Proceedings of the First Annual Northwest Fisheries Science Center Symposium "Looking to the Past to Envision the Future"

December 12–13, 2006
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Steering Committee:

Tom Good (co-chair; rapporteur)
Rohinee Paranpjye (co-chair; rapporteur)
Elizabeth Clarke
Jim Faulkner
Kerri Haught
Vicky Krikelas
Don Larsen
Michelle McClure
Karma Norman
Mark Scheuerell
Ashley Steel
Kevin Williamson

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Introduction

On 12-13 December 2006, the Northwest Fisheries Science Center (NWFSC or Center) sponsored the first Center-wide Science Symposium at the Musuem of History and Industry in Seattle, WA. The goals of the symposium were to foster communication and collaboration among scientists at the Montlake Laboratory and all NWFSC field stations as well as to showcase new and exciting areas of research.

Approximately 250 NWFSC scientists and staff attended the symposium. The two-day symposium consisted of a poster session and short oral presentations by NWFSC staff and scientists. Presentations also included overview talks from the five Division Directors for Conservation Biology (CB), Environmental Conservation (EC), Fish Ecology (FE), Fisheries Resources Analysis and Mointoring (FRAM), and Resource Enhancement and Utilization Technologies (REUT) divisions.

The overarching theme of the symposium, "Looking to the Past to Envision the Future", was selected in commemoration of the Montlake Laboratory's 75th anniversary celebration in 2006. Presentations occurred in a series of sessions highlighting the past, present and future of fisheries research. The presentations were grouped into the following sessions:

- Fisheries analysis
- Emerging technologies/ biotechnology
- Hatcheries and aquaculture
- Humans and the environment/ oceans and human health
- Organismal ecology and behavior
- Population and community dynamics and ecology

Symposium participants expressed an overwhelming appreciation for the information presented and opportunity to collaborate with their colleagues, and also supported conducting a biannual symposium in the future.

Agenda

Day 1: Tuesday, December 12

8:00 am – 8:15 am Welcome (Usha Varanasi)

8:15 am – 8:40 am **REUT Overview** (Walton Dickhoff)

Humans and the Environment/Oceans and Human Health

8:40 am – 9:05 am "From Chemical Warfare, Spies and Drugs, and the Tropics to the Cold North Pacific: A History of Marine Biotoxin Research at the NWFSC" (John Wekell)

9:05 am – 9:30 am "Big Picture, Smaller Snapshot: an Overview and Example of Social Science Research at the NWFSC" (Karma Norman)

9:30 am – 9:55 am "Successful Economic Surveys for Commercial Fisheries Management" (Carl Lian)

9:55 am – 10:10 am **-Break- Refreshments in the MOHAI Auditorium Foyer**

10:10 am – 10:35 am **Conservation Biology Overview (**Mike Ford)

Population and Community Dynamics and Ecology Part I

10:35 am – 11:00 am "Marine Growth Rate of Juvenile Salmon and its Relationship to Adult Return" (Brian Beckman)

11:00 am - 11:25 am "The Columbia River Estuary: Implications for Recent Research for Salmon Recovery" (Kurt Fresh)

11:25 am – 11:50 am "Teasing Demographic Rates out of Abundance Data: Age-Structured Modeling Provides Evidence for a 30-year-old birth rate decline in Stellar Sea Lions" (Eli Holmes)

11:50 am – 12:50 pm Lunch

Population and Community Dynamics and Ecology Part II

12:50 pm -1:15 pm "Linking Models: a Framework for Evaluating Salmon Recovery Strategies in a Puget Sound Watershed" (Krista Bartz)

1:15 pm – 1:40 pm "Salmon Population Viability in an Uncertain Future: Prognosticating Impacts of Climate and Hydropower" (Rich Zabel)

1:40 pm – 2:05 pm "Pacific Salmon Extinctions: Quantifying Lost and Remaining Diversity" (Rick Gustafson)

2:05 pm – 2:30 pm **"**Recolonization of the Cedar River, WA by Pacific Salmon: Genetic to Ecosystem-Level Effects"
(Peter Kiffney)

2:30 pm – 2:45 pm **-Break- Refreshments in the MOHAI Auditorium Foyer**

Fisheries Analysis

2:45 pm – 3:10 pm "Monitoring Catch in the West Coast Commercial Groundfish Fleets: The Key to Bycatch Management" (Jonathan Cusick)

3:10 pm – 3:35 pm "The Perils of Point Estimates: Effective Scientific Presentation in the Face of Uncertainty and Human Psychology" (Ian Stewart)

3:35 pm – 4:00 pm "Looking to the Future -- A Report from the Research Planning Team" (Michelle McClure)

Day 2: Wednesday, December 13

8:00 am – 8:25 am **Fish Ecology Overview (**John W. Ferguson)

Hatcheries and Aquaculture
8:25 am – 8:50 am "Conservation Hatchery Effects on Steelhead in Hood Canal" (Barry Berejikian)

8:50 am – 9:15 am "Effects of Hatchery Rearing on Behavioral Diversity – Some Tests with Rockfish and Steelhead" (Jonathan Lee)

9:15 am – 9:40 am "Multiple facets of a Salmon Bacterial Disease" (Linda Rhodes**)**

9:40 am – 9:55 am **-Break- Refreshments in the MOHAI Auditorium Foyer**

9:55 am – 10:20 am **Fishery Resource Analysis & Monitoring Overview** (Elizabeth Clarke)

Emerging Technologies/Biotechnology
10:20 am – 10:45 am "Molecular Approaches for Understanding Oil Spill Impacts on Nearshore Spawning Fish" (Heather Day**)**

10:45 am – 11:10 am "Assessing Alaskan Killer Whale Feeding Ecology Using Chemical Tracers" (Doug Burrows)

11:10 am -11:35 am **Environmental Conservation Overview** (Tracy Collier)

11:35 am – 1:35 pm **Lunch and Poster Session ****

Organismal Ecology and Behavior
1:35 pm – 2:00 pm "A History of Telemetry Technology Development in the Columbia River Basin and its Use in Evaluating Fish Passage Behavior and Survival" (Eric Hockersmith)

2:00 pm – 2:25 pm "Using Parasites to Study Marine Populations and Ecosystems: an Old Tool Brought to the Future" (Kym Corporon Jacobson)

2:25 pm – 2:50 pm "Using Molecular Genetic Techniques to Address Key Questions for Southern Resident Killer Whale Conservation" (Brad Hanson)

2:50 pm – 3:15 pm "Beyond Birth and Death: the Potential Value of Behavioral Ecology to Ecosystem Management"
(Phil Levin)

*** Speakers are allotted 25 minutes for presentations including time for questions; moderators will keep the overall program on schedule by enforcing time limits.**

**** Posters will be available for viewing from 8 am to 5 pm on Tuesday, Dec. 12,
and from 8 am to 3:15 pm on Wednesday, Dec. 13 in the McCurdy Gallery. Poster presenters will accompany their posters during the 11:35 am – 1:35 pm poster session on Dec. 13.**

Oral Presentations

From chemical warfare, spies, and drugs, and the tropics to the cold north Pacific: A history of marine biotoxin research at the NWFSC

John C. Wekell
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
john.c.wekell@noaa.gov

Work on marine biotoxins here at the Center goes back to the late 1940s by the old Seattle Technological Laboratory. Work then centered on Paralytic Shellfish Poisoning (PSP) and means to reduce or eliminate the toxins from processed shellfish, *e.g.*, through canning. Some of this early work was done in conjunction with a sister laboratory/field station in Ketchikan, AK. In the late 1940s and 1950s, the Ketchikan laboratory provided assistance and support to accumulate PSP toxins for the Chemical Warfare Corp of the U.S. Army in Fort Detrick, MD. This accumulated toxin (which has a story in itself worthy of a "spy novel" since it involved the CIA), consisting mostly of what is now known as saxitoxin, became the standard that is currently used by regulatory labs in the United States. Biotoxin work in Seattle went into hibernation until the late 1960s when we got involved in Ciguatera fish poisoning in Caribbean. This program was a joint effort with the then Bureau of Commercial Fisheries and the Food and Agriculture Organization (FAO). Research on marine biotoxins evolved in the 1970s, to finding useful and potent compounds that might have pharmaceutical value ("Drugs from the Sea"). Biotoxin and marine natural product research went into a hiatus during the late 70s and to the late 80s. Marine Biotoxin research was restarted in the early 1990s when we got Seafood Safety funds. An outbreak of domoic acid poisoning in Monterey Bay shifted our focus away from PSP to domoic acid. At the beginning of the 90s, we focused on analytical methodology and surveillance of domoic acid but later turned to causes of the blooms of *Pseudo-nitzschia* that produced this toxin. Grants from National Center for Coastal Ocean Sciences for monitoring and surveillance and another grant from ECOHAB have now given the program a far better understanding on origins of this noxious diatom.

