Update Report

Period: 2/1/2014 - 1/31/2015

Project: R/OCEH-8 - *Using zebrafish to assess the health effects of persistent pollutants in Pacific salmon*

STUDENTS SUPPORTED

Huizar, Nancy, nhuizar@uw.edu, UW, fisheries and aquatic sciences, status: cont, field of study: Fisheries, advisor: unknown, degree type: BS, degree date: 2015-06-01, degree completed this period: No
Student Project Title:
Laboratory assistant
Involvement with Sea Grant This Period:
Helped maintain zebrafish colony, assisted with zebrafish experiments
Post-Graduation Plans:
Unknown

neves, ana, anapaulapneves@gmail.com, UW Bothell, Continuing education, status: new, field of study: Pharmacy, advisor: Unknown, degree type: BS, *no degree date*, degree completed this period: No
Student Project Title:
Genotyping of transgenic zebrafish
Involvement with Sea Grant This Period:
Ana is an exchange student from University of Curitiba Brazil taking studies abroad coursework at UW Bothell. The current sea grant provided supplies support for her to work with the zebrafish model
Post-Graduation Plans:
Unknown

Williams, Chase, crw22@uw.edu, University of Washington, Environmental and Occupational Health Science, status: cont, field of study: Environmental Toxicology, advisor: E. Gallagher, degree type: PhD, degree date: 2015-12-01, degree completed this period: No
Student Project Title:
Effect of cadmium on salmon olfactory injury
Involvement with Sea Grant This Period:
Continues to maintain zebrafish colony, assist with reading and advise on experimental work
Post-Graduation Plans:
Is working on a new sea grant application to support postdoctoral studies targeting the effects of climate change on salmon olfaction

Yeh, Andrew, ayeh3@uw.edu, University of Washington, Environmental and Occupational Health Science, status: cont, field of study: Environmental Toxicology, advisor: E. Gallagher, degree type: PhD, degree date: 2015-12-01, degree completed this period: No
Student Project Title:
Biomarkers of emerging contaminants in Puget Sound salmon
Involvement with Sea Grant This Period:
Continued laboratory experiments on the effects of omega-3 unsaturated fatty acids

on PBDE toxicity. Completed a publication. Also working on effects of emerging contaminants on Puget Sound salmon
Post-Graduation Plans:
not applicable

CONFERENCES / PRESENTATIONS

GALLAGHER, E.P. 2014. Developing biomarkers of toxicity for Puget Sound salmon. University of Washington-Tacoma, Invited presentation., public/profession presentation, 50 attendees, 2014-09-04

Yeh, A., Meador, J.P., Young, G., Shibata, Y., Marcinek, D.J., Ramsden, R., and GALLAGHER, E.P. 2014. Assessing sublethal effects and generating biomarkers for biomonitoring of contaminants of emerging concern in Puget Sound. Vancouver, B.C., Poster presentation., public/profession presentation, 30 attendees, 2014-11-13

Yeh, A., Kruse, S.E., Marcinek, D.J., and GALLAGHER, E.P. 2014. Effect of omega-3 polyunsaturated fatty acid oxidation products on the cellular and mitochondrial toxicity of BDE 47. Phoenix, Poster presentation., public/profession presentation, 30 attendees, 2014-03-24

GALLAGHER, E., Yeh, A., Meador, J., and Young, G. 2014. Innovative approaches to monitoring chemicals of emerging concern in the Puget Sound. Salish Sea symposium. Seattle, Invited presentation., public/profession presentation, 150 attendees, 2014-05-02

GALLAGHER, E., Wang, L., and Williams, C. 2014. Biomarkers of metal-induced loss of olfactory function in Pacific salmon. Aquatic animals and human health meeting. Austin, Invited presentation., public/profession presentation, 100 attendees, 2014-12-13

ADDITIONAL METRICS

P-12 Students Reached:	**P-12 Educators Trained:**
Participants in Informal Education Programs:	**Volunteer Hours:**
Acres of coastal habitat protected, enhanced or restored:	**Resource Managers who use Ecosystem-Based Approaches to Management:**
Annual Clean Marina Program - certifications:	**HACCP - Number of people with new certifications:**

ECONOMIC IMPACTS

No Economic Impacts Reported This Period

SEA GRANT PRODUCTS

Description	Developed?	Used?	ELWD?	Number of Managers	Names of Managers
Multiplex PCR assay to analyze gene expression in zebrafish targeting oxidative stress as a mechanism of toxicity.	Yes	No	No	0	

