RESEARCH/PD ANNUAL REPORT - FINAL REPORT

2015 annual report - final
Evan Gallagher
Using zebrafish to assess the health effects of persistent pollutants in Pacific salmon
R/OCEH-8
Submitted On: 04/27/2016 01:51:51 PM

METRICS & MEASURES

Metric/Measure	Value	Note
Acres of coastal habitat	0	Not applicable
Fishermen and seafood industry personnel	0	Not applicable
Communities - economic and environmental development	0	Not applicable
Stakeholders - sustainable approaches	0	Not applicable
Informal education programs	0	Not applicable
Stakeholders who receive information	0	Not applicable
Volunteer hours	0	Not applicable
P-12 students reached	18	South Park and Georgetown middles school lab tours
P-12 educators	2	South Park and Georgetown middles school lab tours - part of the Duwamish Valley Youth Corps (DVYC)

REQUESTED INFORMATION

Publications

Olfactory Transcriptional Analysis of Salmon Exposed to Mixtures of Chlorpyrifos and Malathion Reveal Novel Molecular Pathways o
Publication Type: Peer-reviewed: Journals (incl. articles), Books, Proceedings, and Other Documents
Publication Year: 2015
Publication Authors:
Publisher Info: Toxicological Sciences
Notes:
Related URLs:
Keywords: chlorpyrifos malathion salmon olfaction microarray analysis mitochondria
Publication URLs: http://toxsci.oxfordjournals.org/content/149/1/145.long
Abstract: Pacific salmon exposed to sublethal concentrations of organophosphate pesticides (OP) have impaired olfactory function that can lead to loss of behaviors that are essential for survival. These exposures often involve mixtures and can occur at levels below those which inhibit acetylcholinesterase (AChE). In this study, juvenile Coho salmon were exposed for 24 h t either 0.1, 0.5, or 2.5 ppb chlorpyrifos (CPF), 2, 10, or 50 ppb malathion (MAL), or binary mixtures of 0.1 CPF:2 ppb MAL, 0.5 CPF:10 ppb MAL, or 2.5 CPF:10 ppb MAL to mimic single and binary environmental exposures. Microarray analysis of olfactory rosettes from pesticide-

exposed salmon revealed differentially expressed genes involved in nervous system function and signaling, aryl hydrocarbon receptor signaling, xenobiotic metabolism, and mitochondrial dysfunction. Coho exposed to OP mixtures exhibited a more pronounced loss in detection of a predatory olfactory cue relative to those exposed to single compounds, whereas respirometry experiments demonstrated that exposure to OPs, individually and in mixtures, reduced maximum respiratory capacity of olfactory rosette mitochondria. The observed molecular, biochemical, and behavioral effects occurred largely in the absence of effects on brain AChE. In summary, our results provide new insights associated with the sublethal neurotoxic effects of OP mixtures relevant to environmental exposures involving molecular and cellular pathways of injury to the salmon olfactory system that underlie neurobehavioral injury.
Citation: Lu Wang, Herbert M. Espinoza, James W. MacDonald, Theo K. Bammler, Chase R. Williams, Andrew Yeh, Ke'ale W. Louie, David J. Marcinek, and Evan P. Gallagher. Olfactory Transcriptional Analysis of Salmon Exposed to Mixtures of Chlorpyrifos and Malathion Reveal Novel Molecular Pathways of Neurobehavioral Injury. Toxicological Sciences. (2016) 149 (1): 145-157
Citation for Coverpage:
SG can post PDF online?: Yes
Uploaded File: Toxicol._Sci.-2016-Wang-145-57.pdf

Contaminants of emerging concern in a large temperate estuary
Publication Type: Peer-reviewed: Journals (incl. articles), Books, Proceedings, and Other Documents
Publication Year: 2016
Publication Authors:
Publisher Info: Environmental Pollution
Notes:
Related URLs:
Keywords: contaminants of emerging concern, (CECs) Chinook salmon, Pacific staghorn sculpin, pharmaceuticals,
Publication URLs:
Abstract: This study was designed to assess the occurrence and concentrations of a broad range of contaminants of emerging concern (CECs) from three local estuaries within a large estuarine ecosystem. In addition to effluent from two wastewater treatment plants (WWTP), we sampled water and whole-body juvenile Chinook salmon (Oncorhynchus tshawytscha) and Pacific staghorn sculpin (Leptocottus armatus) in estuaries receiving effluent. We analyzed these matrices for 150 compounds, which included pharmaceuticals, personal care products (PPCPs), and several industrial compounds. Collectively, we detected 81 analytes in effluent, 2 analytes in estuary water, and 42 analytes in fish tissue. A number of compounds, including sertraline, triclosan, estrone, fluoxetine, metformin, and nonylphenol were detected in water and tissue at concentrations that may cause adverse effects in fish. Interestingly, 29 CEC analytes were detected in effluent and fish tissue, but not in estuarine waters, indicating a high potential for bioaccumulation for these compounds. Although concentrations of most detected analytes were present at relatively low concentrations, our analysis revealed that overall CEC inputs to each estuary amount to several kilograms of these compounds per day. This study is unique because we report on CEC concentrations in estuarine waters and whole-body fish, which are both uncommon in the literature. A noteworthy finding was the preferential bioaccumulation of CECs in free-ranging juvenile Chinook salmon relative to staghorn sculpin, a benthic species with relatively high site fidelity.
Citation: James P. Meador, Andrew Yeh, Graham Young, Evan P. Gallagher. Contaminants of emerging concern in a large temperate estuary. Environmental Pollution. (2016) 213: 254-267.
Citation for Coverpage:
SG can post PDF online?: Yes
Uploaded File: 1-s2.0-S0269749116300884-main.pdf

