all other photos © Washington Sea Grant.

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Sound

lanes, so Dahl could more easily separate natural sounds caused by wind and waves from noise generated by human-caused sources.

"For my project, I wanted to gather information from which we could characterize background sound levels and how they change over the course of the day," Dahl says. Although only a pilot study, Dahl and his graduate student, David Dall'Osto, were able to estimate what they refer to as the permanent noise — a measure of the background sound level associated with the 100-meter-deep waters north Smith Island. This permanent noise is attributed to both natural sources and more distant anthropogenic sources. It does not, however, represent the transient increase in noise from a nearby vessel.

"How this permanent, or ambient, noise level changes over the course of a year and over the geography of Puget Sound is of interest to regulatory agencies," explains Dahl. For example, "there is much interest in reducing underwater noise from pile-driving during construction of docks and bridges. The value of this permanent background level determines in part how far away from such operations the monitoring of sound is required."

Dahl further notes that different marine mammals may be less directly affected by increases to ambient underwater noise. For example, gray whales often forage in shallow waters, scooping up sediment from the bottom and straining out and swallowing the invertebrates that live in the sand and mud. "These are baleen whales and they use sound primarily for communication, rather than for both communication and food foraging, as with killer whales. It is likely they would respond differently to changes in the acoustic environment," says Dahl.

Dahl and Dall'Osto also measured noise from aircraft flyovers, using a combination of a microphone above the water with a hydrophone below the water. "The Doppler shift associated with a source in motion (such as a passing ambulance) is something we've all experienced," says Dahl. "But the physics of air-water sound transmission lends itself to two types of Doppler shifts, which we have used to isolate aircraft noise from other underwater-generated sounds." Dahl and Dall'Osto are currently working on a model to represent this phenomenon. The model will help to more accurately determine the overall contribution that aircraft make to underwater ambient noise.

Dahl cautions against simple analogies between sound in the air and underwater. "An understanding of underwater sound is best achieved by appreciating the differences between this sound environment and the one we live in," he says. "In our environment, we can walk a half-mile from the busy Interstate 5 corridor and find ourselves in a peaceful, backyard setting. The din of I-5 is still there but the noise levels are much reduced. However, in the underwater environment, sound is trapped between the sea surface and seabed and, thus, does not spread or decay with range like it does in air. At any given underwater location, distant sources contribute to a greater degree, and pockets of quiet, like a backyard environment, are not likely to be found."

For more information, contact Peter Dahl at 206.543.2667 and *dahl@apl.washington.edu* or the "Research" page on the WSG Web site, *wsg. washington.edu/research/index.html*.

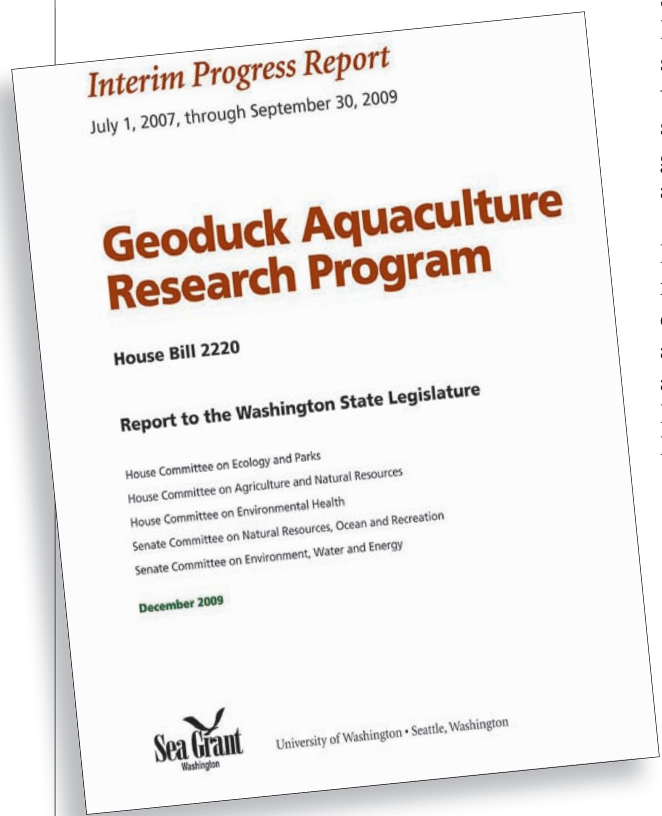
David Dall'Osto readies a hydrophone for deployment beneath the waves.