Big picture, smaller snapshot: An overview and example of social science research at the NWFSC

Karma Norman and Suzanne Russell
Northwest Fisheries Science Center
Socioeconomics Program, Science Division
Seattle, Washington
karma.norman@noaa.gov

Jennifer Sepez and Ron Felthoven
Alaska Fisheries Science Center
Economic and Social Sciences Research Program
Seattle, Washington

At the Northwest Fisheries Science Center (NWFSC), an emergent research focus is on the human dimensions – the people and their institutions and activities – that are a central component of the marine and inland ecosystems of the U.S. west coast. The NWFSC first developed its capacity for economic research by bringing economists into the center and, in recent years, has added both economists and scientists trained in other social science disciplines. Social science research occurs within two groups: 1) economic research supporting the scientific and management information goals of the Fishery Resources Analysis and Monitoring (FRAM) division and 2) social science research that supports scientific and management work for all species within the NWFSC purview. The research example described here elaborates on the approach and conclusions of a single socioeconomic research project – the large-scale rank ordering and profiling of west coast communities methodologically defined as 'dependent on' or 'engaged in' the fisheries of the Pacific Fishery and North Pacific Fishery management regions. Because of the overlap between the communities and fisheries of the Pacific and North Pacific management regions, this research involved collaboration with social scientists at the Alaska Fisheries Science Center. The particular project described also brings together fisheries, economic and demographic data and, as such, represents an apt example of both baseline socioeconomic research and its utility in terms of Pacific fishery management.

Problem

Written into the act governing U.S. fisheries regulation is a reference to communities 'dependent on' or 'engaged in' fishing. However, the Magnuson-Stevens Act's definition of "fishing community" does little to guide social scientists in deciding which among thousands of potential places are fishing communities and which are not. According to the federal register, a fishing community is "a community which is substantially dependent on or substantially engaged in the harvest or processing of fishery resources to meet social and economic needs, and includes fishing vessel owners, operators, and crew and United States fish processors that are based in such community" (16 U.S.C. Ch. 38(I) §1802 3 (16)). Due to these legal mandates, in addition to the broad research goals aimed at placing human communities into the marine ecosystem context, the National Marine Fisheries Service seeks to methodologically illustrate fishing 'dependence' and 'engagement,' and to profile social and economic linkages to fishing at the community level. Scientific consensus on a standard methodological approach to fishing

community definition and profiling has thus far been absent, although multiple approaches have been used (Acheson, et al. 1980; Gilden 1999; Hall-Arber, et al. 2001). For this reason, our project sought a methodology that organized North Pacific and Pacific West Coast fishing data by community, ordered these communities according to level of 'dependence' on and 'engagement' in fishing, and allowed for a standardized profile of each of the most fishing-dependent and fishing-engaged communities.

Methods

The communities were ultimately selected through a process that assessed involvement in commercial fisheries using quantitative data from the year 2000, in order to coordinate with demographic data from the year 2000 U.S. Census. The quantitative indicators were associated with west coast communities that have commercial fisheries landings (indicators: weight and value of landings, number of unique vessels delivering fish to a community) and communities that are home to documented participants in the fisheries (indicators: state and federal permit holders and vessel owners).

Table 1. Fisheries involvement indicators used as "outputs" in the model of dependence, with input values equal to each community's population in 2000.

Type of Indicator	Specific Indicator
Landings	Weight in metric tons of West Coast fish landed in the community.
	Metric tons of North Pacific fish landed in the community.
	Value in U.S. dollars of West Coast fish landed in the community.
	Value in U.S. dollars of North Pacific fish landed in the community.
Vessels Delivering	Number of unique vessels making deliveries to the community as their primary port for landings that were involved in West Coast fisheries.
	Number of unique vessels making deliveries to the community as their primary port for landings that were involved in North Pacific fisheries.
Permits	Number West Coast fisheries permits registered to community residents.
	Number North Pacific fisheries permits registered to community residents.
	Number of individuals holding federal West Coast fisheries permits.
	Number of individuals holding federal North Pacific fisheries permits.
	Number of North Pacific halibut individual fishing quotas (IFQs) registered to community residents.
	Number of North Pacific sablefish IFQs registered to community residents.
	Number of individuals holding state permits for West Coast fisheries.
	Number of individuals holding state permits for North Pacific fisheries.
Vessel Owners	Number of vessels owned by community residents involved in West Coast fisheries.
	Number of vessels owned by community residents involved in North Pacific fisheries.

Once we had gathered the data that could be incorporated into our selected indicators, indicators were assessed in two ways: once as a ratio to the community's population, and in another approach, as a ratio of involvement within a particular fishery. The two different ratios allowed for a methodological definition of the fishing *dependence* and fishing *engagement* concepts described earlier in the references to the legal language governing communities research. In order to compare across all communities and across all indicators, and in order to satisfy the dual

ratio comparison criterion, the research team selected a Data Envelopment Analysis (hereafter, DEA) approach to the data.

DEA produces an efficient frontier representing the greatest level of involvement in fishing based on multiple quantitative indicators. Proximity to that frontier therefore presents a means of comparing each community to the most heavily involved communities -- see Figure 1 below, which displays a frontier in two dimensions. Communities that lie along, or close to, the frontier have demonstrated strong participation according to the indicators. Communities that lie distant from the frontier have demonstrated relatively weaker participation. Distance to the n-dimensional frontier becomes a uniform measure across indicators that we can use to assess all participation. We used OnFront2 software to execute our model.

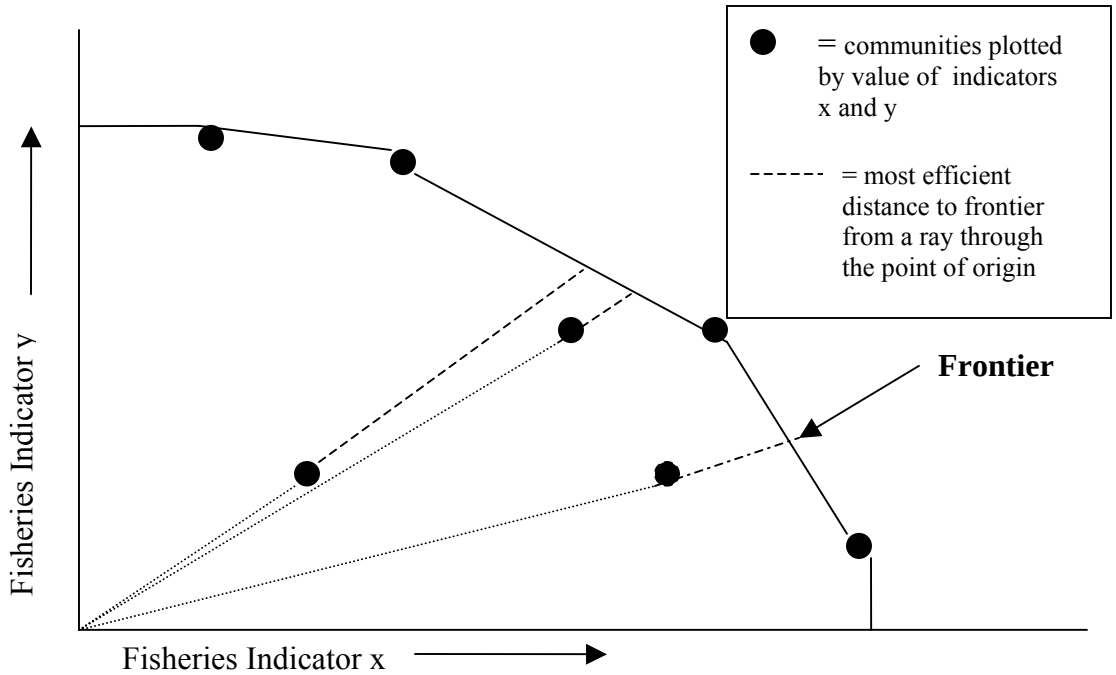

Figure 1. Graphic representation of the Data Envelopment Analysis (DEA) model fisheries involvement frontier for two dimensions.

Results

Six DEA scores were ultimately generated for each community (see Table 2). Communities were analyzed and ranked based upon their dependence scores for dependence on the fisheries of the North Pacific (NP), on the fisheries of the West Coast (WC), and on a combination of the two regions (NP & WC). Likewise, communities were examined according to their engagement scores in the specific fisheries of the NP, the WC, and a combination of the two, and were ranked accordingly.

Table 2. Examples of each DEA score type for five communities (selected for geographic breadth and score variations).

