Update Report

Period 2/1/2013 - 1/31/2014

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 new, field of study Fisheries, advisor unknown, degree type BS, degree date 2015-06-01, degree completed this period No
Student Project Title Not identified

Involvement with Sea Grant This Period maintained zebrafish colony and including breeding

Post-Graduation Plans none

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 2015-06-01, degree completed this period No
Student Project Title Effect of cadmium on salmon olfactory injury

Involvement with Sea Grant This Period PhD student working on effects of environmental chemicals on salmon olfaction

Post-Graduation Plans not applicable

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 2015-06-01, degree completed this period No
Student Project Title biomarkers of emerging contaminants in Puget Sound salmon

Involvement with Sea Grant This Period involved in laboratory and field experiments understanding the effects of PBDEs and other emerging contaminants in salmon.

Post-Graduation Plans not applicable

CONFERENCES / PRESENTATIONS

Wang, L. and GALLAGHER, E. 2013. Role of Nrf2 in cadmium olfactory injury in zebrafish. Society of Toxicology Annual Meeting. Salt Lake City, public/profession presentation, 100 attendees, 2013-03-15

ADDITIONAL METRICS

K-12 Students Reached	Acres of degraded ecosystems restored as a result of Sea Grant

	activities
Curricula Developed	Resource Managers who use Ecosystem-Based Approaches to Management
Volunteer Hours	HACCP - Number of people with new certifications
Cumulative Clean Marina Program - certifications	

PATENTS AND ECONOMIC BENEFITS

No Benefits Reported This Period

TOOLS, TECH, AND INFORMATION SERVICES

Description		Developed	Used	Names of Managers	Number of Managers
A microRNA dataset generated in the olfactory system of zebrafish exposed to metals. R/OCEH-8	Actual (2/1/2013 - 1/31/2014)	1	1		0
	Anticipated (2/1/2014 - 1/31/2015)	0	0		

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2013 - 1/31/2014)
Anticipated (2/1/2014 - 1/31/2015)

Number of fishers using new techniques
Actual (2/1/2013 - 1/31/2014)
Anticipated (2/1/2014 - 1/31/2015)

Sustainable Coastal Development
Actual (2/1/2013 - 1/31/2014)
Anticipated (2/1/2014 - 1/31/2015)

Coastal Ecosystems
Actual (2/1/2013 - 1/31/2014)
Anticipated (2/1/2014 - 1/31/2015)

PARTNERS

Partner Name North Carolina state University, type Academic Institution, scale Federal or National

Partner Name University of Washington

IMPACTS AND ACCOMPLISHMENTS

Title Washington Sea Grant probes the effects of flame-retardant chemicals on fish and human cells

Type accomplishment

Description Relevance Polybrominated diphenyl ether (PBDE) flame retardants are found in many products, including building materials, textiles, plastics and electronics. PBDE levels 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. Increasing levels in marine mammals such as harbor seals that are at the top of the Puget Sound food chain give rise to questions about possible health effects in residents who consume local salmon. Response Washington Sea Grant-supported researchers have been using zebrafish as surrogates to understand human exposure to PBDEs, to develop new biomarkers for PBDEs and other emerging contaminants in fish models, and to develop novel, cost-effective models for studying human risk. They completed a dietary exposure study in zebrafish exposed to a PBDE mixture relevant to salmon, initiated quantitative PCR analysis of gonadal gene expression, and conducted additional studies of PDBE effects in human cells. Results Analysis indicated that the exposed zebrafish bioaccumulated PBDEs, and their reproductive tissues were injured at the cellular level. The researchers also identified PDBE impacts in human cells. Leveraging WSG funding enabled them to establish and validate technology that will be used to help determine the effects of PBDEs and other contaminants on gene expression.

Recap Washington Sea Grant researchers use zebrafish as a low-cost model for studying the environmental and health hazards of toxic PDBEs.

Comments Primary Focus Area OCEH (SSSS) State 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).