Geoduck Aquaculture Interim Report

The Geoduck Aquaculture Research Program Interim Progress Report is now available online.

At the direction of the State Legislature, Washington Sea Grant established the geoduck aquaculture research program in 2007. It funded four projects to examine key uncertainties related to geoduck aquaculture that could have implications for the health of the Puget Sound ecosystem and wild geoduck populations. The projects were initiated in May 2008. The *Interim Progress Report* includes information on each project's status to-date.

The 16-page illustrated report is available for download at *wsg.washington.edu/research/pdfs/reports/GeoduckIntProReport.pdf*. Additional information on each project is available at *wsg.washington.edu/research/geoduck/current_research.html*.



Interim Progress Report

July 1, 2007, through September 30, 2009

Geoduck Aquaculture Research Program

House Bill 2220

Report to the Washington State Legislature

House Committee on Ecology and Parks
House Committee on Agriculture and Natural Resources
House Committee on Environmental Health
Senate Committee on Natural Resources, Ocean and Recreation
Senate Committee on Environment, Water and Energy

December 2009

Sea Grant Washington

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New Hershman Fellows Pursue Passions

Jessica Silver

Starting in January, Jessica Silver and Michael Grilliot are putting their marine science skills to work for Washington state agencies through Washington Sea Grant's Marc Hershman Marine Policy Fellowships.

Silver has just finished her master's degree at the UW School of Aquatic and Fishery Sciences and is working at the state Department of Health. Grilliot is finishing his master's degree at Western Washington University's Huxley College of the Environment. He is working with the state Department of Natural Resources.

Silver's long-term goal is to pursue a career that uses her background in marine ecology to develop environmental policy. As a Hershman Fellow, she is monitoring and assessing information about harmful algal blooms on the Washington coast and in Puget Sound. She also coordinates educational outreach on emerging and historical marine toxins with state, tribal and local health officials.

Grilliot brings his knowledge of coastal processes and marine environmental policy to work on best-use practices and management plans for submerged vegetation, marine reserves and state-owned aquatic lands. He is interested in a career in coastal research and marine policy.

Silver and Grilliot were among 12 applicants for 2009-2010 Hershman Fellowships. Washington Sea Grant created the fellowships to introduce students to ocean and coastal policy and enable state agencies to benefit from the students' knowledge and experience on those subjects. The nine-month paid fellowship places graduate students with Washington State Caucus agencies in Olympia.

Michael Grilliot

In addition to Washington Sea Grant, Caucus members includes the Governor's office and departments of Ecology, Natural Resources, Fish and Wildlife, Health, and Community, Trade and Economic Development, as well as the State Parks and Recreation Commission and Military Department Emergency Management Division.

The fellowship honors Marc Hershman, who taught coastal and ocean law, seaport management and coastal management at the UW School of Marine Affairs and School of Law for more than 30 years. Before his death in 2008, he served on the U.S. Commission on Ocean Policy and was an active member of the Ocean Governance Study Group, an alliance of marine policy leaders at academic institutions across the nation.

For information about the Marc Hershman Maine Policy Fellowship, contact Nancy Reichley, WSG Education Specialist, at 206.685.8302 or *sgfellow@u.washington.edu*.

WSG Selects New Research Projects for 2010-2011

This year, Washington Sea Grant is funding 12 new marine research projects addressing a variety of issues and challenges facing Puget Sound and the state's outer coast. The projects range from a study of the factors inhibiting the establishment of native oyster populations to the impacts of bulkheads and other hard armoring structures on area beaches.

"These projects will explore some of Washington's most critical marine resource concerns," says WSG Director Penny Dalton. "With this round of funding, we're able to continue supporting important ongoing research on harmful algal blooms and other problems while addressing emerging issues such as ocean acidification and the effect of hypoxia on fish movements."

WSG selects, funds, oversees and manages marine-related projects carried out by academic and research institutions throughout Washington. WSG-supported research complements ongoing outreach activities in a broad range of topic areas, including aquaculture, fisheries, water quality, invasive species, coastal economic development, shoreline land use and marine technology training. Outreach staff members share university resources and their own expertise with local communities and user groups.