Copper induced deregulation of microRNA expression in the zebrafish olfactory system
Publication Type: Peer-reviewed: Journals (incl. articles), Books, Proceedings, and Other Documents
Publication Year: 2013
Publication Authors:

Publisher Info:
Notes:
Related URLs:
Keywords: Zebrafish, copper, microRNA, olfaction
Publication URLs: http://www.ncbi.nlm.nih.gov/pubmed/23745839
Abstract:
Citation: Environmental science and technology, 2013 July 2 47 (13) 7466 – 7474
Citation for Coverpage:
SG can post PDF online?: Yes
Uploaded File: nihms751079.pdf

Students Supported

Kevin Hefferen (New Student)
heff2474@uw.edu
University of Washington, Environmental and Occupational Health Sciences

Field of Study: Environmental Toxicology
Advisor: Dr. Evan Gallagher
Degree Type: MS
Degree Year: 2017

Student Project Title:

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): Masters student

Post-Graduation Plans (employer, grad school, etc.): Differential toxicity of contaminants o
major classes of zebrafish olfactory sensory neurons.

Was this thesis/dissertation supported by Sea Grant?: No

Thesis / Dissertation:

New or Continuing?: New

Degree awarded this reporting period?: No

Financially supported?: No

Narratives

SeaGrant final report 2017
Uploaded File: Sea_Grant_final_year_progress_report_April_27_2016.2.pdf

Partners This Period

UW Department of Radiology
Types: Academic Institution
Scale: STATE
Notes: Dr. David Marcinek - Collaborator on mitochondrial studies in relation to Puget Sound emerging contaminants study.

STANDARD QUESTIONS

Community Hazard Resilience

No **Community Hazard Resilience** information reported

Economic Impacts

No **Economic Impacts** information reported

Impacts and Accomplishments

(1)

Type	accomplishment
Title	Washington Sea Grant research uses original technology and a surrogate species to probe health effects of persistent pollutants in salmon
Relevance	Now banned but once widely used, polybrominated diphenyl ethers (PBDEs) persist in the environment, where they may still affect human health. Elevated PBDEs have been found in resident Chinook salmon collected in polluted areas of Puget Sound. High fish consumption in the Pacific Northwest, particularly in Native communities, lent urgency to the question: what are the risks from eating PDBE-contaminated salmon?
Response	Washington Sea Grant-supported researchers investigated the reproductive and behavioral effects of feeding PDBEs to zebrafish, a cost-effective surrogate species. They developed new high-throughput gene-expression technology to determine whether molecular biomarkers would better indicate reproductive injury. The researchers also studied PDBE-associated mitochondrial injury in human cells. Spin-off studies entailed measuring other contaminants of emerging concern in Puget Sound salmon and developing biomarkers to assess their effects.
Results	High PBDE exposure caused minor damage to zebrafish reproductive tissues but no significant behavioral or molecular effects. However, related studies found that salmon harvested near wastewater treatment plants bioaccumulated numerous pharmaceutical and personal-care chemicals at levels harmful to other species. Investigators determined that omega-3 fatty acids found in salmon may provide chemoprotection against mitochondrial injury in human cells caused by PDBEs. These findings, together with other studies noting declining PDBE levels in the marine environment, suggest that other contaminants may be of more concern. Researchers shared these findings with educators, students, citizens' groups and state and federal scientists.
	Washington Sea Grant-supported researchers revealed minor histological damage to a surrogate