Related Partners

PUBLICATIONS

Title Copper-induced deregulation of microRNA expression in the zebrafish olfactory system

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year 2013 Uploaded File es400615q.pdf URL none

Abstract Although environmental trace metals, such as copper (Cu), can disrupt normal olfactory function in fish, the underlying molecular mechanisms of metal-induced olfactory injury have not been elucidated. Current research has suggested the involvement of epigenetic modifications. To address this hypothesis, we analyzed the microRNA (miRNA) profiles in Cu-exposed adult zebrafish olfactory tissue. Our data revealed 2, 10, and 28 differentially expressed miRNAs in response to increased Cu concentration. Moreover, numerous deregulated miRNAs were involved in neurogenesis (let-7, miR-7a, miR-128 and miR-138), indicating a role for Cu-mediated toxicity via interference with olfactory neurogenesis. Putative gene targets of deregulated miRNAs identified in our previously reported microarray data set are involved in various biological processes, including cellular growth and proliferation, cell death, and cell morphology. In particular, numerous deregulated miRNAs (miR-203a, miR-199*, miR-16a, miR-16c, and miR-25) were inversely correlated with the depression of key genes within the OST pathways, which likely contributed to the inhibition of olfactory function. Collectively, our finding provide further insight into the epigenetic regulatory mechanism in heavy metal-induced neurotoxicity of fish olfactory system as well as identifies novel miRNA biomarkers in response to environmental toxicant exposure.

Citation Wang, L., Bammler, T., Beyer, R. and E. GALLAGHER. 2013. Copper-induced deregulation of microRNA expression in the zebrafish olfactory system. Environmental Science and Technology. Jul 2;47(13) 7466-74. PMID 23745839

Copyright Restrictions + Other Notes

Journal Title environmental science and technology

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

Type influenced Period 2013-10-13 2013-10-17Amount $500

Purpose A student, (chase Williams) was awarded travel funding to support from the UW NIEHS Superfund program to attend the annual conference. At the conference, he won second place for his poster presentation on the effects of contaminants on salmon olfaction

Source University of Washington Superfund research program

Type influenced Period 2013-09-30 2017-09-29Amount $1100000

Purpose WSG funds helped develop technologies in zebrafish that led to successful securing of a multi-investigator four year NSF Grant in which our laboratory is using zebrafish to screen for the health effects of green chemicals

Source National Science Foundation

Type influenced Period 2013-02-01 2015-03-31Amount $500000

Purpose WSG funds for PBDE studies over the past three years provided data and technology that helped us secure a two-year grant from the Washington Department of ecology to study emerging contaminants in the Puget Sound

Source Washington Department of ecology

UPDATE NARRATIVE

Uploaded File Gallagher_5538_update_....7.pdf

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 2/1/2013 – 1/31/2014

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. In this regard, zebrafish (*Danio rerio*) 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 developmental and reproductive toxicity. Our screening strategy can be applied to assess other emerging contaminants in Pacific salmon. Our ongoing work will be accomplished through a series of experiments in two specific aims:

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. We prepared a zebrafish diet 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. We worked with our collaborator Dr. Heather Stapleton at Duke University who used gas chromatography mass spectrometry in electron capture negative ionization mode (GC/ECNI–MS) to validate the experimental diets. Following validation of the experimental diets, adult pre-spawning AB strain zebrafish of mixed sexes received static dietary exposures just below satiation (e.g., 3 % body weight/d) so that all food was consumed. Control animals received zebrafish food containing evaporated methylene chloride. Exposures occurred for 28-56 days with 50% water changes daily, and the animals were monitored for overall health, signs of toxicity, and mortality. Survival and body weights are recorded throughout the experiment.