City	North Pacific (NP) Dependence	West Coast (WC) Dependence	WC NP Combined Dependence	North Pacific (NP) Engagement	West Coast (WC) Engagement	WC NP Combined Engagement
Eureka, CA	0.0064 (rank: 573)	0.6993 (rank: 31)	0.6993 (rank: 31)	0.0091 (rank: 456)	0.9009 (rank: 22)	0.9009 (rank: 30)
Ferndale, WA	0.0412 (rank: 256)	0.1608 (rank: 110)	0.1927 (rank: 104)	N/A	0.3367 (rank: 55)	0.0050 (rank: 88)
Port Orford, OR	0.0055 (rank: 609)	1.0000 (rank: 7)	1.0000 (rank: 13)	0.0075 (rank: 523)	1.0000 (rank: 16)	1.0000 (rank: 10)
Seattle, WA	1.0000 (rank: 23)	1.0000 (rank: 19)	1.0000 (rank: 17)	1.0000 (rank: 7)	1.0000 (rank: 4)	1.0000 (rank: 1)
Seaford, VA	0.0060 (rank: 582)	N/A	0.0060 (rank: 1011)	1.0000 (rank: 1)	N/A	1.0000 (rank: 4)

Communities with DEA scores at least one standard deviation above the mean were selected for more detailed profiling. Additionally, communities could be ranked according to their DEA scores. Once the ranked lists for each DEA score type were combined, repeated appearances by certain communities were recognized. The complete list was then reduced to a list of only those communities which scored at least one standard deviation above the mean for one or more score types, and we were left with 125 communities potentially dependent upon and/or engaged in the fisheries of the North Pacific and Pacific West Coast, in rank order. The 125 selected communities were then profiled in detail in a document to be released as a 2007 NOAA Technical Memorandum entitled *Community Profiles for West Coast and North Pacific Fisheries—Washington, Oregon, California, and other U.S. States*.

The profiles are given in a narrative format that includes four sections: *People and Place, Infrastructure, Involvement in West Coast Fisheries,* and *Involvement in North Pacific Fisheries*. *People and Place* includes information on location, demographics (including age and gender structure of the population, racial and ethnic make up), education, housing, and local history. *Infrastructure* covers current economic activity, governance (including city classification, taxation, and proximity to fisheries management and immigration offices) and facilities (transportation options and connectivity, water, waste, electricity, schools, police, public accommodations, and ports). *Involvement in West Coast Fisheries* and *Involvement in North Pacific Fisheries* detail community activities in the fisheries of these two regions, utilizing the fisheries data accumulated and organized in the community selection process.

Discussion

DEA modeling can be applied in regions where there is very little quantitative fisheries data, and regions where there is a great deal of data, though the advantage in regions with substantial data is obvious. Having many different indicators enabled us to explore the multi-dimensionality of

community involvement in commercial fisheries, instead of limiting us to one prominent dimension, such as landings or total number of fishermen.

The resultant document profiles 125 communities linked to fishing in Washington, Oregon, California, and other U.S. states, with basic information on social and economic characteristics. Various federal statutes, including the Magnuson-Stevens Fishery Conservation and Management Act and the National Environmental Policy Act, among others, require federal agencies to examine the social and economic impacts of policies and regulations. These profiles can serve as a consolidated source of baseline information for assessing community impacts in these states.

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Successful economic surveys for commercial fisheries management

Carl Lian
Northwest Fisheries Science Center
Fisheries Resource Analysis and Monitoring Division
Seattle, Washington
carl.lian@noaa.gov

The Magnuson Act requires fisheries managers to consider the economic, social, and biological impacts of regulatory measures. A lack of economic data has placed severe constraints on our ability to conduct economic analyses of West Coast commercial fisheries. Low response rates to previous economic surveys of commercial vessel owners and processors have been a key reason for this lack of economic data. A recent data collect effort has been much more successful. This presentation describes the design of the survey and discusses which factors have produced a high survey response rates to date. The FRAM Economics Team has led the implementation of a program to collect needed economic data on the federally managed groundfish and salmon fisheries. This data will be used in research addressing important economic performance measures such as profitability, production efficiency, regional economic impacts, and bioeconomic models. The first step in this program has been the development and implementation of an economic survey of the limited entry fleet. The presentation will also provide a preliminary analysis of survey data.

The Columbia River estuary: Implications of recent research for salmon recovery

Kurt L. Fresh and Ed Casillas
Northwest Fisheries Science Center
Fish Ecology Division
Seattle, Washington
kurt.fresh@noaa.gov

Dan Bottom
Hatfield Marine Science Center
Fish Ecology Division
Newport, Oregon

Charles Simenstad
University of Washington
School of Aquatic and Fisheries Sciences
Seattle, Washington

This presentation summarizes recent research on juvenile salmon use of estuaries in the Pacific Northwest, focusing on the Columbia River estuary and Chinook salmon. Collectively, this work demonstrates that juvenile salmon use of estuaries is directly linked to population viability and resilience. Use of estuarine habitats varies with estuary, species, population, life history strategy, and size of fish. In the Columbia River, juvenile Chinook salmon are present year round with individual residence times ranging up to 140 days. Fish size and occupation of tidal wetland systems are related, with the smallest size classes of juvenile Chinook salmon (< 90mm) most abundant in these wetland habitats. Larger size classes of Chinook salmon (e.g., yearlings) also benefit indirectly from wetland habitats as a result of food web linkages because the food sources of most of the primary prey of juvenile Chinook salmon are largely plant material from shallow water areas (e.g., benthic algae and wetland plants). Construction of dikes and levees and altered flows has eliminated a large amount of the shallow wetland habitats important to both yearlings and sub-yearlings. The distribution of Chinook salmon life history strategies available to use the estuary has also been significantly altered. However, research in the Salmon River (OR) estuary demonstrates that habitat restoration can help recover life history diversity. In conclusion, to help recover salmon and steelhead, connectivity of the entire habitat landscape (spawning grounds to ocean rearing areas) must be reestablished, of which the estuary is an important part.

Marine growth rate of juvenile salmon and its relationship to adult return

Brian Beckman and Brad Gadberry
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington
brian.beckman@noaa.gov

Cheryl Morgan
Hatfield Marine Science Center
Cooperative Institute for Marine Resources Studies
Newport, Oregon

Kathleen Cooper and Paul Parkins
University of Washington
School of Aquatic and Fishery Sciences
Seattle, Washington

Joe Fisher
Oregon State University
College of Oceanic and Atmospheric Sciences
Corvallis, Oregon

Growth is an important ecological trait and there is a great deal of interest in developing markers for growth rate. A good candidate for an index of growth rate is insulin-like growth factor-I (IGF-I) - a peptide hormone that directly stimulates cell division and growth. We have examined the relationship between plasma IGF-I and body growth of individually tagged coho salmon in several laboratory experiments. Overall, a significant and biologically relevant linear relationship between individual growth and plasma IGF-I in juvenile salmon was found. Food supply (nutrition), growth, and plasma IGF-I levels were clearly linked in these experiments. In addition, growth - IGF-I relations were similar for fish maintained under differing temperature regimes, suggesting that IGF-I may be used as a growth index for salmon under environmentally relevant conditions. Over the past several years we have monitored plasma IGF I levels in post-smolt coho salmon collected during a series of research cruises off the Oregon-Washington coast. We found significant differences in mean June plasma IGF-I levels between years, suggesting that growth of salmon after ocean entry differs interannually (2000–2006). A significant relation was also found between plasma IGF-I levels and an index of relative food abundance (salmon prey biomass/salmon abundance). Furthermore, a positive relationship was found between mean June IGF-I level and survival of adult coho salmon from the Oregon Production Index in the following year. This suggests that inter-annual variation in ocean conditions results in altered growth rate of juvenile coho salmon and that these growth differences affect subsequent adult survival.

Teasing demographic rates out of abundance data: Age-structured modeling provides evidence for a 30-year birth rate decline in Stellar sea lions

E.E. Holmes
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington
eli.holmes@noaa.gov

Lowell W. Fritz
Alaska Fisheries Science Center
National Marine Mammal Laboratory
Seattle, Washington

Anne E. York
York Data Analysis
Seattle, Washington

Kathryn Sweeney
University of Washington
School of Aquatic and Fisheries Sciences
Seattle, Washington

Since the mid-1970s, the western stock of Stellar sea lion (*Eumetopias jubatus*) inhabiting Alaska waters from Prince William Sound west through the Aleutian Islands has declined by over 80%. Concurrently, other apex predators in the North Pacific Ocean also experienced large declines. In 1990, Stellar sea lions were listed as threatened under the U.S. Endangered Species Act, and in 1997, the western stock was uplisted to endangered. Over $120 million in federal funding was allocated between 2001 and 2004 for research on factors hypothesized to impact the population, but their complexity, indirectness and cumulative impact have made it difficult to associate abundance changes to specific impacts. In this study, we used age-structured models to analyze abundance and juvenile fraction data for Stellar sea lions in the central Gulf of Alaska and found strong support for a steady 30-year decline in birth rate concurrent with a steady increase in survivorship after a severe drop in the early 1980s. This result was robust to variations on the underlying Leslie matrices, including a generic matrix, and the model predictions concur with the limited available field data on survivorship, age-structure and fecundity. These results suggest that (1) direct mortality is currently low and is not the primary threats to recovery for the western Stellar sea lion, and (2) new research efforts should focus on the reproductive ecology and energy budgets of adult female Stellar sea lions.