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Number of stakeholders modifying practices:

Sustainable Coastal Development

# of coastal communities:

PARTNERS

Partner Name: Duke University

Partner Name: University of Washington

IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant investigates the effects of persistent environmental pollutants on salmon and human health**
Type: accomplishment
Description:
Relevance: Although use of polybrominated diphenyl ether (PBDE) flame-retardants has been banned, these toxic chemicals are environmentally persistent and can affect human health. Elevated PBDE levels have been found in Puget Sound salmon and in the marine mammals that eat them, lending urgency to questions about possible health implications for local residents who eat PDBE-contaminated salmon.

Response: Washington Sea Grant-supported researchers investigated PBDE impacts by conducting long-term feeding studies using zebrafish, a cost-effective surrogate for other fish and human subjects. They analyzed the reproductive effects on adult zebrafish of dietary PBDE exposure and developed new screening methods to assess molecular damage. They also used human cells to test for mitochondrial injury and explored the chemical protection of omega-3 fatty acids, which are associated with salmon consumption.

Results: Long-term PBDE exposures resulted in microscopic damage to zebra fish

reproductive tissues. By contrast, there were no behavioral effects in zebrafish that were fed PBDEs. A new technology developed for rapid gene-expression analysis will be used to assess other contaminants in salmon. Project results have been incorporated into outreach programs for use by local people and organizations working with vulnerable Puget Sound communities including regional Environmental Protection Agency scientists, the University of Washington TEACH program that provides Native Americans with environmental health information, and the Duwamish River Clean Up Coalition.
Recap:
Recap: Washington Sea Grant researchers confirm that zebrafish exposed to persistently high levels of PBDEs showed damage to reproductive tissues, suggesting that humans who eat contaminated salmon might be at risk.
Comments:
Primary Focus Area: OCEH (SSSS)
Associated Goals: Reduce toxic, nutrient and pathogen pollutants in water and the marine food web and address their relationships to and impacts on human health. (SSSS, Consumers)
Partners:
Duke University
Environmental Protection Agency
Duwamish River Clean Up Coalition
Related Partners: *none*

PUBLICATIONS

Title: **Effect of omega-3 fatty acid oxidation products on the cellular and mitochondrial toxicity of BDE 47**
Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2015
Uploaded File: *none*
URL: http: //www.ncbi.nlm.nih.gov/pubmed/25659769
Abstract:
High levels of the flame retardant 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) have been detected in Pacific salmon sampled near urban areas, raising concern over the safety of salmon consumption. However, salmon fillets also contain the antioxidants eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whose oxidation products induce cellular antioxidant responses. Because oxidative stress is a mechanism of BDE 47 toxicity, we hypothesized that oxidized EPA and DHA can ameliorate the cellular and mitochondrial toxicity of BDE 47. HepG2 cells were treated with a mixture of oxidized EPA and DHA (oxEPA/oxDHA) at a ratio relevant to salmon consumption (1.5/1 oxEPA/oxDHA) followed by exposure to 100μM BDE 47. Pretreatment with oxEPA/oxDHA for 12h prior to BDE 47 exposure prevented BDE 47-mediated depletion of glutathione, and increased expression of antioxidant response genes. oxEPA/oxDHA also reduced the level of reactive oxygen species production by BDE 47. The oxEPA/oxDHA antioxidant responses were associated with partial protection against BDE 47-induced loss of viability and also mitochondrial membrane potential. Mitochondrial electron transport system functional analysis

revealed extensive inhibition of State 3 respiration and maximum respiratory capacity by BDE 47 were partially reversed by oxEPA/oxDHA. Our findings indicate that the antioxidant effects of oxEPA/oxDHA protect against short exposures to BDE 47, including a protective role of these compounds on maintaining cellular and mitochondrial function.
Citation:
Yeh, A., Kruse, S.E., Marcinek, D.J., and E.P. GALLAGHER. 2015. Effect of omega-3 fatty acid oxidation products on the cellular and mitochondrial toxicity of BDE 47. Toxicology In Vitro. Jun;29(4): 672-80.
Copyright Restrictions + Other Notes:
Journal Title: Toxicology In Vitro

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

Type: influenced Period: 2014-11-12: : 2014-11-15Amount: $1200
Purpose:
Travel funding for graduate student to attend annual Superfund program conference to present a poster on his work targeting the effects of metals on salmon physiology.
Source: UW NIEHS Superfund program

UPDATE NARRATIVE

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Section I. A report that describes progress made towards meeting project objectives during **THIS REPORTING PERIOD**, that includes activities carried out, participants, results, challenges encountered, any changes in project direction, etc. **Length**: Two to three pages, include tables and figures as relevant.