Recap	fish species fed high levels of polybrominated diphenyl ethers and developed new technology to investigate molecular effects.
Comments	
Primary Focus Area	Sustainable Fisheries and Aquaculture
Secondary Focus Areas	
Goals	Seafood consumers understand the health benefits, safety and environmental sustainability of their seafood choices.
Partners	Duwamish River Clean Up Coalition Environmental Protection Agency, Region 10 University of Washington, Department of Radiology, School of Medicine (UW) Washington State Department of Ecology
PI Draft	* Type accomplishment * Title Effects of contaminated fish on salmon and human health: Using zebrafish to assess the health effects of persistent pollutants in Pacific salmon * Relevance Despite the banning of polybrominated diphenyl ethers (PBDEs found in flame retardants, textiles, plastics, and electronics), they still persist and can still affect human health. Elevated PBDEs have been found in resident Chinook salmon collected near polluted areas of Puget Sound. Fish consumption is high in the Northwest and highest in Native communities, lending urgency to the question, what are the effects of eating PDBE-contaminated salmon? * Response Washington Sea Grant-supported researchers investigated these effects in long-term PBDE feeding studies using zebrafish, a cost-effective surrogate for other fish and humans with high consumption rates of local salmon species. They analyzed the reproductive effects of subchronic dietary PBDE exposures in adult zebrafish and developed new methods to facilitate screening for molecular injury. They have also studied the effects of PBDEs on mitochondrial injury using human cells, and identified some chemoprotective effects of the omega-3 fatty acids associated with salmon consumption on PBDE mitochondrial cell injury. They are using new technologies in rapid gene expression to better understand the effects of environmental contaminants. Parallel spin-off studies supported in part from Sea Grant addresses the occurrence of other contaminants of emerging concern in Puget Sound salmon, and developing biomarkers to assess their effects. The aforementioned studies were accompanied by outreach to educators and students in the community, and project updates to the Duwamish River Clean Up Coalition and scientists from the EPA region 10 and the WA Department of ecology. * Results Long term PBDE exposures resulted in histological damage to zebrafish

	reproductive tissues that was associated with bioaccumulation of two common PBDE congeners in zebrafish receiving high PBDE exposures associated with extensive fish consumers. By contrast, there were no behavioral effects in zebrafish fed PBDEs or effects on molecular biomarker genes. A high throughput gene expression technology was developed to analyze subtle PBDE effects at the molecular level, and this technology is being evaluated to assess other contaminants found in Puget Sound salmon. Studies in Puget Sound salmon harvested near wastewater treatment plants revealed bioaccumulation of a number of pharmaceuticals and personal care products to levels associated with adverse effects in other species. * Recap Zebrafish exposed to persistently high levels of PBDEs showed damage to reproductive tissues, and researchers developed new technologies to investigate for other adverse effects to help understand the risks for humans that consume high levels of PBDE-contaminated salmon. Comments Primary Focus Area Healthy Coastal Ecosystems Secondary Focus Areas Resilient Communities and Economies Goals Seafood consumers understand the health benefits, safety and environmental sustainability of their seafood choices. Partners Duwamish River Clean Up Coalition, EPA region 10 and the WA Department of Ecology

Leveraged Funds

No **Leveraged Funds** information reported

Meetings, Workshops, Presentations

(1)

Type of Event	Public or professional presentation
Description	Yeh, A., Marcinek, D., Meador, J.P., and E. Gallagher. Inhibition of Mitochondrial Electron Transport System Function in Fish from Sites Impacted by Contaminants of Emerging Concern. Society of Toxicology Annual Meeting. 3/22/15-3/26/15. San Diego, CA.
Event Date	03-23-2015
Number of Attendees	200

(2)

Type of Event	Public or professional presentation
Description	Gallagher, E. Role of Nrf2 in Regulating Cellular Antioxidant Responses of Fish. Society of Toxicology Annual Meeting. 3/22/15-3/26/15. San Diego, CA.

Event Date	03-23-2015
Number of Attendees	200

(3)

Type of Event	Public or professional presentation
Description	Wang, L., Espinoza, H.M., MacDonald, J.W., Bammler, T.K., Williams, C.R., Yeh, A., Louie, K.W., and E. P. Gallagher. Transcriptional and Biochemical Effects of Chlorpyrifos and Malathion and Their Mixtures on Neurobehavioral Function in Coho Salmon. Society of Toxicology Annual Meeting. 3/22/15-3/26/15. San Diego, CA.
Event Date	03-24-2015
Number of Attendees	200

(4)

Type of Event	Sea Grant-sponsored/organized event
Description	Tours of zebrafish facility and lectures for South Park and Georgetown middle school students
Event Date	03-16-2015
Number of Attendees	20

Tools, Technologies, Information Services / Sea Grant Products

(1)

Description	Multiplex PCR assay to analyze gene expression in zebrafish targeting oxidative stress as a mechanism of toxicity and reproductive parameters.
Developed (in the reporting period)?	Yes
Used (in the reporting period)?	Yes
Used for EBM?	No
ELWD product?	No
Number of managers	0
Description/Names of managers	N/A