Linking models: A framework for evaluating salmon recovery strategies in a Puget Sound watershed

Krista K. Bartz, Mark D. Scheuerell, James Battin, Mary H. Ruckelshaus,
Hiroo Imaki, Kerry M. Lagueux, and Timothy Beechie
Northwest Fisheries Science Center
Seattle, Washington

Ray Hilborn
University of Washington
School of Aquatic and Fishery Sciences
Seattle, Washington

Matthew W. Wiley, Richard N. Palmer, and Elizabeth Korb
University of Washington
Department of Civil and Environmental Engineering
Seattle, Washington

Andrew D. Haas
Surface Water Management Division, Snohomish County
Public Works Department
Everett, Washington

Kit Rawson
The Tulalip Tribes of Washington
Tulalip, Washington

One of the most significant challenges associated with recovering imperiled species is identifying the set of actions needed to ensure species' persistence. Here we outline a spatially and temporally explicit framework for translating proposed restoration actions into projected improvements in four population parameters: abundance, productivity, spatial structure, and life history diversity. We applied this framework iteratively to a threatened population of Chinook salmon (*Oncorhynchus tshawytscha*) in the Snohomish basin of Puget Sound. For the first iteration, we used statistical models to relate restoration actions to salmon productivity and capacity, which served as inputs to a multi-stage Beverton-Holt model along with hatchery and harvest variables. For the second iteration, we incorporated potential effects of climate change by replacing some of the statistical models with mechanistic climate and hydrology models. Results from this second iteration suggest that climate change will lead to increased peak stream flows during winter, decreased base stream flows during summer, and increased water temperatures throughout the year, particularly in high-elevation subbasins. These impacts to stream flows and temperatures are likely to be detrimental to salmon survival and reproduction. Model projections suggest that climate change may cause Chinook population declines of 20% to 40%. Planned restoration actions are predicted to lessen these declines, but meeting population recovery targets is likely to be more difficult when climate change is taken into account.

Methods

Four suites of restoration actions (hereafter called "land-use scenarios") were developed by a local scientific advisory group prior to our analysis (SBSRTC 2004). For the purposes of this presentation, the scenarios will be labeled minimum restoration, moderate restoration, maximum restoration, and historical (Table 1). They depicted the Snohomish basin landscape in terms of target levels for eight characteristics (i.e., road density, impervious cover, riparian forest cover, total forest cover, anthropogenic barriers, edge habitat, off-channel habitat, and habitat structures). We quantitatively linked these land-use scenarios to salmon viability parameters via an iterative analysis based on empirical data. We used the 62 subbasins that comprise the Snohomish basin as our unit of analysis.

Table 1. Brief summary of the four land-use scenarios.

Scenario	Description
Minimum	Assumed current rates of habitat protection, restoration, and degradation continue for the next 25 years
Maximum	Assumed the implementation of protection and restoration actions that are hypothesized to achieve recovered status for Chinook in the basin
Moderate	Calculated target levels using the following equation: $\text{target}_{mod} = ((\text{target}_{max} - \text{target}_{min}) \cdot 0.75) + \text{target}_{min}$
Historical	Assigned target levels so as to reproduce conditions prior to European settlement (~1850)

Detailed methods for the first iteration of the analysis are provided in Bartz et al. 2006 and Scheuerell et al. 2006. In short, we estimated changes in the quality and quantity of salmon habitat using the target levels for each scenario (Fig. 1). Subsequently, habitat quality and quantity were related to salmon survival and capacity at various life stages. Survival and capacity, in turn, served as inputs to a life cycle model called Shiraz, capable of integrating the effects of habitat, harvest, and hatcheries on salmon populations.

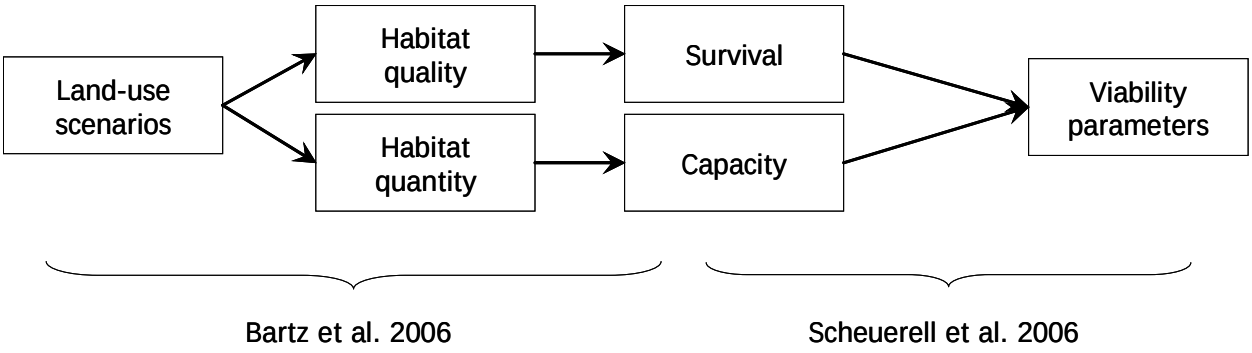

Figure 1. Conceptual diagram of the analytical steps taken in the first iteration of our analysis. Arrows represent models used to link variables.

Methods for the second iteration of the analysis are described in Battin et al. in press. Essentially, we used the outputs of two climate models (GFDLR30 and HadCM3), and land

cover data based on the scenarios, as inputs to a hydrology model (DHSVM; Fig. 2). The hydrology model produced spatially explicit water temperatures and flows, which subsequently were linked to survival at various life stages through the same functional relationships used in the first iteration. Capacity was estimated as in the first iteration; then survival and capacity were entered into Shiraz.

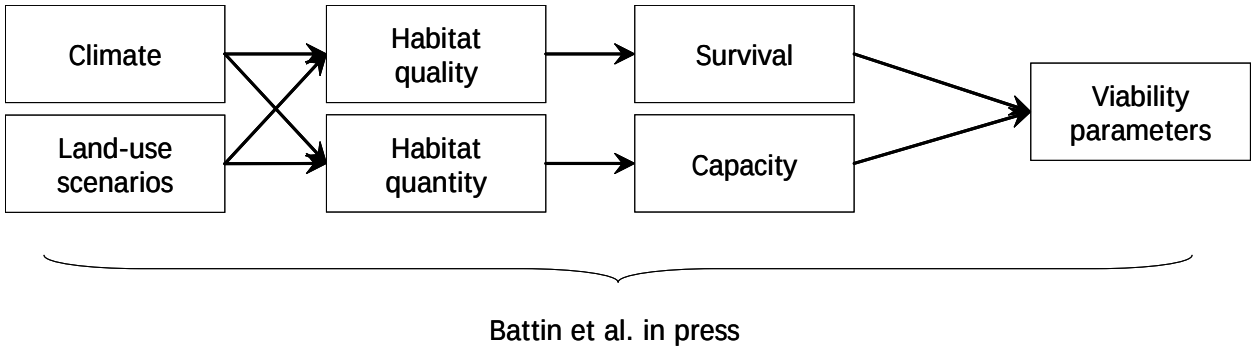

Figure 2. Conceptual diagram of the analytical steps taken in the second iteration of our analysis. As in Figure 1, arrows represent models used to link variables.

Results

The Shiraz model provided estimates of four criteria for describing viable salmon populations: abundance, productivity, spatial structure, and diversity. Here, we presented only abundance and spatial structure results under particular scenarios (minimum, maximum, and historical for the first iteration; minimum and moderate for the second iteration). For additional results, see Scheuerell et al. 2006 and Battin et al. in press.

First Iteration

The abundance and spatial distribution of wild spawners across the landscape under the minimum restoration scenario differed considerably from our best approximation of that under historical conditions (Figure 3). Overall, we estimated that the Snohomish basin would produce only 17% of the historical number of spawners if the minimum restoration scenario were implemented. Although only 34 of 62 subbasins supported spawners under this scenario, we estimated that 37 of them did historically. The six additional subbasins that supported spawners under minimum restoration, but not historically, resulted from an active management decision by WDFW to truck spawners above Sunset Falls, a naturally occurring anadromous barrier.

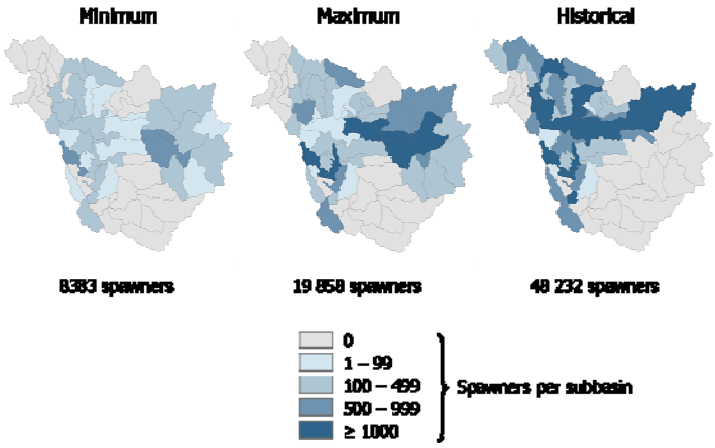

Figure 3. Maps of the spatial distribution of the predicted number of wild Chinook spawners under the minimum, maximum, and historical scenarios. An estimate of the equilibrium spawner abundance is listed beneath each map.

