Mechanisms of petroleum hydrocarbon toxicity in fish early life history stages (Year 3)

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Hypothesis and objectives: Studies prompted by the Exxon Valdez oil spill, which contaminated spawning grounds for Pacific herring (Clupea pallasi) and pink salmon (Oncorhynchus gorbuscha), identified a common syndrome of developmental abnormalities in both species induced by petroleum-derived polycyclic aromatic hydrocarbons (PAHs) [1-9]. An extensive literature has linked the toxicity of petrogenic PAHs to activation of the aryl hydrocarbon receptor (AhR) pathway and cytochrome P4501A (CYP1A) induction [10, 11]. In this model of PAH toxicity, harmful effects could be due to direct consequences of AhR activation, or to indirect effects of toxic PAH metabolites resulting from CYP1A activity. Since exposure of fish embryos to either petrogenic PAH mixtures or potent AhR ligands such as dioxins produces superficially similar syndromes, it has been generally held that these two classes of compounds act on developing fish by a common pathway. However, such a commonality has yet to be demonstrated. Knowledge of the precise mechanisms of PAH toxicity are important, because CYP1A activity in fish is generally the indicator used to assess exposure to oil, and this assay has been central to the debate over lingering effects of the Exxon Valdez spill [12, 13].

To address this problem we have used zebrafish (Danio rerio), a major model system for the study of vertebrate development at the molecular and genetic level. Studies conducted Years 1 and 2 of this grant identified AhR-independent disruption of embryonic cardiac function and morphogenesis as a primary pathway of toxicity for weathered crude oil in developing zebrafish [14]. Exposure of zebrafish embryos to weathered crude oil by two different methods produced a syndrome of embryolarval toxicity that was distinct from the AhR-dependent effects of dioxins. Instead, weathered crude oil caused early cardiac function defects that were consistent with the effects of the most abundant tricyclic PAHs (fluorenes, dibenzothiophenes, and phenanthrenes) expected from our work on individual model 3-ring PAHs [15]. Embryos in which either the AhRs or CYP1A were inactivated with antisense morpholino oligonucleotides (MOs) were actually more sensitive to weathered crude oil toxicity, indicating that the AhR/CYP1A pathway actually provides a measure of protection against petrogenic PAHs, rather than playing a causal role in toxicity. Therefore, direct targets of PAHs are most likely intrinsic to cardiomyocytes. In parallel work, recently funded by the NOAA/NWFSC Internal Grants Program and to be initiated in May 2005, we are using the commercially available (Affymetrix) zebrafish DNA microarray to identify potential cardiac targets of petrogenic PAHs by gene expression profiling of embryos exposed to phenanthrene or weathered crude oil and with genetically-derived cardiac dysfunction (see Appendix). The primary objective for Year 3 of OWCN funding is to groundtruth the cumulative findings from zebrafish studies in Pacific herring early life history stages through the following specific aims:

1. Conduct a detailed phenotypic analysis of Pacific herring embryos exposed to weathered Alaska North Slope (ANS) crude oil.
2. Identify and knockdown the AhR and CYP1A genes in Pacific herring embryos.

Experimental Plan Aim 1: Although the effects of weathered ANS oil on Pacific herring embryos are well-documented [6], those studies analyzed primarily late end points of toxicity in hatching stage larvae. Because there is considerable overlap of the appearance of the late-stage effects with classic AhR-mediated dioxin toxicity, a close
Incardona