WASHINGTON SEA GRANT PROGRESS REPORT

for the period 2/1/2014 – 1/31/2015

1. PROJECT OBJECTIVES.

Background. Waterborne contaminants in Washington's coastal areas threaten marine and estuarine ecosystems and can negatively impact human health. Of concern in the Pacific Northwest is the presence of persistent organic chemicals in seafood species such as Pacific salmon. Toxic chemicals of current concern include brominated flame retardants (BFRs), a group of persistent global environmental contaminants that include the polybrominated diphenyl ethers (PBDEs). PBDEs have been massively produced and used in widespread consumer applications leading to detection of increasing concentrations in fish, wildlife and human tissues. Of relevance to Washington Sea Grant are PBDE residues in resident Puget Sound Chinook salmon (*Oncorhynchus tshawytscha*) from certain contaminated sites that have been shown by NOAA investigators to be high relative to the levels of PBDEs detected at other sites and in other salmon species. Chinook are an important ecological and food fish in Washington State, and contribute to the livelihood of commercial and tribal fishermen. From the standpoint of seafood consumption, Chinook remain a predominant food source for some underrepresented minorities such as Tribal Nations who have special dietary practices that can lead to significant exposures to persistent pollutants found in fish and shellfish. Accordingly, there is public health concern surrounding the consumption of contaminated salmon. We are using zebrafish as a novel, cost-effective biological model as a surrogate to better understand health effects of these contaminants. Zebrafish are a small laboratory fish model that has been widely accepted by the biomedical community for use in toxicological studies of relevance to human health.

The **overall goal of this project** is to use toxicological approaches in zebrafish to better understand if dietary exposure of PBDEs found in Chinook salmon from polluted waterways pose a risk for reproductive toxicity. Our screening strategy can be applied to assess other emerging contaminants in Pacific salmon.

Specific aim 1. Determine the effects of PBDE mixtures found in Chinook from urbanized and non-urbanized areas of the Puget Sound on reproductive and trans-generational toxicity in zebrafish. Based upon these results, determine if salmon from urbanized locations contain contaminant levels that may result in adverse reproductive outcomes.

Specific aim 2. Translate the results of our studies in a Community Education and Research Translational (CERT) project component involving local stakeholders and K-12 students and Educators.

2. PROJECT PROGRESS

Specific Aim 1 progress and results. In our previous reporting period, we completed our exposures of zebrafish to diets consisting of the 3 predominant PBDE congeners representative of certain resident Puget Sound Chinook from contaminated areas. This included a PBDE mixture comprising 50% BDE 47, 40% BDE 99, 10% BDE 100, and at concentrations to model PBDE exposures associated with extensive and moderate consumers of contaminated salmon based on

prior reports on blackmouth salmon contamination in the Duwamish waterway. We used estimates of high consumers to be 620 grams of salmon consumption per day, and moderate consumers averaging 40 grams/salmon/day. Our collaborator, Dr. Heather Stapleton (Duke University) analyzed bioaccumulation of PBDEs exposures over the 28-56 day exposure periods which confirmed bioaccumulation of BDE47 and BDE 100, but poor bioaccumulation of BDE99. As reported previously, no adverse neurobehavioral effects of PBDE consumption at the dietary levels used were observed. However, dietary exposure to the high dose of the PBDE mixture negatively affected oocyte production in reproductively active female zebrafish and adverse histopathological effects were observed on the reproductive tissues. Quantitative PCR analysis of gene expression in the adult zebrafish receiving the chronic exposures was inconclusive will be being reanalyzed using different gene targets and methods. To help achieve this goal, we worked with Affymetrix Incorporated and developed a multiplexed PCR panel for rapid analysis of 15 genes that will detect oxidative damage, a mechanism of reproductive toxicity, in the tissues of PBDE exposed zebrafish. The gene panel is designed to allow for incorporation of other genes of interest as needed using the Affymetrix *QuantiGene* platform.