At 28 and 56 d of exposure, 3 male and 3 female fish (based upon phenotypic inspection and identification of females via uro-genital papilla) were euthanized and analyzed for PBDE bioaccumulation by Dr. Stapleton. Analysis of PBDE residues confirmed bioaccumulation of BDE47 and BDE 100, but poor bioaccumulation of BDE99. During these exposures, we analyzed a subset of adult zebrafish for potential PBDE induced neurobehavioral injury. No adverse neurobehavioral effects of PBDE consumption at the dietary levels used was observed. To assess the effects of dietary PBDE exposures on zebrafish reproduction, adult spawning zebrafish from the above exposures were allowed to undergo weekly spawning for 4 weeks. These fish were exposed for 28 d prior to the induction of spawning, and PBDE exposures were continued. Our intent was to create a scenario mimicking sub-chronic human exposures prior to, and during, pregnancy. However, there was no reproduction or egg release in any of the experimental groups, including controls. The poor reproductive success might have been associated with the stress of the chronic static renewal water exchanges. By contrast, our preliminary analysis indicates that dietary exposure to the high dose of the PBDE mixture negatively affected oocyte production and reproductively active female zebrafish (**figures 1 and 2**). Gonadal samples from female zebrafish were harvested for quantitative PCR analysis which is continuing.

Specific Aim 2 progress and results

Several activities associated with aim 2 research translational aims were accomplished in this reporting period. 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. Throughout the reporting period, the research team continued to provide tours of the zebrafish facility to junior high school students and faculty. In addition, the PI and graduate student (Williams) continued active participation in collaborative activities among our Center for ecogenetics and environmental health, research translation core, and DRCC partners. Chase Williams provided a lecture on a boat trip of the Duwamish waterway in April 2013 that was co-sponsored by the DRCC and our NIEHS center for ecogenetics and environmental health. Participants included members of the DRCC, local area teachers, graduate students and researchers. Graduate student Chase Williams discussed pollution aspects, seafood safety, and our approaches using zebrafish to understand human risk from consumption of contaminated seafood. A second trip was sponsored in the summer 2013 by the CEEH in which the PI (Gallagher) as well as graduate student (Williams) gave presentations to a second group of educators on the history of pollution in the Duwamish River and also harbor island, and how using novel approaches such as zebrafish and biomarkers of pollutants in salmon, can help understand the risks of pollution in the ecosystem, but also how we can determine the effectiveness of site remediation.

Summary of results. A sub chronic feeding exposure was carried out with preliminary results suggesting reproductive tract injury in adult female zebrafish exposed to high levels of PBDEs associated with extensive consumption of contaminated blackmouth salmon. The reproductive tract injury was not associated with other neurobehavioral outcomes. Other biochemical analyses are continuing.

3. ACTIVITIES CARRIED OUT.

In addition to completing the laboratory investigations of the hypotheses of Aim 1 and research translational activities associated aim 2, Sea Grant research was presented at two scientific meetings. Two new partnerships were developed for this project, including a new partnership with Dr Wu Dong, a histopathologist at the University of Mississippi, who is analyzing reproductive tract injury in adult zebrafish at no cost to Sea grant. In addition, a partnership developed in the last reporting period with Dave Marcinek (UW Department of Physiology).

4. CHALLENGES

Two challenges arose during this reporting period. The PI experienced personnel turnover in the laboratory which delayed achieving our goals, and resulting in an application for a no-cost extension. Specifically, our primary research scientist, postdoctoral researcher, and a research technician moved on to other positions. The research technician (Ke'ali Louie) moved on to dental school. The postdoctoral researcher supervised Mr. Louie on key aspects of this project. The PI is working with these former staff by e-mail to continue analysis of existing data, and will assign a new graduate student or technician to continue the project during our no-cost extension. The second challenge encountered was associated with the poor spawning success of adult zebrafish undergoing the 28-56 day PBDE exposures. Because the poor reproduction was also observed in control animals not receiving exposures to PBDE's, no analysis of the offspring could be conducted. The PI is considering options on how to deal with this particular setback, including a possible repeat of exposures and using new experimental animals.

5. CHANGES IN PROJECT DIRECTION.

No major changes in project direction occurred. However, we continue to leverage our Sea grant funding to investigate new technologies for studying environmental chemical effects in zebrafish. These included bringing to fruition a publication on small non-coding microRNAs in the control of zebrafish gene expression in fish exposed to pollutants. This paper was published in *Environmental Science and Technology*. Additionally, we continued our investigations on novel aspects of BDE 47 toxicity to human cells, with a focus on impacts to cellular mitochondrial function.

