Update Report

GALLAGHER, Evan

Period: 2/1/2012 - 1/31/2013
Project: R/OCEH-8 - *Using zebrafish to assess the health effects of persistent pollutants in Pacific salmon*

:: STUDENTS SUPPORTED

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:
Use of Zebrafish to Understand Mechanisms of Environmental Chemical Toxicity

Involvement with Sea Grant This Period:
continuing PhD student

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:
Effect of omega-3 PUFAs on PBD toxicity

Involvement with Sea Grant This Period:
continuing graduate student

Post-Graduation Plans:
not applicable

:: CONFERENCES / PRESENTATIONS

Gallagher, E.P. 2012. Mechanisms and biomarkers of PBDE toxicity. SETAC Australia. Brisbane, Australia, public/profession presentation, 75 attendees, 0001-03-12

:: ADDITIONAL METRICS

K-12 Students Reached: 140

Based on instructional material provided to 7 K-12 high school teachers, and to student:faculty ratio of 20:1, 140 K-12 students were reached

Curricula Developed: 0

Acres of degraded ecosystems restored as a result of Sea Grant activities: 0

not applicable

Resource Managers who use Ecosystem-Based 0

Approaches to Management:

none during this reporting period

Volunteer Hours: 0

HACCP - Number of people with new certifications: 0

none during this reporting period

N/A

Cumulative Clean Marina Program - certifications: 0

N/A

:: PATENTS AND ECONOMIC BENEFITS

No Benefits Reported This Period

:: TOOLS, TECH, AND INFORMATION SERVICES

Description		Developed	Used	Names of Managers	Number of Managers
Analysis of small noncoding RNAs (miRNAs) using an Agilent microRNA platform to identify microRNAs in the zebrafish olfactory system. R/OCEH-8	**Actual** (2/1/2012 - 1/31/2013) :	1	1		0
	Anticipated (2/1/2013 - 1/31/2014) :	0	1		

:: HAZARD RESILIENCE IN COASTAL COMMUNITIES

Name of coastal community	County		Number of resiliency trainings / technical assistance services*provided*	Was community hazard resiliency improved (e.g., via changes in zoning ordinances) ?
		Actual (2/1/2012 - 1/31/2013) :		n/a
		Anticipated (2/1/2013 - 1/31/2014) :		n/a

:: ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2012 - 1/31/2013) : 0

Number of fishers using new techniques
Actual (2/1/2012 - 1/31/2013) : 0

Anticipated (2/1/2013 - 1/31/2014) : 0	**Anticipated** (2/1/2013 - 1/31/2014) : 0
Sustainable Coastal Development	Coastal Ecosystems
Actual (2/1/2012 - 1/31/2013) : 0	**Actual** (2/1/2012 - 1/31/2013) : 0
Anticipated (2/1/2013 - 1/31/2014) : 0	**Anticipated** (2/1/2013 - 1/31/2014) : 0

:: PARTNERS

Partner Name: Dr. Heather Stapleton, type: academic, scale: local

:: IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant researchers use zebrafish to investigate the health risks of consuming PBDE-contaminated salmon, and educate the public about these risks**

Type: accomplishment

Description:
Relevance: Polybrominated diphenyl ethers (PBDEs) are persistent organic compounds widely used as flame retardants. Despite a 2007 ban on the use of PBDEs, high levels have been found in some human milk and salmon, especially in salmon from polluted sites such as resident Chinook in heavily impacted Puget Sound. In laboratory studies, PDBEs are associated with reproductive and developmental injury in animals, but the human health effects of consuming fish containing small quantities are poorly understood.
Response: Washington Sea Grant-supported researchers are using zebrafish as surrogates for human exposure to PBDEs. The fish were dosed for two months with a PBDE mixture relevant to salmon in order to account for bioaccumulation.
Results: At 28 days the exposed adult zebrafish showed no behavioral abnormalities. Gene expressions are now being analyzed. The project seeks to improve public understanding of PDBEs and seafood risks by sharing its results with regulators, educators, high school students, and residents along the polluted Duwamish waterway, and by undertaking a multi-pronged community engagement plan.

Recap:
Washington Sea Grant researchers use zebrafish as a low-cost model for studying the human health hazards of contaminated seafood, together with public education about seafood pollutant hazards.

Comments:
Primary Focus Area – OCEH (SSSS)

Associated Goal: 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:

National Institute of Environmental Health Sciences, National Institutes of Health, US Department of Health and Human Services (US HHS)
University of Washington (UW), Research Translation and Outreach Core Program, Superfund Research Program, Department of Environmental and Occupational Health Sciences, School of Public Health
University of Washington, Department of Environmental and Occupational Health Sciences, School of Public

Health (UW)
US Environmental Protection Agency (US EPA)

:: PUBLICATIONS

No Publications Reported This Period

:: OTHER DOCUMENTS

No Documents Reported This Period

:: LEVERAGED FUNDS

Type: influenced Period: 2012-04-10::2013-01-13Amount: $6979

Purpose:
Salary support for the research scientist Michael Espinoza and graduate student Chase Williams for work on the project. NOT REPORTED

Source: PIs Murphy chair endowment and Department of Environmental and Occupational Health Sciences

Type: influenced Period: 2012-03-15::2013-01-01Amount: $13265

Purpose:
quantitative computer behavioral tracking system purchase and installation for subsequent analysis of effect of PBDEs on zebrafish behavior. NOT REPORTED

Source: Supplemental award from the Department of Environmental and Occupational Health Sciences for zebrafish research

Type: influenced Period: 2012-02-15::2013-01-01Amount: $7780

Purpose:
Supplement for supplies needed money for laboratory research (biochemicals, reagents) and zebrafish husbandry (animals, replacement tanks, sensaphone system operations required by animal care, zebrafish water system purification contract). NOT REPORTED

Source: PIs indirect cost returned to the investigator by the department from other grants.