Specific Aim 2 progress and results. Several activities associated with aim 2 research translational aims were accomplished in this reporting period. In support of **specific aim 2,** we leveraged our outreach activities with our NIEHS Center for Ecogenetics and environmental health. Specifically, we conducted tours of our zebrafish facility and provided lectures associated with our Sea Grant activities to 14 area high school teachers associated with The Academy for Teaching About Health and Environment Associations (ATHENA). This was conducted in May 2012, and also in August 2014, the latter tour in which the teachers returned to the UW to share their successes with one another and UW faculty and staff during a follow-up session. On April 19 2013, the PI provided a lecture to environmental health science professors on the use of biomarkers and remediation in the Puget Sound developed in support from SeaGrant activities. On March 28, 2014, the PI provided a tour and lecture to 7 Native American health scientists with the Native Tradition, Environment And Community Health (TEACH) Project. This project began in 2008 with a collaborative grant supplement funded by the National Institute for Environmental Health Sciences (NIEHS) which identified Native researchers from the Puget Sound region to hold discussions about environmental health in their communities. The tribal professionals were most interested in seafood safety issues and our approaches with zebrafish. In addition, project updates were continually shared with Katie Frevert of the UW Superfund Sciences research translation core who met regularly with the Duwamish River cleanup coalition (DRCC) and scientists from EPA region 10. Ms. Frevert is assisting the PI and will work with WA Sea Grant staff during the no-cost extension on continuing research translational activities.

Summary of results. A subchronic feeding study of PBDE congeners in zebrafish which involved mass-spec analysis of experimental diets and bioaccumulation of PBDE congeners in adults, as well as analysis of histological and behavioral effects after exposures. These results confirmed bioaccumulation of PBDE congeners in our long-term feeding study, and histological injury in the gonads of adult female zebrafish receiving the dietary PBDE exposures. By contrast, there were no behavioral effects in zebrafish fed diets PBDEs relevant to levels of human exposures of salmon representative of polluted areas. Other biochemical analyses are continuing, including application of a new technology involving rapid gene expression analysis. Community engagement activities targeted local Native American health scientists, DRCC, high school area teachers, and EPA region 10 scientists.

3. ACTIVITIES CARRIED OUT.
In addition to completing the laboratory investigations and research translational activities associated aim 2, Sea Grant research was presented at two scientific meetings. In addition, leveraging from Sea Grant research leveraged our NSF grant, which involves co-investigators from George Washington University, Baylor, and Yale University. Our NSF grant is using zebrafish to assess the effects of green chemicals. In addition, our Sea Grant studies on PBDEs have helped us develop biomarkers for assessing emerging contaminants in Puget Sound salmon on a grant funded by the Washington Department of ecology. Partnerships with scientists at University of Mississippi, Duke University, and with Dave Marcinek (UW Department of Physiology) are continuing.

4. CHALLENGES
The PI experienced significant personnel turnover in the laboratory in 2013 and also disruption of activities while reestablishing a new zebrafish colony in 2013-2014, which resulted in a one-year no-cost extension. However, we have filled the vacated positions and work is now continuing. Because of the stress of the subchronic study design on zebrafish offspring, we had to abandon analysis of the effects of transgenerational effects of PBDEs. In lieu of this issue, we will conduct a more in-depth molecular analysis on the subchronic PBDE exposures on the adults using the multiplexed PCR system that we have designed in collaboration with Affymetrix Incorporated

CHANGES IN PROJECT DIRECTION. As discussed, the lack of poor reproduction due to the stress of the long-term static renewal protocol in zebrafish has forced us to refocus our reproductive effects on the adults. No other major changes in project direction occurred other than those associated with the above challenges.

6. PARTICIPANTS

1. Evan Gallagher: PI

2. Dr. Margaret Mills (postdoctoral researcher) joined our lab in late 2014 and is working on our zebrafish studies. Dr. Mills received her PhD from the UW and conduct her PhD research at the Fred Hutchison Cancer Research Institute.

3. Chase Williams is a senior PhD student in the laboratory who continued to provide translational activities and laboratory tours.

4. Andrew Yeh is a senior PhD student the laboratory who continued investigations on the effects of PBDEs congeners that are a focus of the present study but using fish cell lines. Andrew published a Sea Grant supported manuscript in the journal *toxicology in vitro* based on this work.

5. Dr Wu Dong (University of Mississippi), collaborator

6. Dr. Heather Stapleton (Duke University), collaborator

7. Dr. David Marcinek (University of Washington Department of Physiology), collaborator