WASHINGTON SEA GRANT FINAL PROGRESS REPORT

for the period 2/1/2012 – 1/31/2016

1. PROJECT OBJECTIVES.

Background. Waterborne contaminants in Washington's coastal areas threaten marine and estuarine ecosystems and can negatively impact human health. Toxic chemicals of current concern include brominated flame retardants (BFRs), a group of persistent global environmental contaminants that include the polybrominated diphenyl ethers (PBDEs). PBDE residues in resident Puget Sound Chinook salmon (*Oncorhynchus tshawytscha*) from certain contaminated sites are 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. Chinook remain a predominant food source for some underrepresented minorities such as Tribal Nations members, leading 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. 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. We are evaluating if this strategy can be applied to assess the effects of other emerging contaminants in the Puget Sound.

Specific aim 1. Determine the reproductive and trans-generational toxicity that consumption of the PBDE mixtures found in Chinook from urbanized and non-urbanized areas of the Puget Sound cause 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. Our initial goal for aim 1 was to expose zebrafish to PBDEs in a scenario representative of human consumption. This included a PBDE mixture comprising three of the predominant PBDE congeners measured in salmon from polluted sites in Puget Sound, including the congeners BDE 47, BDE 99 and BDE 100. Makeup of experimental diets was to include the following ratios: 50% BDE 47, 40% BDE 99, 10% BDE 100, and at concentrations to model PBDE exposures associated with high and moderate consumption of contaminated salmon based on prior reports on blackmouth salmon contamination in the Duwamish waterway. High consumption is estimated to be 620 grams of salmon per person per day, and moderate consumers average 40 grams of salmon per day; these amounts were scaled (μg/kg/day) to select the "high" and "low" PBDE treatment levels used in this study. GC-mass spectrometry (GC-MS) analysis of experimental diets by our collaborator, Dr. Heather Stapleton (Duke University) confirmed that individual congener and total PBDE concentrations were in a good agreement with the desired, environmentally relevant levels targeted in the project description (**Table 1**). Following validation of the test diets, sub-chronic exposures were initiated with sampling points at 28 and 56 days, and an 18 day depuration at 72 days. Because of the lack of flow-through exposure capacity in our zebrafish facility, we used daily static renewal exposures in 20L aquaria over the course of exposures. As shown in **Table 2, we** observed bioaccumulation of BDE47 and BDE 100 over the 28-56 day exposure periods consistent with our target concentrations, but very

low or no bioaccumulation of BDE 99. Although we could not experimentally confirm the reason for the limited bioaccumulation of BDE 99, this may have been due to metabolism or debromination, which has been reported by us in experiments of *in vitro* metabolism in hepatic fractions from Chinook salmon (Browne et al., 2009).

Table 1. **Tandem mass spectrometry analysis of dietary BDE-47, 99, 100 concentrations in experimental diets**. Intended PBDE concentrations were calculated from a model incorporating human consumption data and on PBDE residues in Puget Sound Chinook salmon (Sloan et al., 2010. (ng/g diet)

Exposure group	BDE-47 (ng/g)	BDE-99 (ng/g)	BDE-100 (ng/g)	Total measured PBDE congeners (ng/g)	Total intended PBDE Concentration (ng/kg)
Control	<BDL*	0.31	<BDL*	0.31	<0.50
Low dose	14	3	8	25	16
High dose	862	122	569	1553	1063

*Below analytical limit of detection.

Table 2. Tandem mass spectrometry analysis of BDE-47, 99, 100 by wet weight in pooled zebrafish samples. (ng/g wet weight tissue)

Diet ID	Time	BDE-47 (ng/g)	BDE-99 (ng/g)	BDE-100 (ng/g)	Total PBDE Concentration (ng/g)
Control	Day 28	8.03	<BDL	0.85	8.88
Low		13.99	<BDL	1.49	15.48
High		505.39	6.76	51.26	563.41
Control	Day 54	8.64	<BDL	1.05	9.69
Low		20.14	<BDL	2.36	22.5
High		948.33	3.02	103.39	1054.74
Control	Day 72	5.09	<BDL	0.59	5.68
Low		15.53	<BDL	1.67	17.20
High		801.35	<BDL	85.67	887.02

Effects of PBDE exposures on zebrafish swimming behavior. Adult zebrafish receiving the subchronic dietary PBDE exposures were analyzed for CNS toxicity effects as measured by analysis of swimming ability and anxiety. These were assessed using a battery of behavioral measures, including distance traveled, average swimming velocity, time spent in upper versus lower zones of aquaria (top