analysis over the full developmental period is warranted, with particular attention to early cardiac function. Pacific herring embryos will be exposed to weathered ANS crude oil using the oiled gravel effluent method [6, 7, 14] with filtered seawater at the NOAA Mukilteo field facility near Seattle. Ripe herring are available in Puget Sound January through April, and will be obtained by gill netting with gear and small boats maintained by the NWFSC Environmental Conservation Division. Stripped gametes will be fertilized in the lab, and the naturally adherent eggs deposited on glass microscope slides for exposure in oiled gravel effluent. Examination of early developmental stages will be performed after manual dechorionation with fine iris scissors. Analysis of cardiac function will involve videomicroscopy of live animals, as well as structural characterization using cardiac chamber-specific antibodies [14, 15]. In addition, we anticipate that new cardiac-specific markers of PAH effects will be identified through the parallel zebrafish microarray study. Thus a second component of this aim is to clone the homologous markers from Pacific herring as they are characterized in zebrafish. Because only fragments of cDNA are required for the synthesis of in situ hybridization probes, PCR-based cloning with redundant primers should be sufficient, and generation of a herring cDNA library unnecessary. Methods for whole mount antibody labeling and in situ hybridization for zebrafish embryos are easily adapted to herring (figure above).

Aim 2: Our findings in zebrafish combined with prior studies on a range of other fish species (herring, salmon, mummichog, medaka, rainbowfish [6, 7, 16-18]) are most consistent with a common response of teleost embryos to petrogenic PAHs. However, to determine unequivocally whether PAHs in crude oil act through the same toxic mechanism in divergent species such as herring, we plan to replicate the AhR pathway knockdown studies in Pacific herring. Antisense morpholino (MO) knockdown is now a widely used technique in diverse anamniote embryos other than zebrafish, including sea urchin, frog, and ascidian. Importantly MO knockdown was demonstrated to work in fish species with much more prolonged development at cold temperatures. Effective gene inhibition at 15 to 30 days post-fertilization was demonstrated in rainbow trout [19, 20], which hatch in roughly 30 days at 12-15˚C. Therefore, it is most likely that herring embryos, which develop in roughly 15 days at 10˚C, are amenable to the technique. The overall development of Pacific herring and zebrafish are quite similar (when accounting for temperature differences); for example, the heartbeat begins at roughly one-third of development for both species. Therefore, we anticipate that the relationship of petrogenic PAH-induced cardiac dysfunction in herring—if observed—to AhR pathway activation should be readily dissected via MO knockdown. Herring AhRs (presumably AhR1 and AhR2 homologs) and CYP1A coding sequences will be obtained through a PCR strategy using redundant primers [21] based on conserved regions in published sequences from zebrafish, medaka, mummichog, tomcod and rainbow trout. Design of translation-blocking MOs can be based on coding sequence derived from PCR-amplified cDNA. Microinjection of herring embryos will be performed with similar methods as zebrafish, although it may require prevention of the egg envelope hardening reaction. This reaction generally involves protein cross-linking and was prevented in several species with glutathione [19, 20]. If egg hardening is insurmountable, injection could be achieved with quartz needles rather than the standard borosilicate glass. The main drawbacks to this are higher cost and inconvenience, as a special needle puller is required, which is available at the nearby University of Washington campus.

Significance to oiled wildlife health: Forage fish such as herring are an extremely important component of Northeast Pacific ecosystems, providing a major prey base for marine mammals, birds, and larger fish species such as rockfish and lingcod. Three of the major regional forage fish species, Pacific herring (Clupea pallasi), surf smelt (Hypomesus pretiosus) and sand lance (Ammodytes hexapterus), have demersal eggs that adhere to vegetation or gravel/sand substrate in nearshore or intertidal zones. These zones are generally the most heavily impacted by oil spills, dramatically demonstrated in Prince William Sound after the Exxon Valdez spill. Although some studies have
documented long term, chronic effects of persistent oil on the Prince William Sound ecosystem, there is still considerable debate over the veracity of these findings [12, 13, 22, 23], which has impacted the response to contemporary spills such as the November 2002 Prestige incident [24]. Despite the scale and depth of analyses applied to a range of biological systems as a consequence of Exxon Valdez, there is still no clear understanding of the mechanisms through which low-level PAH exposure adversely impacts organisms, in particular developing fish embryos. Guidelines for the protection of fish spawning habitat from anthropogenic inputs of PAHs will remain uncertain without filling these apparent data gaps concerning PAH toxicity.