Type: influenced Period: 2012-04-01::2012-06-07Amount: $1653

Purpose:
Tuition shortfalls for PhD students Andrew Yeh and Chase Williams not covered by SeaGrant. NOT REPORTED

Source: Department of Environmental and Occupational Health Sciences

Type: influenced Period: 2012-02-01::2013-01-01Amount: $17870

Purpose:
Partial salary support offset for the PI to devote more supervision to researchers and graduate students and project coordination time associated with the project. NOT REPORTED

Source: Department of Environmental and Occupational Health Sciences

PROGRESS REPORT NARRATIVE

For the period 2/1/12 - 1/31/13

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

Project Summary

The goal of this project is to use an *in vivo* surrogate model species (zebrafish) to determine if exposure to levels of polybrominated diphenyl ether (PBDE) congener mixtures relevant to the human consumption of Puget Sound salmon (in particular, Chinook or blackmouth salmon) causes toxicological effects in laboratory exposed specimens. We are analyzing the effect of dietary PBDE exposures on sublethal and reproductive injury in adult zebrafish and on developmental injury in offspring. We are using the zebrafish model to accomplish this goal and aim to address gaps in the risk assessment of PBDEs in salmon. An additional goal of this project is to share our scientific approaches and findings with three regional audiences who are stakeholders in this work: residents of the lower Duwamish waterway (LDW), local regulatory agencies, and Seattle area high school educators and their students. Our multi-pronged Community Engagement Plan (CEP) will ensure that the innovative tools generated by this project are applied in a way that maximizes the public's understanding of the risks associated with PBDEs.

Completed Activities

The following major activities were completed during the reporting period:

1. Validation of experimental diets. Tandem mass spectrometry readings confirmed that PBDE congener levels within zebrafish experimental diets remained relevant to human consumption models and to NOAA Fisheries data on PBDE residues in Puget Sound Chinook salmon.

2. Analysis of the behavioral effects of PBDEs in the initial phase of exposures, i.e. 28 days

3. Chronic (67 day) zebrafish exposures to dietary PBDEs.

4. Upon termination of the long term exposures, collection of behavioral recordings and samples for gene expression analysis in adult zebrafish.

5. Initiation of research translational activities with high school educators

Participants

Several participants were involved in the study. The majority of project-related activities were performed by a postgraduate research technologist; contributions were also made by the laboratory manager

(Michael Espinoza), a postdoctoral researcher (Dr. Lu Wang), and 2 graduate students (Chase Williams and Andrew Yeh). The PI oversaw the studies.

Results

Specific Aim 1. Use zebrafish to determine the potential for reproductive and developmental toxicity of PBDE mixtures in Puget Sound Chinook under conditions of dietary exposures

Chemical residue analysis of the experimental diets (i.e. zebrafish flakes supplemented with the three primary PBDE congeners measured in local Chinook salmon (BDE 47, 99, and 100)) confirmed that both individual congener and total BDE concentrations were within general agreement to the desired, environmentally relevant levels targeted in the project description (**Table 1**). Following validation of the chemical diets, sub-chronic exposures were initiated with two intended sampling points at 28 and 56-70 days. This was accomplished through daily static renewal exposures in 20L aquaria. Adult zebrafish were analyzed for potential effects of the dietary PBDE mixtures on CNS toxicity as measured by swimming behaviors. As shown in **figure 1**, there were no significant differences in adult zebrafish behavior following 28 days of dietary PBDE exposure. We terminated the experiments at 67 days of dietary exposure. Behavioral data were collected and are currently under analysis. In addition, fish were harvested for analysis of PBDE bioaccumulation, histopathology, and gene expression. Other fish are undergoing a 14 day depuration period for analysis of the aforementioned endpoints (in progress). We also installed a computer-driven behavioral system that will help us analyze the effects of PBDEs on zebrafish behavior.

Due to poor spawning in adults, there was no embryonic data to report (see "Challenges Encountered"). In spinoff experiments during this time period, we optimized a protocol using the Agilent microRNA genomics platform to analyze small noncoding RNA (miRNA) expression and used bioinformatics approaches to identify microRNAs in the zebrafish olfactory system. This work was leveraged by our NIEHS Superfund studies that are using the zebrafish to understand effects of metals on microRNA profiles in the zebrafish olfactory system. This approach may be used in our year 2 studies for PBDEs. A manuscript was generated on the microRNA work and is currently in review (*Environmental Science and Technology*).