6. PARTICIPANTS

Evan Gallagher: PI

Ke'ali Louie (research technician) worked in our lab for several years, originally as an undergraduate researcher and then as a research technician following his completion of a BS degree from the school of fisheries and aquatic sciences.

Dr. Lu Wang was a former postdoctoral researcher in the laboratory that received partial support from Sea grant to help oversee these studies.

Chase Williams is a senior PhD student in the laboratory who assisted with the neurobehavioral studies in zebrafish and continued to provide translational activities.

Dr Wu Dong (University of Mississippi), collaborator

Dr. Heather Stapleton (Duke University), collaborator

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

Figures

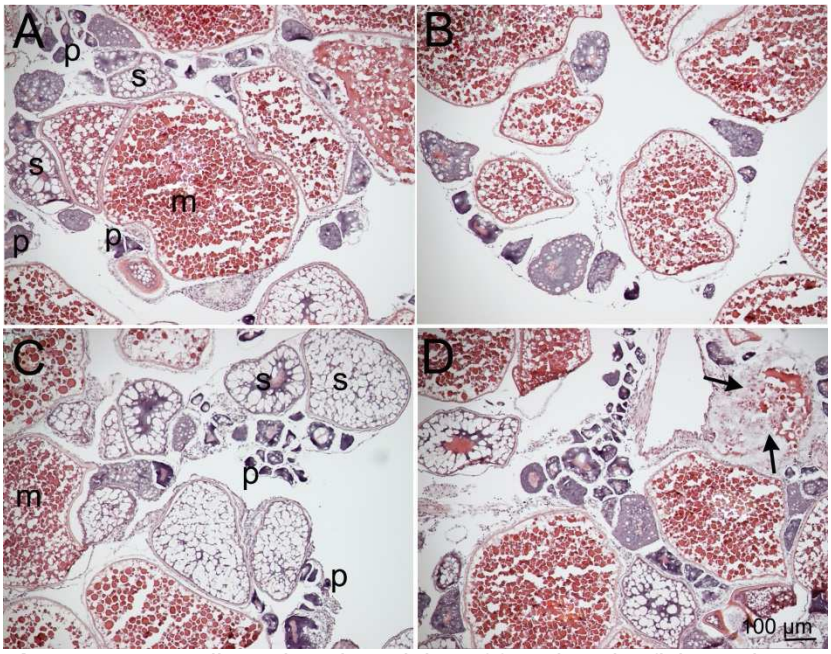

Fig. 1. *Hematoxylin and eosin staining of female zebrafish ovaries, A, B, C and D indicate that control group, low dose group, high dose and high dose group, respectively. m, Mature oocytes; s, Secondary oocytes; p, Primary oocytes. Black arrow indicated degenerate oocyte.*

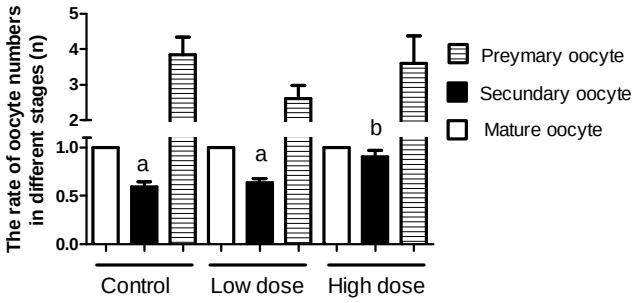

Fig. 2. Effects of dietary PBDE mixture exposures on zebrafish oocyte development. Ovaries of adult female zebrafish exposed to PBDE's (and controls) were stained by H&E, and oocytes counted by different development stage. As indicated, exposure to high concentrations of PBDE mixtures significant increased the rate of secondary oocytes in different oocyte stages. Different letters indicate significant treatment effects at ($P<0.05$). n=10 slides/5 ovaries examined.