**Project Duration:** These aims represent work proposed for the final year of a 3-year study. The broader goals of this project are to more accurately determine the impacts of oil on marine species. We plan to use knowledge of fundamental mechanisms of toxicity for PAHs identified in zebrafish to develop technology that ultimately will be transferred to West Coast marine and anadromous species. Already the first two years of this study have provided considerable insight into the poorly characterized mechanisms underlying PAH toxicity in fish early life history stages. We anticipate that the proposed work should have a major impact on the direction of future research on the effects of PAHs on aquatic ecosystems.
Estimated Budget:

Full-time salary for new molecular biology technician: $40,000

morpholinos 1800
Travel 1200
Indirect costs 6450

Total budget 49,450

Literature cited:


Appendix:

NWFSC Internal Grant Proposal, funded March 2005 (attached)

**NWFSC Internal Grants 2005**

**Identification of physiologically relevant biomarkers of PAH exposure for fish early life history stages using the zebrafish DNA microarray**

Principal investigator: John Incardona, ECD
Junior Track
Budget: $21,149
Start/end dates: May-Nov 2005
Key words: toxicology, oil spill, embryo, heart development, cardiovascular
SUMMARY:

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants in nearshore marine and aquatic habitats. Fish early life history stages (embryos and larvae) exposed to PAHs suffer detrimental effects, both lethal and sublethal. While PAH contamination from environmental catastrophes such as the Exxon Valdez oil spill have clear and dramatic effects on the early life history stages of native fish, a more insidious and less conspicuous source comes from fossil fuel consumption. Due to urbanization and suburban sprawl, PAHs are a ubiquitous contaminant in stormwater runoff, which was identified as an increasing threat to the health of coastal habitats by both the Pew Oceans Commission and the U. S. Commission on Ocean Policy. PAHs are thus a major class of contaminant that will increase in aquatic habitats in conjunction with population increases projected for the Pacific Northwest over the next several decades.

Studies following the Exxon Valdez spill identified a common syndrome that occurs in fish embryos exposed to PAHs in weathered crude oil, and documented sublethal effects that reduced the marine survival of pink salmon that were exposed to PAHs during embryogenesis but appeared externally normal as smolts. The very low levels of PAHs to which these animals were exposed are often exceeded in urban watersheds, so it is highly likely that PAHs associated with urban growth have effects on adjacent fish populations. The long-term goal associated with this pilot project is to identify biomarkers of PAH exposure in fish early life history stages that are predictive of health effects of exposure. Prior studies demonstrated that the current biomarker for PAH exposure, cytochrome P4501A (CYP1A), is a poor indicator of the deleterious effects of PAHs on fish embryos. Using the zebrafish model system, we have identified the embryonic heart as the most important target tissue for PAH exposure. Here I propose to identify potential cardiac-specific biomarkers that reflect the physiological changes induced by PAH exposure, using gene profiling with oligonucleotide microarrays.
RESEARCH QUESTION: The early life history stages (embryos and larvae) of fish can be especially sensitive to environmental contaminants due to the rapid proliferation, differentiation, and growth of tissues. Polycyclic aromatic hydrocarbons (PAHs), derived largely from fossil fuel consumption, are pervasive toxic contaminants in rivers, lakes, and nearshore marine habitats. Since the embryonic and larval stages of many fish species are physically associated with substrates which trap PAHs, they may be particularly susceptible to PAH exposure, as demonstrated dramatically by the 1989 Exxon Valdez oil spill [1-4]. Locally, tests conducted by the Puget Sound Ambient Monitoring Program detected PAHs in Pacific herring eggs from spawning sites, and PAHs are common contaminants in urban streams undergoing major habitat restoration. Population projections indicate that consumption-related sources of PAHs (e.g. automobiles) will continue to rise, and oil spills continue to be a problem in the Puget Sound basin. Understanding the effects of PAHs on fish development is thus especially important for the protection of marine resources and recovery of threatened and endangered salmonids. Yet fifteen years after the Exxon Valdez spill, the pathophysiology behind the lethal and sublethal consequences of PAH exposure is still unclear.