The abundance and spatial structure of spawners increased considerably under the maximum restoration scenario (Figure 3). We projected a 137% increase in abundance of wild fish, resulting in spawner levels that would be 41% of our historical estimate. Additionally, we found spawners expanding their range into a total of 36 subbasins, including those above Sunset Falls, as we assumed that the active management would continue as currently implemented.

Second Iteration

To isolate the effects of climate change on water temperatures and flows, we first applied the hydrologic model with current land cover held constant. Simulations based on both climate models projected basin-wide increases in incubation peak flows and pre-spawning temperatures and decreases in spawning flows (Figure 4). Differences between climate models in predicted precipitation and seasonal climate patterns caused large differences in resulting projections of water temperature and flow variables. Modeled climate effects on both stream flow variables were greatest in the more easterly, higher-elevation subbasins where the effects of warmer winter temperatures on snow accumulation were expected to be most pronounced.

To assess the effects of climate change and habitat restoration on Chinook salmon, we ran the Shiraz model with the water temperature and flow variables derived from the hydrologic model in conjunction with capacity estimates for each land-use scenario. All simulations based on the GFDL climate model produced declines in salmon populations (Fig. 5), although the moderate restoration scenario limited declines to 5%. In contrast, the scenarios based on the HadCM3 model projected an increase in mean salmon abundance of 19% under moderate restoration (Fig. 5), despite the negative impacts of climate change. Under both climate models, minimum restoration failed to balance the effects of climate change by 2050. Model projections indicated that climate change may cause Chinook population declines of 20% to 40% in the absence of restoration (i.e., current land-use; data not shown).

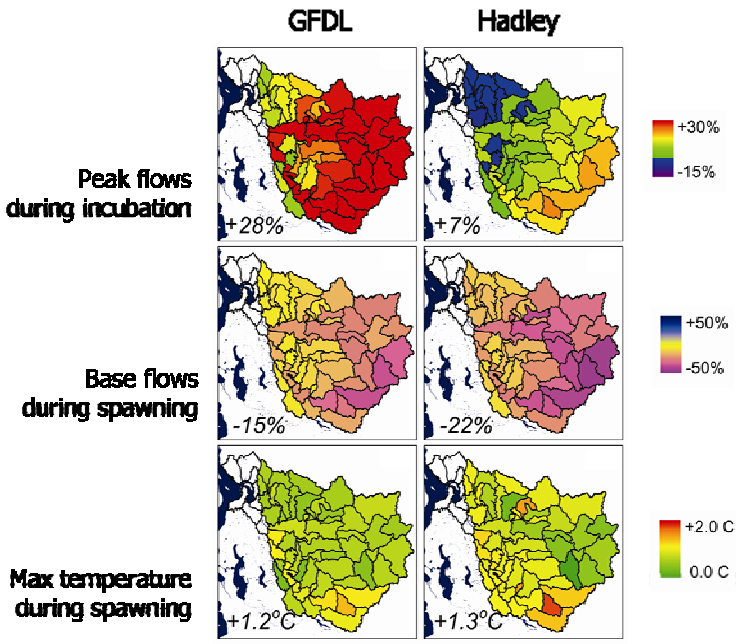

Figure 4. Climate impacts on three hydrologic variables using two climate models. The number in the lower left corner of each panel indicates the basin-wide average impact.

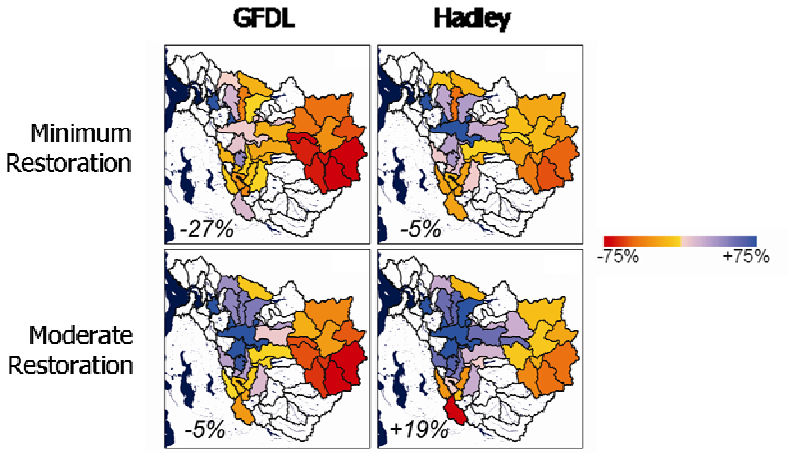

Figure 5. Predicted changes in the spatial distribution of wild Chinook spawners by 2050 under two climate models and two land-use scenarios. The number in the lower left corner of each panel indicates the basin-wide total change in spawning Chinook.

Model results suggest that, because climate impacts on hydrologic variables affect the highest-elevation areas of the Snohomish watershed disproportionately and restoration impacts are concentrated in lower-elevation basins, the combined effect of climate change and restoration will be to cause a shift in salmon distributions to lower elevations. The eastern-most subbasins, which drain high-elevation areas in the Cascade Mountains, exhibited the largest projected declines in salmon numbers by 2050—often in excess of 50%—regardless of land-use scenario (Fig. 5). In contrast, salmon abundance in lower-elevation sites was projected to show relatively modest declines or even to increase, especially under moderate restoration.

Discussion

One of the challenges associated with recovering imperiled species, such as Chinook salmon, is identifying a set of actions that will ensure species' persistence. By using a combination of empirical data and modeling efforts and exploring the outcomes of very different suites of actions, we were able to present the decision makers in the Snohomish basin with clear choices for possible restoration strategies.

Our results from the first iteration of the analysis indicated that the maximum restoration scenario would result in approximately 41% of the estimated historical spawner abundance. This population level would not reach the recovery goal of 80% of historical abundance adopted by the planning group (SBSRTC 2004). Nevertheless, we expected that we underestimated the potential response of salmon to restoration actions, with climate held constant. With the effects of climate change incorporated in the second iteration of our analysis, we found higher water temperatures, lower spawning flows, and, most importantly, increased magnitude of winter peak flows and consequent bed-scour, all of which are likely to increase salmon mortality in the Snohomish basin and in hydrologically similar watersheds throughout the region. The resulting downward pressure on salmon populations is liable to make the attainment of recovery goals more difficult. Therefore, explicit consideration of future climate conditions may improve the long-term effectiveness of restoration planning and modeling for salmon throughout their range.

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Salmon population viability in an uncertain future: Prognosticating impacts of climate and hydropower

Rich Zabel, James Faulkner, Steven Smith, Mark Scheuerell, Lisa Crozier, Michelle McClure, John Williams, and Milhouse Van Houten
Northwest Fisheries Science Center
Fish Ecology Division
Seattle, Washington
rich.zabel@noaa.gov

Population Viability Analysis (PVA) based on stage-structured life-cycle models is a powerful tool for assessing the impacts of changes in specific survival rates on future population viability. We developed stochastic life-cycle models for populations of Snake River spring/summer Chinook salmon that contain density-dependent recruitment and climate relationships in freshwater and the ocean. We used these models as a framework to ask how changes in climate and in hydrosystem operations and configuration will affect our ability to recover these threatened populations. We found that future ocean conditions will play a huge role in population viability. Also, the role of freshwater climate will become increasingly important due to deteriorating hydrological conditions related to decreasing snow pack. To address hydropower impacts, we, along with scientists throughout the region, developed the Comprehensive Passage (COMPASS) model that predicts juvenile survival through the hydrosystem in relation to alternative scenarios of hydrosystem operation. The COMPASS model also characterizes latent effects related to hydrosystem passage based on several alternative hypotheses. The magnitude of this latent mortality largely determines the efficacy of hydrosystem related mitigation actions for recovering Snake River salmonid populations.