For 2010-2011, WSG has about $1.2 million per year for research. In January, scientists submitted 79 preliminary proposals seeking more than $14 million collectively over the biennium. The selected projects emerged after a rigorous 10-month review process by peer reviewers, two external scientific panels and the WSG Advisory Committee and staff.

Among the new projects being funded:

Effect of Salmon Omega-3 Fatty Acids on PBDE Toxicity
Evan Gallagher, UW Department of Environmental Health
Levels of polybrominated diphenyl ether (PBDE), a once-common flame retardant for textiles, have increased in fish, wildlife and human tissues during the past decade and PBDE residues in resident Puget Sound Chinook salmon are high, relative to many other species. On the other hand, salmon consumption has demonstrated health benefits from nutrients such as omega-3 polyunsaturated fatty acids. This project will study the biochemical interactions among these compounds at the cellular level, thus facilitating a better understanding of the risks and trade-offs associated with eating salmon that contains both persistent organic chemicals and beneficial omega-3s.

Non-Invasive Physiological Monitoring of Southern Resident Killer Whales
Samuel Wasser, UW Center for Conservation Biology
Washington's endangered southern resident killer whale population experienced an unexplained 20-percent decline in the late 1990s. The study will use detection dogs aboard boats to locate fresh orca scat on the surface of the water. This noninvasive approach will analyze hormone and toxin levels in the killer whale scat to test three potential population threats: declines in Chinook salmon (a major dietary component), disturbance by vessel traffic and the presence of persistent organic pollutants.

Impacts of Armoring on Puget Sound Beaches
Megan Dethier, UW Biology Department and Friday Harbor Laboratories
Surprisingly little is known about the effects of shoreline armoring in Puget Sound, even though about 30 percent of the Sound's shorelines are already armored. Erosion related to sea-level rise will increase the demand for shoreline protection. Extensive armoring could disrupt many natural processes, resulting in cumulative physical and biological impacts. This research will examine changes in some shoreline physical features and ecosystem functions resulting from removal of armoring at a local park.

Factors Influencing Recruitment Variability in Estuarine Bivalves
Jennifer Ruesink, UW Biology Department
Washington state, particularly Willapa Bay, has experienced dramatic declines in oyster recruitment for the past four years. Commercial production of oysters and clams by hatcheries has also declined, and harvests continually exceed seed renewal rates. This project will evaluate more than 80 years of historical data on oyster and clam reproduction in Willapa Bay, Puget Sound and British Columbia and use these data to guide research directed at determining the key mechanisms controlling reproduction in Washington's cultured shellfish.

Visit the WSG Web site (*www.wsg.washington.edu/research*) for descriptions and contact information for the 12 new projects, along with updated information about ongoing projects.

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SPRING 2010

SALMON STRESSORS
UNDERWATER AMBIENT NOISE
NEW HERSHMAN FELLOWS

Friday Harbor Triumphs in 2010 Orca Bowl

A team from Friday Harbor High School was the winner in Orca Bowl 2010, edging last year's champion in a nail-biting finish.

Orca Bowl 2010's winning team (from left to right): Nick Roberts, Audrey Olshefsky, Stewart Bell, Hannah Snow and Matthew Skeels. Not pictured: Coach Max Maliska.

Orca Bowl is a rapid-fire ocean sciences competition for high schoolers from across the state. This year's contest took place Feb. 6 on the Seattle campus of the University of Washington. In an exciting finale to 11 rounds of play, Friday Harbor A defeated defending champion ExCEL, a home-schooled team from Washougal, by two points.

The Friday Harbor team receives an all-expenses-paid trip to St. Petersburg, Fla., to compete against 24 other teams in the National Ocean Sciences Bowl, April 23-25.

A team from Garfield High School in Seattle finished third and a second team from Friday Harbor finished fourth. The top four teams won opportunities to participate in ocean science research activities, courtesy of regional sponsors. All 15 participating teams received prize packages, including ocean-related reference books.

Following the competition, all teams participated in an awards dinner and celebration at the Seattle Aquarium.

Orca Bowl is hosted annually at the University of Washington by Washington Sea Grant and the UW School of Oceanography. The National Ocean Sciences Bowl is a program of the Consortium for Ocean Leadership. Approximately 2,000 students from more than 400 high schools participate in 25 regional competitions.