Historically, the study of PAH toxicity has focused on the aryl hydrocarbon receptor (AhR) pathway. The AhR is a ligand-activated transcription factor that controls the expression of a battery of genes involved in the metabolism and elimination of xenobiotic compounds, including PAHs. PAHs bind the AhR and thus induce their own metabolism by enzymes such as cytochrome P4501A (CYP1A). CYP1A generally is the sole biomarker used to assess PAH exposure in field samples, and it is widely held that PAH toxicity is mediated through toxic intermediates and oxidative stress resulting from CYP1A catalytic activity. However, studies carried out using the zebrafish model during my tenure as an NRC associate showed that the AhR pathway and CYP1A do not play causal roles in PAH toxicity, and that CYP1A levels in fish embryos and larvae are unrelated to the negative impacts of PAH exposure. Instead, embryonic cardiac function is the primary target of petrogenic PAH toxicity [5, 6]. These findings undermine much of the utility of CYP1A as a biomarker. As exemplified by the still highly contentious debate over potential lingering effects more than a decade after the Exxon Valdez spill [4], there remains an urgent need for biomarkers that are indicative of the health status of fish that were spawned in PAH-contaminated habitats.

I propose to address this problem with the following specific aim: Identify new biomarkers that reflect PAH-induced changes in cardiac function using gene expression profiling with a zebrafish microarray representing 14,900 transcripts. Conventional studies limited to one or a few genes have already demonstrated changes in gene expression in response to cardiac dysfunction in zebrafish embryos [7, 8], and it is well established in human heart disease. The zebrafish model is particularly well suited for microarray studies, because the genetic capabilities overcome one of the biggest challenges of interpreting microarray data: identifying which genes are truly biologically relevant. The pilot study proposed here is a simple microarray experiment utilizing 15 array chips and the follow-up PCR analysis to confirm changes in the expression of candidate genes. Future studies would determine the pathophysiological role of genes identified in this screen, and ultimately develop homologous probes/markers for analyzing embryos from field studies targeting species of concern for NOAA Fisheries. Three features of the zebrafish model make this long term goal feasible: (1) the traditional forward genetics approach utilized in the mid 1990s to generate a large collection of mutants that affect the development of virtually every organ system in zebrafish; (2) the ability to “knock-down” gene expression in zebrafish embryos by injection of highly stable antisense morpholino oligonucleotides at early cleavage stages; and (3) the ability to overexpress cloned genes in zebrafish embryos by injection of in vitro transcribed RNA. Thus changes in gene expression detected on microarrays can be readily translated into biological relevance.
METHODS AND RESEARCH PLAN

Overall experimental design: The goal is to compare gene expression profiles in cardiac tissue under three conditions at a single time point during development (48 hpf): Wild type embryos exposed to weathered crude oil (effluent from an oiled gravel column); wild type embryos exposed to phenanthrene, a tricyclic PAH representing the most abundant class of PAHs in weathered crude oil; and embryos with cardiac dysfunction due to a mutation in a cardiac-specific gene. Two control conditions will include wild type embryos reared in an incubator (in parallel with phenanthrene exposure and the cardiac mutant embryos), and wild type embryos reared in the effluent of a control (unoiled) gravel column. The rationale for analyzing a cardiac-specific mutant is that I anticipate that the study could identify two classes of genes; those whose expression is changed directly as a consequence of PAH exposure, and those that are changed secondarily as a response to cardiac dysfunction. Either type of gene has a potential role as biomarkers for PAH exposure, and the inclusion of a cardiac function mutant will allow the unequivocal identification of transcriptional responses to changes in cardiac physiology. Of the zebrafish cardiac function mutants that have been characterized at the molecular level, and are known to be cardiac specific genes, *weak atrium* (*wea*) is probably the best candidate. Mutations in *wea* disrupt the *atrial myosin heavy chain* gene, which is required for normal myofiber structure and contractile function of the atrium, and produces a cardiac function phenotype with features that overlaps with early phases of PAH-induced cardiac dysfunction. There are also changes in ventricular function and structure in *wea* mutants, which are secondary and represent a response to atrial dysfunction. Therefore, it is likely that there would be some overlap between changes in cardiac gene expression between PAH-exposed embryos and *wea* embryos.