Pacific salmon extinctions: Quantifying lost and remaining diversity

Rick Gustafson, Jim Myers, Laurie Weitkamp, Orlay Johnson, and Jeff Hard
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington

Robin Waples
Northwest Fisheries Science Center
Seattle, Washington

Greg Bryant
Southwest Fisheries Science Center
Protected Resources Division
Arcata, California

Our objectives in this paper were to systematically enumerate historical Pacific salmon populations and characterize lost ecological, life-history, and genetic diversity types among six species of Pacific salmon in the western contiguous United States. In the present context, we used the term "Pacific salmon" to refer to Chinook salmon *Oncorhynchus tshawytscha* (Walbaum), sockeye salmon *O. nerka* (Walbaum), coho salmon *O. kisutch* (Walbaum), chum salmon *O. keta* (Walbaum), and pink salmon *O. gorbuscha* (Walbaum), as well as the anadromous form of rainbow trout, commonly known as steelhead *O. mykiss* (Walbaum). Although previous investigators [e.g., Nehlsen et al. (1991)] have produced partial lists of extinct stocks of Pacific salmon in the western contiguous United States, these studies did not define "stocks" or "populations" in a consistent way and did not attempt to quantify the magnitude of lost biodiversity associated with these extinctions. Here, we used a standardized population definition (McElhany et al. 2001) to identify both extant and extinct Pacific salmon populations and estimated population losses by region and species since substantial Euro-American contact (approximately AD 1800 in the Pacific Northwest and AD 1770 in California). In addition, we characterized each population following the hierarchical matrix of salmon diversity first proposed by Waples et al. (2001) and assessed the magnitude of lost biodiversity in the form of major levels of ecological, life-history, and genetic diversity.

Material and Methods

We organized historical populations of Pacific salmon within ecological regions that encompassed parts of Washington, Oregon, Idaho, Nevada, and California and portions of the Canadian Province of British Columbia that share transboundary ecological regions with U.S. salmon populations (Fig. 1). We incorporated historical populations identified by technical recovery teams (TRTs) into our analysis, which accounted for 36% of our total population list. In areas where the TRTs have not identify populations we used five general criteria to identify extinct populations: (1) documented historical presence, (2) basin area (km^2) and structure (e.g., barrier waterfalls), (3) environmental characteristics, (4) temporal isolation (different run or spawn timing), and (5) distance between potential spawning aggregates (e.g., high-elevation

refuges for adult stream-maturing fish). To identify extant populations we used the above criteria as well as known genetic attributes, phenotypic characteristics, dispersal distance and rates, and population dynamics and size. In areas where salmon no longer occur, we first examined primary (e.g., archaeological reports and accounts of early explorers, surveyors,

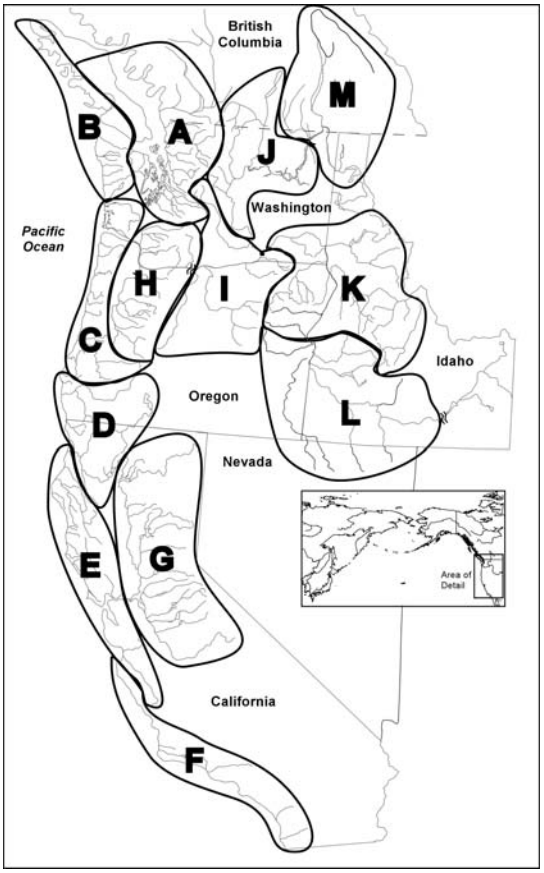

Figure 1. Map of the study area showing level-I ecological provinces (uppercase letter designations as presented in Table 1) that historically supported spawning populations of Pacific salmon.

fur trappers, missionaries, and settlers) and secondary (agency fisheries reports, journal articles, and ethnographic reports) sources to identify historical distributions of Pacific salmon in the Pacific Northwest and California. We then analyzed these watersheds for potential population isolating mechanisms such as seasonal or complete migration barriers, ecologically distinctive tributary habitats, and distance between potential spawning aggregates.

We classified populations as extinct or extirpated if the population no longer occurs in its historical habitat, the population has been replaced by a nonindigenous population, or the anadromous component of the population no longer exists, even if a potential remnant gene pool of resident fish still survives. We used criteria explained in detail in Waples et al. (2001) to assign each population to three hierarchical levels (I, II, III) of ecological, life-history, and genetic diversity, with level-I representing major groups. Here, we limited our analyses to level-I diversity categories, with one exception: both Chinook salmon and steelhead have two adult

maturation strategies (designated as stream- and ocean-maturing in reference to the location where the final maturation of adults takes place). Because the stream-maturing life-history strategy makes these populations much more vulnerable to anthropogenic threats, we compiled separate extinction data for the two types.

Results and Discussion

Descriptions of the hierarchical diversity characteristics, a list of biogeographical sources, and a detailed list of extant and extinct populations and their hierarchical diversity assignments are online at: http://www.nwfsc.noaa.gov/publications/displayallinfo.cfm?docmetadataid=6570.

Nearly 1,400 Pacific salmon populations historically occurred in the study area, and an estimated 29% have gone extinct since substantial Euro-American contact (Table 1). Patterns of population extinction within the 13 major ecological regions in our study area were strongly biased geographically (Table 1; Fig. 2). Each species has been extirpated from a minimum of two, and a maximum of five, major ecological regions; overall, the six species no longer occur in about one-third of the regions they once occupied (Table 1; Fig. 2).

Table 1. Estimated number of extant and (extinct) Pacific salmon populations within each species and region and in the entire study area..

	Populations extant (extinct)														
Ecological Region	**Steelhead**		**Chinook**		**Sockeye**		**Coho**		**Chum**		**Pink**		**Total**		**Ext. (%)**
Georgia Basin	69	(2)	40	(15)	14	(6)	50	(0)	56	(3)	36	(6)	265	(32)	11
Coastal Rainforest	84	(1)	32	(2)	21	(1)	23	(0)	14	(1)	6	(0)	180	(5)	3
Northern Coastal	43	(1)	37	(6)	-		24	(6)	15	(7)	-		119	(20)	14
Klamath Mountains Province	28	(3)	22	(6)	-		11	(1)	1	(1)	0	(1)	62	(12)	16
Northern California	34	(2)	10	(7)	-		15	(4)	0	(1)	0	(1)	59	(15)	20
Southern California	60	(28)	0	(2)	-		0	(3)	-		-		60	(33)	31
California Central Valley	40	(41)	19	(32)	-		0	(2)	0	(1)	0	(1)	59	(77)	57
Willamette/Lower Columbia River	24	(7)	23	(11)	-		12	(7)	3	(8)	-		62	(33)	35
Mid-Columbia	16	(4)	11	(9)	0	(5)	0	(10)	0	(1)	-		27	(29)	52
Upper Columbia	11	(8)	10	(15)	2	(5)	0	(10)	-		-		23	(38)	62
Lower Snake River	27	(4)	33	(18)	1	(6)	0	(7)	-		-		61	(35)	36
Upper Snake River	0	(23)	0	(25)	0	(3)	-		-		-		0	(51)	100
Columbia Headwaters	0	(7)	0	(11)	0	(8)	-		-		-		0	(26)	100
Total	436	(131)	237	(159)	38	(34)	135	(50)	89	(23)	42	(9)	977	(406)	
Extinct (%)		23		40		47		27		21		18	29		

The estimated proportion of extinct historical populations was relatively low in coastal drainages from Vancouver Island to northern California (≤ 20%), but increased dramatically in southern California (31%), the California Central Valley (57%), and the interior Columbia River Basin (35–62% in areas still accessible to Pacific salmon) (Table 1). Paired *z*-test comparisons across the 13 ecological regions indicated that the Coastal Rainforest (area B) had a significantly lower proportion of population loss than any other region (12 paired *z*-tests, $z \geq 2.4$, $p < 0.004$). Overall, there was a highly significant difference in the proportion of population extinctions between combined data for coastal (areas A–F, 0.14 extinct) and interior (areas G–M, 0.55 extinct) regions ($z = 15.8$, $p < 0.001$). These patterns can be attributed to a number of causes including differences in regional distribution of species or maturation-types, regional differences in human activities (e.g., dam-building and land-use practices), and perhaps differing regional impacts of climate change. All historical anadromous populations were extirpated from the Upper Snake River (area L) and Columbia River Headwaters (area M) regions following installation of impassable dams.