0.5L and bottom 1.0L sections of the tank), as well as the number of transitions between upper and lower zones in the tank, and the length of time before the first transition to the upper zone (Cachat et al. 2010). The behavioral trials were conducted on zebrafish (n=7-9/exposure group) on days 54 and 72, as well as for a subset of 3 fish on day 28. Briefly, individual fish were placed in 1.5L tanks (Pentair Aquatic Eco-Systems, Inc.) containing 1L of freshly reconstituted water and were filmed through the side of the tank for 6 minutes after a 1-minute acclimation period. Behavioral metrics were quantified using EthoVision XT (Noldus) video tracking software. As shown in **Figure 1**, there were no significant differences in adult zebrafish behavior after 54 days of dietary PBDE exposure or an additional 18 days of depuration, as measured by total distance traveled, average velocity, or zonal transitions. However, in the high exposure group, there were trends toward the diminishment of velocity, distance traveled, and number of zone transitions relative to low exposure and controls. Similarly, there was a trend towards a reduction of time spent in the upper portion of the tanks for fish receiving the high PBDE exposures and then depuration (**Figure 1**). However, due to interindividual variation in these analyses, the effects were not statistically significant at $p<0.05$. Not shown are the no-effect data on a subset of fish analyzed for distance traveled and average velocity for day 28.

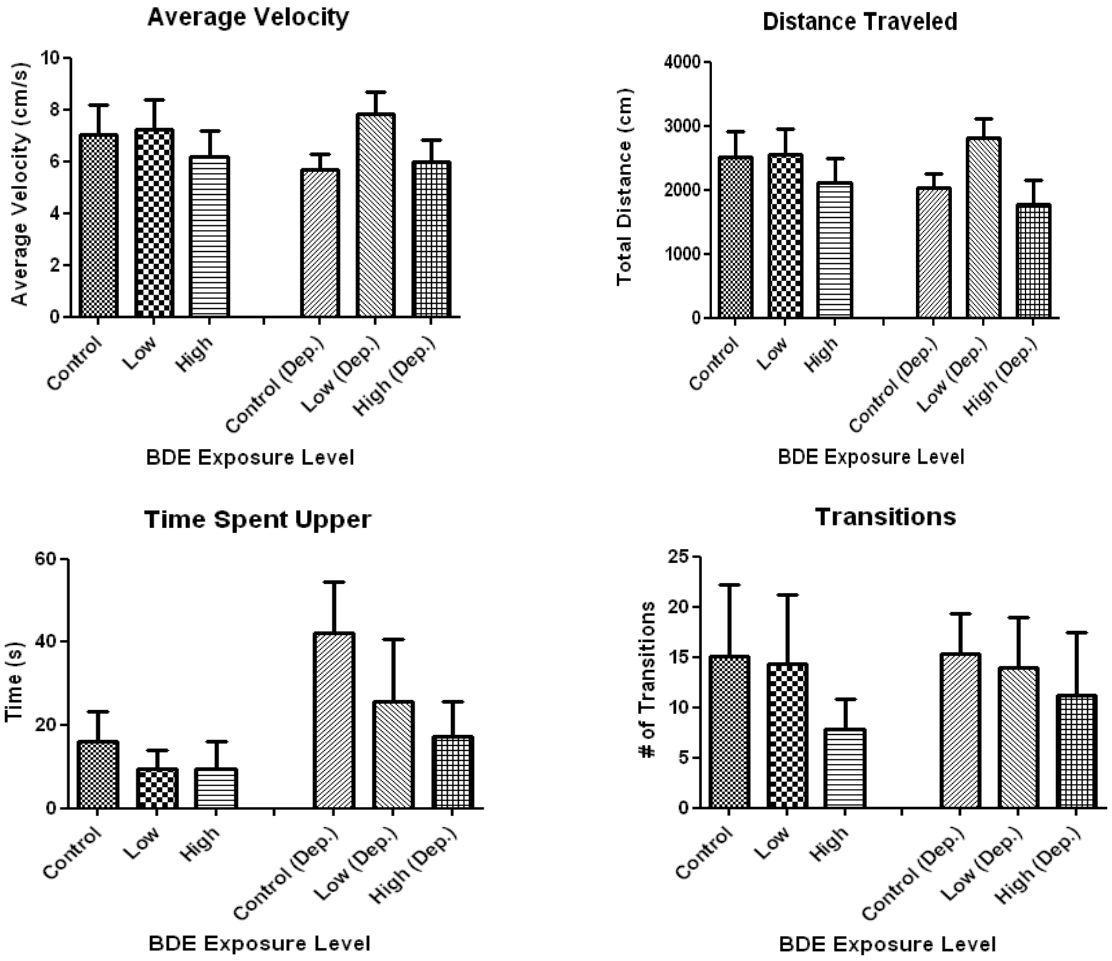

Figure 1. Swimming behavior of adult zebrafish receiving 54 day PBDE exposures and following an 18-day depuration. Behavioral trials were conducted over a five-minute period and analyzed by computer imaging software. Upper left panel: average velocity (cm/s); upper right panel: distance

traveled (cm); lower left panel: time spent in the upper zone (0.5L) of aquaria; lower right panel, number of transitions between upper and lower zones. There were no statistically significant effects of dietary PBDE on any of these behaviors at $p<0.05$ relative to control diets.