The proposed work will be carried out in collaboration with the laboratory of Dr. Scott Argraves at the Medical University of South Carolina (MUSC), and will utilize the commercially available zebrafish oligonucleotide gene array (the Affymetrix GeneChip® Zebrafish Genome Array). Dr. Argraves runs the microarray core facility at MUSC, and is known from a collaboration stemming from the PI’s previous position prior to joining the NWFSC. MUSC and NOAA-NOS are jointly involved in operations of the Hollings Marine Lab and the East Coast OHH Center of Excellence, and Dr. Argraves’ facility also provides support for genomics projects at NOS. Zebrafish embryo exposures, dissection of hearts, and RNA preparation will be carried out by the PI at the NWFSC. Microarray hybridizations and subsequent data analysis will be carried out at MUSC. Follow up real time quantitative PCR will be carried out by the PI at NWFSC using the Center’s facilities overseen by Dr. Penny Swanson.

Preparation of total RNA from dissected hearts: The array experiment will be performed in triplicate, thus utilizing 15 Affymetrix oligonucleotide microarray chips (3X3 experimental, 2X3 controls). A single 48 hpf embryo has roughly 0.5 µg RNA. The MUSC microarray facility is routinely working with pg quantities of RNA, so we anticipate that problems of target RNA dilution by non-target tissue can be markedly reduced by collection of hearts isolated from as few as 50 embryos. Thus a single array replicate would entail dissection of and RNA isolation from 150 hearts from the three classes of embryos. Experimental and control hearts dissected and placed into RNAlater reagent (Qiagen) to stabilize RNAs during the time required for dissection. Hearts will then be homogenized with Rnase-free micropestles and Qiashredders (Qiagen). If necessary, homogenates will be stored at -70°C to await further tissue collection. We anticipate that an RNA amplification step will be required, and performed using the Arcturus RiboAmp® RNA Amplification Kit (recommended for 10-40 ng total RNA).

Quality control of total RNA preparations and cRNA target preparation: Quality and purity of total RNA preparations will be assessed by A260:A280 ratios and by quantification of 28S:18S ribosomal RNA ratios as measured by an Agilent 2100 Bioanalyzer. Total RNA preparations with A260:A280 ratios of ≥1.7 and 28S:18S ratios of ≥1.6 are typically deemed acceptable. Biotin-labeled, fragmented cRNA targets will be prepared from total RNA samples using protocols established for the Affymetrix
GeneChip System (Santa Clara, CA; [http://www.affymetrix.com/](http://www.affymetrix.com/)). Detailed protocols for biotin labeling and fragmentation of cRNA targets can be downloaded as a PDF from [http://www.affymetrix.com/products/reagents/specific/cleanup.affx](http://www.affymetrix.com/products/reagents/specific/cleanup.affx). Fragmented cRNA samples will be evaluated with the Agilent 2100 Bioanalyzer to ensure appropriate fragment length distribution (~35-200 bases).

**Hybridization of cRNA targets to Affymetrix GeneChips:** Labeled and fragmented cRNA targets will be hybridized to Affymetrix genomic oligonucleotide arrays. Target cRNAs will be submitted to the MUSC Proteogenomics Facility for all GeneChip hybridization steps, including GeneChip hybridization, post-hybridization washing, staining and scanning. The facility, which employs Affymetrix GeneChip technology almost exclusively, is now in its fourth year of operation and has become proficient in microarray experimentation [9, 10]. Target preparations will be hybridized to Affymetrix Test3 arrays to assess target quality (e.g., 3' : 5' labeling ratios) and performance (e.g., detection of housekeeping genes, and absence of problematic background hybridization signals). Targets will then be hybridized to Affymetrix GeneChip® Zebrafish Genome Arrays that contain representations of over 14,900 *Danio rerio* transcripts. This array was constructed using information from RefSeq (July 2003), GenBank (release 136.0, June 2003), dbEST (July 2003), and UniGene (Build 54, June 2003).