Extinctions were also nonrandom with respect to species and major maturation types (Tables 1-2, Fig. 3). Coho salmon once occupied a range almost as large as that of Chinook salmon and steelhead, which historically occupied all ecological regions, but native coho salmon populations have disappeared from large portions of California and the Columbia River Basin (Table 1; Fig. 2). Coho salmon may be particularly at risk due to their lengthy (greater than one year) juvenile freshwater residence (where they are exposed o freshwater habitat alterations) and a nearly fixed three-year life cycle (providing less of a buffer against year-class failure than most other salmon species). Almost half of historical lacustrine sockeye salmon populations have been lost (Table 1, Fig. 3), no doubt a result of that species' almost exclusive dependence for juvenile rearing on lake habitats, which have often been blocked by impassable dams. At the other extreme, pink and chum salmon had relatively low levels of population extinction (18 and 21%, respectively; Table 1), which can be attributed to the fact that the majority of these historical populations occurred in northern coastal portions of our study area, where overall extinction rates were relatively low (Table 1; Fig. 2) and to these species' short juvenile residence in freshwater (<1–2 months). Stream-maturing steelhead had significantly more proportional population losses than ocean-maturing steelhead ($z = 3.0$, $p = 0.003$), and stream-maturing Chinook salmon had a significantly higher proportion of population losses than ocean-maturing Chinook salmon ($z = 6.6$, $p < 0.001$) (Table 2). Higher losses of stream-maturing populations are likely due to widespread loss of crucial high-elevation (generally >500 m) holding habitats and to their vulnerability during the prespawning holding period.

The loss of major genetic groups (27%) was nearly as high as extirpation from ecological regions (33%), but the loss of major life-history types was less extensive (an estimated 15%) and perhaps confined to two species (chum and coho salmon) (Table 3).

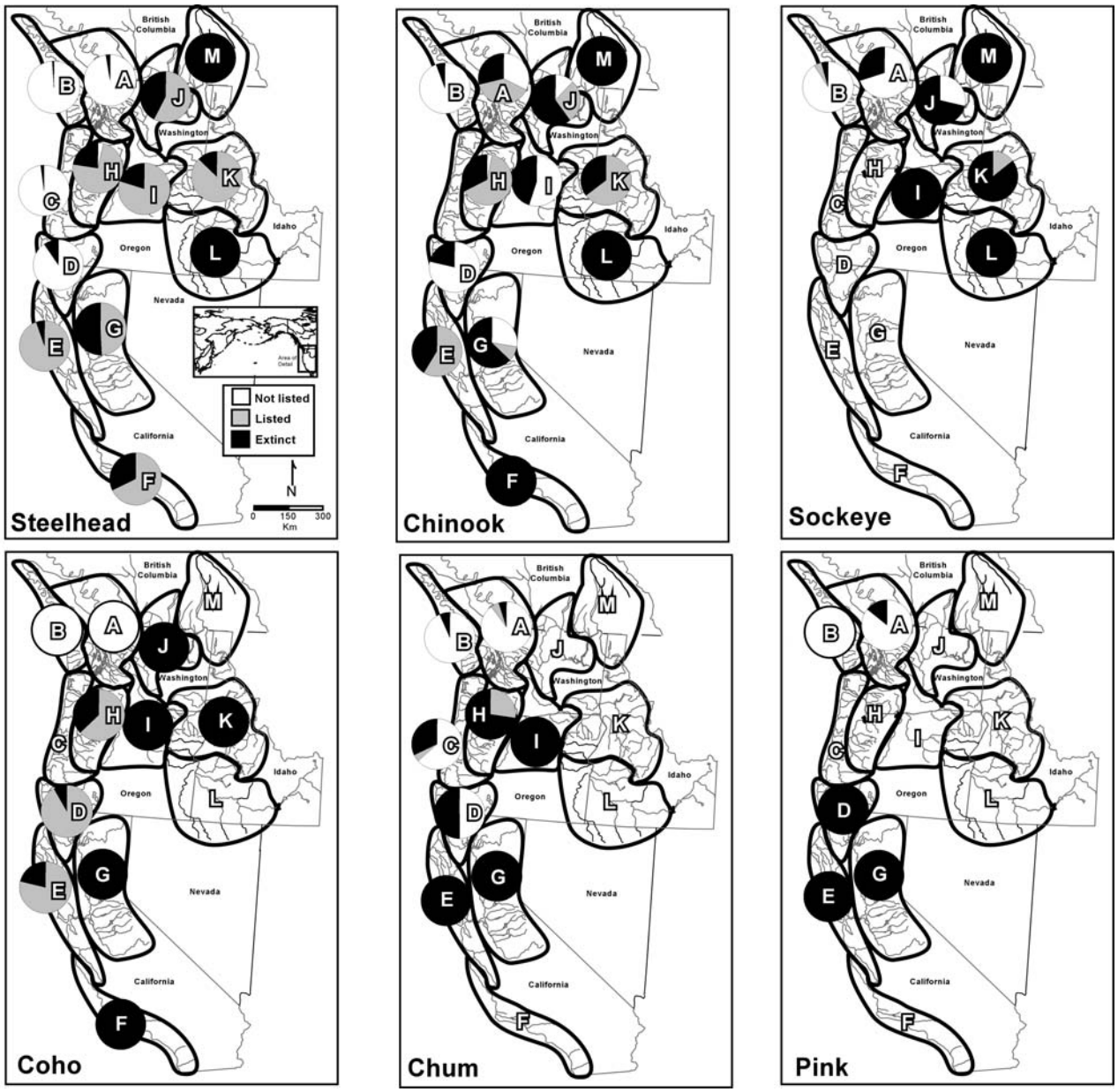

Figure 2. The relative proportion of populations of each species within each historically occupied ecological region (uppercase letter designations as presented in Table 1) that are extinct, extant but included in ESA-listed ESUs, or extant but not listed under the ESA.

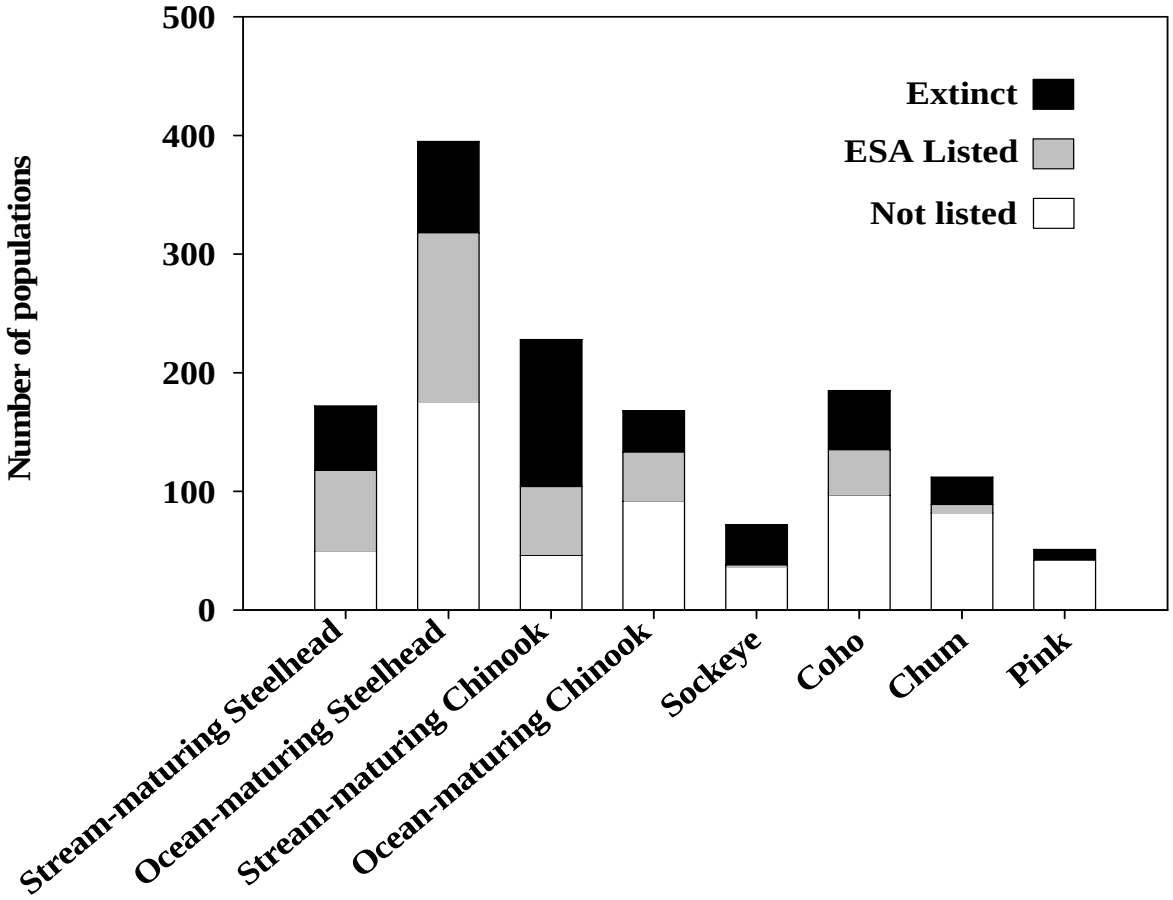

Figure 3. Total number of extinct and extant Pacific salmon populations in each species and major maturation type that are extinct, ESA-listed, or extant and unlisted.