Effects of PBDE dietary exposures on zebrafish gonadal histology. Ovarian tissues (n=3 females /treatment) were harvested at two time points (day 54 and 72) and placed in 4% paraformaldehyde at 4°C for 24 hours following behavioral testing. These samples were then cryoprotected, at room temperature and with gentle rotation, in PBS/sucrose solutions as follows: 3x 5% sucrose for 10min, 10% for 30min, 12.5% for 30min, 15% for 30 min, and 20% at 4°C for 24 hours. Samples were further infiltrated with the following ratios of 20% sucrose/PBS solutions to OCT (Tissue-Tek) for 30min a piece: 100% OCT, 1:2 sucrose to OCT, 1:1 sucrose to OCT, and 2:1 sucrose. Tissues were finally embedded in a 2:1 sucrose to OCT solution before sectioning at 5um on a cryostat machine set to -24°C. The resultant slides were stained with hematoxylin and eosin and analyzed by our collaborator Dr. Wu Dong at Duke University. Dietary exposure to the high dose of the PBDE mixture caused adverse histopathological effects in the reproductive tissues (**Fig. 2**), and caused a statistically significant increase in the number of secondary oocytes present in reproductively-active female zebrafish (**Fig. 3**).

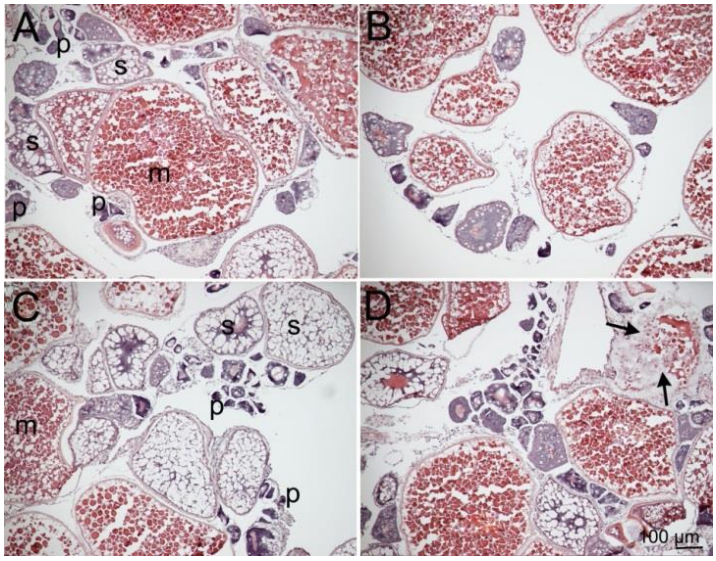

Figure 2. Adult female zebrafish ovaries at day 54 of PBDE exposure, **H&E staining**. Sections of stained ovary tissue from fish fed control (A), low (B), and high (C and D) BDE diets. m, Mature oocytes; s, Secondary oocytes; p, Primary oocytes. Black arrows indicate degenerate oocytes in the high PBDE dose group.

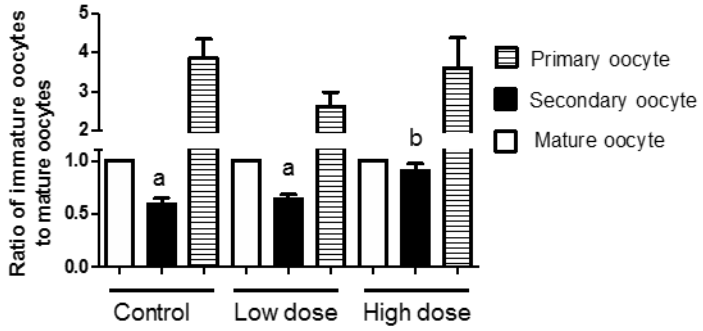

Figure 3. Morphological analysis of PBDE effects on zebrafish oocyte development. Oocytes in H&E stained zebrafish ovaries at day 54 of exposure were counted and categorized by developmental stage. The high PBDE-dietary exposure group had a significantly higher ($p<0.05$) ratio of secondary oocytes to mature oocytes than controls or low exposure group (n=10 slides/5 ovaries).