**Normalization of hybridization data and determination of differentially expressed genes:** Many steps in the analysis of DNA microarray data will be executed using the R statistical environment to implement packages available from the Bioconductor open source software project ([http://www.bioconductor.org/](http://www.bioconductor.org/)). Normalization and quality assessment of hybridization data are critical aspects of DNA microarray analysis. There are many different algorithms and methods currently available for these processes, and each may result in subtle or perhaps significant differences in the interpretation of gene expression [11, 12]. Raw hybridization data will be normalized among GeneChips using the R environment/BioConductor implementation of Robust MultiChip Average (RMA; [13-15]). RMA normalized expression values will be compared between control and experimental samples and differentially expressed genes will be assessed according to the following criteria: fold change ≥2, p-value for unpaired t-Test ≤0.05, and false discovery rate (assessed by random iterations of permuted sample assignments) <0.05.

**Hierarchical clustering and functional categorization of differentially expressed genes:** Hierarchical clustering of differentially expressed genes will be performed using the R environment/BioConductor implementation of previously described methods [16, 17] Descriptive information, gene ontology information and associated annotations for differentially expressed genes will be collected and assembled using the BioConductor package AnnBuilder. Differentially expressed gene sets will be evaluated for groups of functionally related genes using Gene Ontology classification as the primary determinant. To augment these tasks we will employ dbSieve, a web-based program developed by the MUSC Proteogenomics Facility in association with Array Genetics, Inc. ([http://www.arraygenetics.com/](http://www.arraygenetics.com/)).

**Confirmation of candidate genes by Quantitative Real Time PCR:** We plan to confirm changes in gene expression detected in the microarray study by QPCR. Up to six candidate genes will be analyzed, with reagents designed for a seventh housekeeping gene as an internal control. QPCR will be performed using the fluorescence-based TaqMan assay, using the NWFSC QPCR system based in the Swanson lab in REUT. The TaqMan assay requires the design of a hybridization probe in addition to amplification primers, which requires sequence data from the target gene, as well as additional expense. We anticipate that sufficient sequence data for most candidate genes will be available in the zebrafish genome or EST database.
EXPECTED DIFFICULTIES: Microarray experiments with zebrafish embryos are uncommon to date. One of the biggest challenges in microarray studies is reducing variability in apparent gene expression levels that result from confounding, trivial factors. For example, dissection errors could contribute to apparent changes in gene profiles. Similarly, it may be difficult to detect changes in gene expression if genes of interest are diluted out by a high background of total RNAs. Most microarray studies have thus utilized tissues (from adult animals) that can be easily dissected, collected, or measured (e.g. sections of an organ like liver, blood). Given the very small size of zebrafish embryos (~2.5 mm at 48 hpf), dissecting individual tissues in sufficient quantities is a challenge. It is not yet clear if total RNA isolated from whole embryos can yield useful tissue-specific information in microarray studies. However, the zebrafish heart is one tissue that is readily accessible and easy to dissect in a consistent manner. Although we expect that the RNA amplification step should compensate for the amount of tissue, we do not yet know precisely what yields of RNA can be obtained from isolated hearts. Nevertheless, this difficulty is not insurmountable. It may mean that larger numbers of embryos (which are not limiting) will need to be exposed, and hearts dissected and frozen from multiple exposures before RNA isolation. Similarly the time spent dissecting may limit the number of embryos for RNA isolation from a given exposure, requiring multiple exposures. Although tedious, the cardiac dissection is much less challenging than other microdissections that were integral to the PI’s previous post-doctoral work in chick and mouse embryos. Ideally, the studies would be performed on single exposures as another means to reduce variability.