<u>Specific Aim 2</u>: Involve three stakeholder communities (LDW residents, regulatory agencies, and Seattle area high school teachers and students) in the project through a Community Engagement Plan (CEP) that leverages existing relationships and outreach networks.

The K-12 educational and community outreach activities included in this specific aim are primarily translational in nature and are to be expanded in project year 2. However, in year one, we leveraged our existing relationship with the research and translational core of our NIEHS center and initiated our activities by working with high school health teachers to integrate environmental health science content into the curriculum. Seven teachers from the Bellevue School District underwent several "modules" of content 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, among other technical approaches, a demonstration of zebrafish behavioral software and paid particular attention to PBDEs as persistent pollutants.

Challenges Encountered

Several challenges were involved in carrying out this project. Project initiation was delayed due to turnaround times associated with receiving UW IACUC compliance approvals for zebrafish dosing. Also, because we could not maintain our zebrafish husbandry room at the desired temperature (28^{o}C), we had to change our dosing protocol to a static renewal approach involving temperature manipulation of individual tanks. The subsequently observed failure of zebrafish to produce sufficient numbers of embryos for analysis affected experimental endpoints and negatively impacted the analysis of pollutant-induced embryonic developmental defects.

Project Alterations

Adherence to the originally proposed goals and aims remained a priority despite issues with animal numbers. The shortage of available zebrafish specimens due to poor spawning necessitated a reduction in the number of experimental doses from five, as originally proposed, to three: control, low, and high. The "low" dose represented the average human consumption of fish with a moderate contaminant load while the "high" dose represented the conspicuous consumption of fish with a high contaminant load (**Figure 1**). Regardless, the important human health applications of the project remained intact; selected doses were representative of BDE intake rates observed in humans consuming Puget Sound Chinook salmon.

The major impact from the poor spawning was an inability to conduct the study's trans-generational aim in a timely fashion. We are approaching this problem on several fronts. Although these are extremely laborious exposures (i.e. static renewal over 56 days), we may repeat the chronic dietary experiment in year 2 to determine if we achieve, under modified experimental conditions, better spawning success. However, this may rely on upgrades to our zebrafish facility that are in progress and are being supported by non-Sea Grant resources leveraged by the PI. In lieu of the transgenerational study in year 1, we added a histological study targeting the effects of PBDEs on reproductive tissues in adults. The histological study will provide an additional phenotypic anchor to our behavioral analysis, will help us understand the mechanisms of PBDEs' impacts on the reproductive system, and will strengthen associations drawn from our upcoming gene expression analysis also intended to provide molecular biomarkers of PBDE reproductive injury.

Table 1. **MS analysis of dietary BDE-47, 99, 100 concentrations in experimental diets**. Desired food concentrations were calculated from a model incorporating human consumption data and NOAA Fisheries data[1] on PBDE residues in Puget Sound Chinook salmon.

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

MDL-below maximum limit of detection by MS analysis .

[1] Sloan, C. A.; Anulacion, B. F.; Bolton, J. L.; Boyd, D.; Olson, O. P.; Sol, S. Y.; Ylitalo, G. M.; Johnson, L. L., Polybrominated diphenyl ethers in outmigrant juvenile Chinook salmon from the lower Columbia River and Estuary and Puget Sound, Washington. *Arch Environ Contam Toxicol* **2010**.*58*, (2), 403-14.

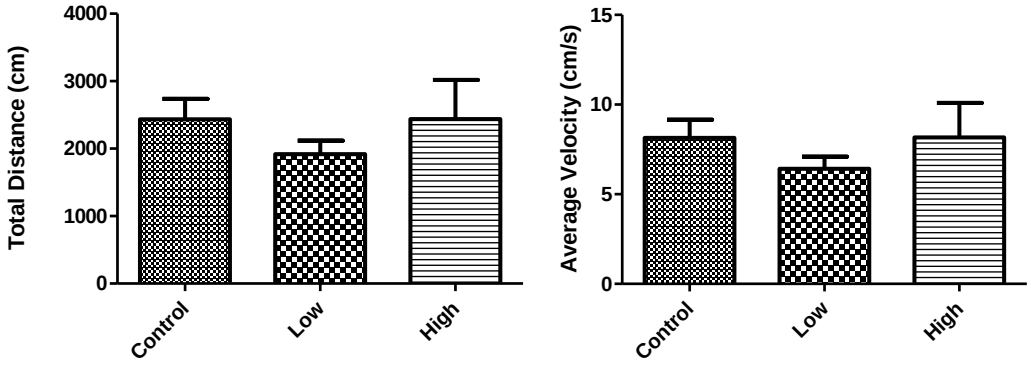

Figure 1. Swimming behavior analysis of adult zebrafish receiving 28 day exposures to PBDEs of relevance in Puget Sound Chinook salmon. (A, left) Total distance traveled (cm) over a 5 minute period by zebrafish exposed to a PBDE-containing diet for 28 days (n=3). (**B, right**) Average velocity (cm/s) over a 5 minute period by zebrafish exposed to a PBDE-containing diet for 28 days (n=3). There were no statistically significant dietary effects on behavior