Gene expression analysis of ovarian tissues. Because dietary exposure to PBDEs affected oocyte formation and maturation in female zebrafish, we examined whether molecular biomarkers reflected the histological injury we observed. To achieve this goal, in 2015 we used an array of gene probes on the QuantiGene Plex platform that we developed to look at oxidative stress (Mills and Gallagher, in prep), a mechanism of PBDE toxicity, and we augmented our array with a set of endocrine genes. The molecular biomarker array included ten genes that respond to cellular oxidative stress and oxidative DNA damage (*hmox1*, *gclc*, *gstp1*, *nqo1*, *prdx1*, *gpx1a*, *sod1*, *sod2*, *hsp70*, *gadd45bb*) two genes involved in steroidogenesis (*cyp19a1a*, *star*), two steroid receptors (*esr2a*, *esr2b*), and two gonadotropin receptors (*fshr*, *lhcgr*). We used this platform to analyze gene expression in the ovarian tissues of a subset of three adult female zebrafish from each experimental group. Analysis of the panel of 16 oxidative damage and reproductive genes were normalized to two reference genes that were stably expressed in our adult ovary samples (*actb1*, *gapdh*). Our analysis indicated that neither the 54-day dietary exposure to PBDEs, nor the subsequent 18 days of depuration, significantly affected expression of any of the genes examined in zebrafish ovaries (data not shown). This may have been due to small sample size associated with limitations of our static renewal exposures.

Despite the lack of significant changes in gene expression caused by exposure to these levels of dietary PBDEs, we are using this QuantiGene Plex technology in studies of the effects of environmental chemicals in zebrafish larvae, and a similar platform is under development for salmon exposed to Puget Sound pollutants.

Collectively, our results indicate that female zebrafish fed an environmentally relevant mixture of PBDEs have adverse impacts on oocyte formation, and a trend towards adverse swimming behavioral effects. We could not ascertain effects on fecundity or transgenerational effects of the dietary PBDE exposures due to poor reproductive success in all of our zebrafish, including non-treated controls.

Specific Aim 2 progress and results. In years 1-2 of the project we initiated translational activities by working with high school health teachers to integrate environmental health science content into the curriculum. Seven teachers from the Bellevue School District participated in several "modules" including, on 9/12/12, a presentation and tour by graduate student Chase Williams regarding pollution issues with salmon and the utility of zebrafish models to our understanding of toxicological issues in salmon. The lecture and tour included a demonstration of zebrafish behavioral software and other tools for use in zebrafish toxicology research, and paid particular attention to PBDEs as persistent pollutants. In year 2, 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). These learning experiences were conducted in May 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 Sea Grant 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 research programs research translation core who met regularly with the Duwamish River cleanup coalition (DRCC) and scientists from EPA region 10. Ms. Frevert assists the PI on continuing research translational activities associated with sea grant, as well as our NIEHS Superfund program. On March 16 2015 we conducted two tours of our zebrafish facility and provided lectures associated with our Sea Grant activities to 18 "at-risk" middle school students and their teachers from South Park and Georgetown middle schools. We were also contacted by Dr. Kathleen Halligan at York College (PA) with regards to incorporating zebrafish into her undergraduate biology curriculum, and provided a tour of the fish room and discussed with her using zebrafish as a model for doing environmental health studies in undergrad teaching labs (Nov 18, 2015).

The PI has developed strong ties through the course of this project with local agency staff working on salmon and fish consumption issues, including staff from Region 10 EPA, ATSDR, the Washington Department of Ecology. He continued to be involved in meetings with these groups during this reporting period. The agency work is leveraged through our NIEHS Superfund grant, with the goal of informing research results that may have an impact on community health or agency regulations.

Summary of results. A sub-chronic feeding study of PBDE congeners in zebrafish which involved GC-MS analysis of experimental diets and bioaccumulation of PBDE congeners in adults was completed. These results confirmed bioaccumulation of 2 predominant PBDE congeners found in Puget Sound salmon in our long-term feeding study but not a third congener, which may have been the result of debromination *in vivo*. The dietary PBDE exposures associated with abnormal oocyte development in the gonads of adult female zebrafish receiving the dietary PBDE exposures. By contrast, the dietary PBDE exposures did not elicit behavioral effects in the adult zebrafish fed diets PBDEs relevant to levels of human exposures of salmon as measured by swimming behaviors. We applied a new technology involving rapid gene expression analysis to determine if molecular biomarkers were more sensitive indicators of reproductive injury. However, this was not the case, as no changes in molecular endpoints associated with PBDE toxicity in reproductive injury were observed. Because our fish did not reproduce due to the apparent stress of the static renewal exposures, it is unclear of the impact of these PBDEs on zebrafish reproduction. However, our results suggest that these approaches in zebrafish could still be useful to help assess risk for contaminants in Puget Sound salmon, with the caveat of necessity for using a different exposure scenario that does not involve the stress of static renewal exposures. A new technology involving rapid gene expression analysis for examining the effects of environmental chemicals in zebrafish targeting two key biochemical pathways was developed, and a manuscript summarizing the results of the feeding study is currently being drafted. In addition, three manuscripts were published during this project from spinoff studies that were supported in part by Sea Grant funding (see 3 below). Community engagement activities targeted local Native American health scientists, DRCC, high school area teachers and at risk students, and local agencies.