GUIDELINES STATEMENT: The mission of the Northwest Fisheries Science Center is to “provide the scientific basis to meet NOAA’s stewardship role to conserve and manage living marine resources and their habitat with emphasis on the Pacific Northwest”. Included in this vision is to make meaningful contributions to the stewardship of living marine resources, and to support the NMFS Strategic Plan. A major component of the NMFS Strategic Plan is to “protect and maintain the health of coastal marine habitats”. Inherent to these goals is a greater understanding of the anthropogenic impacts on fish populations. The work proposed here will contribute directly to the implementation of these goals. This is a pilot project that is unlikely to be funded by another source, and would otherwise not be completed. However, the pilot data would serve to determine whether microarray analysis should be incorporated (and appropriately funded) as a main tool used by the Ecotoxicology program to address potential impacts of the complex array of contaminants that will encroach upon fish habitat in the Pacific Northwest over the next several decades.

BUDGET:

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<td>Applied Biosystems TaqMan probes, 7 genes</td>
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<td>Stereomicroscope for dissection</td>
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Total budget 21,149
Literature cited:


CURRICULUM VITAE

John Patrick Incardona

Degrees:
MD Case Western Reserve University, Cleveland, OH 1996
PhD Genetics, Case Western Reserve University, Cleveland, OH 1995
BS Honors Biology, Indiana University, Bloomington, IN 1988

Employment/Research Experience:
♦ Nov 2004-present Research Toxicologist, NMFS/NOAA Northwest Fisheries Science Center, Environmental Conservation Division, Fish Neurobiology and Development Team
♦ 2002-2004 National Research Council Senior Associate, NMFS/NOAA Northwest Fisheries Science Center, Environmental Conservation Division (T. Collier). Analysis of environmental contaminant effects on fish development. Use of zebrafish as a model system for assessing the effects of pollutants on embryonic and larval development, physiology, and behavior.
♦ 1999-2001 Senior Fellow, Department of Biological Structure, University of Washington (H. Roelink). Cellular aspects of Sonic Hedgehog (Shh) signaling in neural development and mechanism of action of Shh-inhibitory teratogens; roles of cholesterol in Shh signaling.
♦ 1989-1994 Ph.D. Thesis in Genetics, Department of Pharmacology, Case Western Reserve University, Cleveland, OH (T. Rosenberry). Genetic Analysis of Glycolipid Anchor Function Using Drosophila Acetylcholinesterase as a Model Protein. Molecular biology, generation of transgenic flies; protein expression, purification, and generation of antibodies; enzyme kinetics; subcellular fractionation/cellular biochemistry; microdissection and culture of primary neurons; immunocytochemistry and immunoelectron microscopy; behavioral assays.

Fellowships:
2002-present National Research Council Senior Associateship, National Atmospheric and Oceanic Administration, Seattle
1996-1999 National Research Service Award Fellowship, Medical Teratology Training Program, University of Washington, Seattle
1987-1996 National Research Service Award Pre-doctoral Fellowship, Medical Scientist Training Program, Case Western Reserve University, Cleveland, OH

Journal Reviewer:
Mechanisms of Development
Birth Defects Research (formerly Teratology)
Competitive Grants Awarded:

Project title: **Evaluating the effects of forestry herbicides on early life history stages of fish.**
Funding agency: U.S. Forest Service, Pesticide Impact Assessment Program
Co-Investigator: Nat Scholz, NWFSC
Project Duration: 2 years, beginning FY03
Total Award: $104,518

Project title: **Mechanisms of petroleum hydrocarbon toxicity in fish at early life history stages**
Funding agency: Oiled Wildlife Care Network
Co-investigator: Nat Sholz, NWFSC
Project duration: 3 years, beginning FY03
Total award: $48,559 (year 1); $49,394 (year 2)

Project title: **Identification of physiologically relevant biomarkers of PAH exposure for fish early life history stages using the zebrafish DNA microarray**
Funding Agency: NOAA Northwest Fisheries Science Center Internal Grants Program
Project duration: 1 year
Total award: $21,149

Peer-reviewed Publications:


CURRICULUM VITAE

Nathaniel L. Scholz

Degrees:
Ph.D.  Zoology, University of Washington, Seattle, WA  1997
M.A.  Biology, Boston University Marine Program, Woods Hole, MA  1991
B.A.  Marine Biology with Distinction, Boston University Marine Program  1991

Fellowships and Awards:
◆ Special Act Award, National Oceanic and Atmospheric Administration, 2002
◆ Sustained Superior Performance Award, National Oceanic and Atmospheric Administration, 2001

Employment:
◆ Postdoctoral Associate, 1998-1999, National Oceanic and Atmospheric Administration (E. Casillas). Investigated the effects of current use pesticides on the neurobiology and behavior of Pacific salmon. Found that the insecticide diazinon interferes with predator avoidance and homing behaviors in chinook salmon.
◆ Research Zoologist, 1999-present, National Oceanic and Atmospheric Administration. Supervising a research laboratory at the Northwest Fisheries Science Center in Seattle. Our research focuses on environmental contaminants and their impacts on the neurobiology and development of protected fish species.

Teaching (TA):
◆ Completed Preparing Future Faculty program, 1996, University of Washington and Pew Charitable Trusts
Chemosensory Biology, Woods Hole (22 students, marine science majors)
Experimental Cell Biology, Friday Harbor (12 students, graduate)
Introductory Physiology, Seattle (18 students, freshmen and nonmajors)
Marine Invertebrate Zoology, Friday Harbor (15 students, graduate)
Natural History of Marine Invertebrates, Seattle (28 students, juniors and seniors)
Physiology and Development, Seattle (24 students, sophomores and juniors)
Sea Education Semester, Lesser Antilles (24 students, juniors and seniors)

Journal Reviewer:
American Zoologist
Comparative Biochemistry and Physiology
Journal of Comparative Neurology
Pesticide Biochemistry and Physiology

Presentations at Professional Meetings:
Georgia Basin/Puget Sound Research Conference (2003)
Society for Behavioral Toxicology (2002)
Society for Environmental Toxicology and Chemistry (1999)
Society for Integrative and Comparative Biology (1999)
Invited Lectures and Seminars:
Pacific Northwest Threatened and Endangered Species Conference, Yakima, 1999
Society for Integrative and Comparative Biology, Session on Nitric Oxide, 2000
Pacific Northwest Agriculture and Water Quality Conference, Eugene, 2000
Oregon Graduate Institute, Winter Seminar Series, 2001
Duke University Integrated Toxicology Program, Scholar Seminar Series, 2001
University of Wisconsin-Madison, Zoology Department, Spring Seminar Series, 2002
Behavioral Toxicology Society, National Meeting, Research Triangle Park, NC, 2002
Washington State University, Cooperative Extension Workshop, Pullman, 2002
Northwest Fisheries Science Center, Winter Seminar Series, Seattle, 2003

Popular Media:
“Pesticides enter salmon picture”, Seattle Post-Intelligencer, Jan. 30, 2001
“When it rains, it pours pollutants into the waters”, Seattle Post-Intelligencer, Nov. 20, 2002.

Competitive Research Grants Awarded:

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<td>Rapid phenotypic screening in zebrafish</td>
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<td>Sublethal effects of the carbamate insecticide, carbaryl, on coastal cutthroat trout in Willapa Bay, Washington</td>
<td>UFSWS, Environmental Contaminants Program</td>
<td>Jay Davis, USFWS, Western Washington Office</td>
<td>4 years, beginning FY02</td>
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<td>Effects of algal toxin exposure in early life history stages of fish</td>
<td>Ecology and Oceanography of Harmful Algal Blooms (ECOHAB)</td>
<td>Kathi Lefebvre and Vera Trainer, NWFSC</td>
<td>3 years, beginning FY02</td>
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<tr>
<td>Evaluating the effects of forestry herbicides on early life history stages of fish</td>
<td>U.S. Forest Service, Pesticide Impact Assessment Program</td>
<td>John Incardona, NWFSC</td>
<td>2 years, beginning FY03</td>
<td>$104,518</td>
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</tbody>
</table>
Refereed Publications:


Book Chapters and Other Publications