3. ACTIVITIES CARRIED OUT. Support for our Sea Grant studies on PBDEs was leveraged in other studies during the course of this project to help us understand the effects of Puget Sound pollutants in salmon, and also to develop zebrafish as a model to facilitate an understanding of how chemicals of relevance to the Puget Sound impact fish physiology. These activities led to 3 peer-reviewed publications.

In the first study (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) we used genomics techniques to understand the mechanisms of how

copper causes injury to the olfactory system of zebrafish. Copper is a prevalent stormwater contaminant in the Puget Sound region that is hazardous to salmon, and in previous studies we identified genes in the olfactory system of these fish that are negatively affected by copper. Copper inhibition of olfaction in fish has been linked to a loss of survivorship. In this paper, we identified microRNAs that were targets of copper and involved in the dysregulation of copper-mediated olfactory gene expression.

In the second study (Wang, L. Bammler, T., Beyer, R., McDonald, J., Yeh, A., Williams, C. and GALLAGHER, E. 2015. *Transcriptional and biochemical effects of binary organophosphate mixtures on the Coho salmon olfactory system*. *Toxicol. Sciences.* Epub October 21), we reported molecular changes in novel pathways of cellular injury in the olfactory system of coho salmon exposed to a binary mixture of common pesticides. The molecular affects were associated with loss of neurobehavioral function. In general, exposure to multiple chemicals can be a common exposure scenario in certain life stages of Pacific salmon. The effects of multiple chemical exposures are largely understudied.

A third, and most recent, paper resulted from a study extending our work on PBDEs to new contaminants of emerging concern in the Puget Sound (Meador, J., Yeh, A., Young, G., and E.P. GALLAGHER. 2016. Contaminants of emerging concern detected in a large temperate estuary. *Environmental Pollution*. In press e-pub Jan 2016). The study was part of our initiative in assessing emerging contaminants in Puget Sound salmon associated with the project largely funded by the Washington Department of ecology. This particular study is a logical follow-up to the sea grant studies, as the PBDE fire retardants have been phased out of production and there's evidence that the levels of these compounds are decreasing in the environment. This recent publication reported bioaccumulation of pharmaceuticals and personal care products in juvenile salmon near wastewater treatment plants in the South Puget Sound, and is leading to follow-up research. The paper has been widely cited, including local newspaper and television:

http://www.seattletimes.com/seattle-news/environment/drugs-flooding-into-puget-sound-and-its-salmon/ Seattle Times http://www.seattlepi.com/local/science/article/Juvenile-salmon-brains-anti-depressants-puget-6861890.php Seattle PI

http://legacy.king5.com/story/tech/science/environment/2016/02/24/wastewater-entering-puget-sound-contains-drugs-like-prozac/80866726/ King 5 News

Sea Grant research was also leveraged with an existing National Science Foundation grant to the PI 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, and supported the initial validation of the high throughput gene expression assay system used in our Sea Grant studies.

In addition to our laboratory investigations and research translational activities associated aim 2, Sea Grant research was presented at meetings. Partnerships with scientists at University of Mississippi, Duke University, and with Dave Marcinek (UW Department of Radiology) were established. Funding from Sea Grant supported four publications during the course of the Sea Grant project with the goal of assessing the effects of pollutants in zebrafish and Puget Sound salmon. An additional manuscript on the feeding study is in preparation.

4. CHALLENGES

Because of the stress of daily static renewal water changes during the subchronic PBDE feeding study, our zebrafish did not spawn and we could not analyze for fecundity or transgenerational effects.

5. CHANGES IN PROJECT DIRECTION. As discussed, the lack of poor reproduction due to the stress of the long-term static renewal protocol in zebrafish let us to refocus our reproductive effects on

the adults. This led us to the alternative strategy of assessing molecular biomarkers in the ovaries of adult female fish using a high-throughput gene expression assay. No other major changes in project direction occurred, and leveraging from other NIH funded studies augmented our Sea Grant to assess effects of emerging contaminants of relevance to the Puget Sound in salmon, as well as further developing the zebrafish model for applicability to Puget Sound studies.

6. PARTICIPANTS

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2. Dr. Margaret Mills, postdoctoral researcher
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8. Dr. Heather Stapleton (Duke University), collaborators
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10. Dr. James Meador (NOAA Fisheries and affiliate UW DEOHS faculty), collaborator
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