Extinction of all coho and chum salmon populations in the interior Sacramento and Columbia River basins has resulted in the loss of a major life-history type for each species in both basins. Coho and chum salmon that spawned east of the Cascade Mountains in the Columbia River basin would have had an unusually extensive freshwater migration (longer than any other coho and chum salmon populations in the study area), and Sacramento River populations of either species would have had unique adaptations for survival in the Central Valley of California. Sockeye salmon accounted for more than 75% of the lost major genetic units of Pacific salmon (Table 3), which is likely a result of the discontinuous occurrence of lake habitats suitable for sockeye rearing, a resulting high degree of reproductive isolation that has led to strong genetic differentiation and local adaptations, and the proliferation of human-made dams that block anadromous access to most historical lake habitats.

Table 2. Estimated number of stream- and ocean-maturing steelhead and Chinook salmon populations extant and (extinct) in each region and in the entire study area.

	Steelhead				Chinook			
Ecological region	**Stream**		**Ocean**		**Stream**		**Ocean**	
Georgia Basin	12	(0)	57	(2)	12	(9)	28	(6)
Coastal Rainforest	14	(0)	70	(1)	8	(0)	24	(2)
Northern Coastal	4	(1)	39	(0)	12	(1)	25	(5)
Klamath Mountains Province	20	(3)	8	(0)	6	(6)	16	(0)
Northern California	8	(2)	26	(0)	0	(6)	10	(1)
Southern California	-		60	(28)	-		0	(2)
California Central Valley	-		40	(41)	4	(15)	15	(17)
Willamette and Lower Columbia rivers	6	(2)	18	(5)	8	(9)	15	(2)
Mid-Columbia River	16	(4)	-		11	(9)	-	
Upper Columbia river	11	(8)	-		10	(15)	-	
Lower Snake River	27	(4)	-		33	(18)	-	
Upper Snake River	0	(23)	-		0	(25)	-	
Columbia River Headwaters	0	(7)	-		0	(11)	-	
Total	118	(54)	318	(77)	104	(124)	133	(35)
Extinct (%)	31		19		54		21	

Table 3. Estimated number of extant and (extinct) major (level-I) diversity categories of Pacific salmon in the entire study area.

Species	**Ecology**		**Life-history**		**Genetics**	
Steelhead	11	(2)	7	(0)	7	(0)
Chinook salmon	10	(3)	7	(0)	10	(0)
Sockeye salmon	4	(3)	6	(0)	23	(13)
Coho salmon	6	(5)	1	(2)	2	(2)
Chum salmon	5	(3)	1	(2)	2	(1)
Pink salmon	2	(3)	1	(0)	2	(1)
Total	38	(19)	23	(4)	46	(17)
Extinct	33		15		27	

Conclusions

Our analyses indicated that Pacific salmon in this region retain substantial evolvability as demonstrated by the persistence of over two-thirds of historical populations (Table 1) and substantial levels of biodiversity (Table 3). However, high diversity losses in some geographic areas and the large fraction of remaining populations that are currently listed under the ESA (Fig. 3) are particularly troubling. Here, it is apparent that to preserve biodiversity at multiple scales in wild Pacific salmon, both the local population and its habitat (freshwater and marine) must become the basic unit of conservation.

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Recolonization of the Cedar River, Washington, by Pacific salmon: Genetic to ecosystem-level effects

Peter Kiffney and George Pess
Northwest Fisheries Science Center
Watershed Program, Environmental Conservation Division
Seattle, Washington
peter.kiffney@noaa.gov

Joseph Anderson and Tom Quinn
University of Washington
School of Aquatic and Fishery Sciences
Seattle, Washington

Removal of barriers is one of the primary methods to restore anadromous fish populations. Documenting the ecological consequences of barrier removal, however, is rarely done. The city of Seattle constructed a fish passage facility at the Landsburg diversion dam in 2003 to allow anadromous fish access to the upper Cedar River, Washington, and 27 km of relatively high quality habitat. This dam has blocked migration of anadromous fish for over 100 years. In 2000, we initiated a long-term study to document effects of salmon at different levels of ecological organization. These studies include reproductive success of salmon using genetic techniques; growth, survival and movement of trout and salmon populations; effects of juvenile salmon on resident fish communities; and effects of salmon carcasses on nutrient dynamics using stable isotopes. Between 2003 and 2005, 35 coho and 44 Chinook redds were identified: 81% were located within a 4 km reach immediately above the dam. To date, dispersal by juvenile coho has been a significant component of the overall colonization process, as they have dispersed into a major tributary of the Cedar River that was passed over by adult coho. We have no evidence that salmon carcasses have affected nutrient dynamics; however, N^{15} levels in fish and other organisms in locations historically accessible by salmon (before 1906) were higher compared to locations above a natural barrier suggesting a "salmon legacy."

Monitoring catch in the West Coast commercial groundfish fleets: The key to bycatch management

Jonathan Cusick, Janell Majewski, and Vanessa Tuttle
Northwest Fisheries Science Center
Fishery Resource Analysis and Monitoring Division
Seattle, Washington
jonathan.cusick@noaa.gov

The U.S. West Coast commercial groundfish fishery is diverse. There are more than 80 fish species in the Groundfish Fishery Management Plan, vessels utilize multiple gear types such as trawl, longline and pot and vessels range in size from 18-foot skiffs to trawlers in excess of 300 feet. While retained catch data are collected via landing receipts and, in the case of the at-sea hake fleet, processor logs, independently verifiable bycatch and discard data can only be collected through at-sea monitoring programs. Without at-sea monitoring programs, landing receipts only provide a partial picture of total removals. Currently, the FRAM division deploys field biologists (observers) and electronic technology to collect bycatch and discard data to provide an estimate of total removals. Observers collect data on fishing operations (set dates, times, locations, etc.), in addition to estimating total catch weight and speciation of the bycatch and discard. The FRAM division is also testing electronic technology to confirm maximized retention by installing systems to record fishing operations with GPS, winch counter, hydraulic pressure sensors and video cameras. Depending on the specifics of the fishery monitored, data is provided to managers on a real time or regular schedule to be incorporated into estimates of total removals, thus providing managers the tools to manage the fishery within set OYs with greater assurance.

The perils of point estimates: Effective scientific presentation in the face of uncertainty and human psychology

Ian J. Stewart
Northwest Fisheries Science Center
Fisheries Resource Analysis and Monitoring Division
Seattle, Washington
ian.stewart@noaa.gov

Effective presentation of scientific uncertainty is one of the top challenges facing fisheries scientists and managers. We perform computationally intensive analyses to accurately represent the uncertainty associated with point estimates, but may fall short in delivering these results due to human psychology. When faced with uncertainty, people start from an initial value and then adjust their perception based on available information. However, this adjustment is usually insufficient, and therefore different initial values can yield different conclusions. This is phenomenon is called "anchoring." Because of anchoring, people tend to underestimate the probabilities of failure in complex systems. Understanding anchoring, and the conditions that affect it, may help us to more effectively convey our scientific results. I present some simple examples of anchoring and suggest the use of probabilistic statements, informative graphics and caution when presenting point estimates.

Conservation hatchery effects on steelhead in Hood Canal

Barry Berejikian
Northwest Fisheries Science Center
Manchester Research Station
Resource Enhancement and Utilization Technologies Division
Manchester, Washington
barry.berejikian@noaa.gov

Don VanDoornik
Northwest Fisheries Science Center
Manchester Research Station
Conservation Biology Division
Manchester, Washington

Thom Johnson
Washington Department of Fish and Wildlife
Olympia, Washington

Rick Endicott and Joy Lee
Long Live the Kings
Seattle, Washington

Conservation hatcheries for anadromous salmonids must strike a balance between increasing production and minimizing genetic, ecological and demographic risks. A conservation hatchery program for Hood Canal steelhead has caused a significant increase in the number of redds in the supplemented Hamma Hamma River compared to the pre-supplementation period. Three monitored control populations (non-supplemented) either remained stable or declined over the same period. The strategy of rearing steelhead full-term to sexual maturity and releasing them for natural spawning accounted for the greatest proportion of redd abundance increases with a negligible 'mining' impact on the natural population. Environmentally induced differences in spawn timing between the adult release group and anadromous adults (wild adults and those released as smolts from the hatchery program) may explain why the adult release group and anadromous adults assortatively formed pairing combinations on the spawning grounds. The level of genetic variation in the juvenile steelhead (*Oncorhynchus mykiss*) population during the supplementation period appears similar to the pre-supplementation period. Genetic analyses indicate that the steelhead population in the Hamma Hamma River is a mixture of native steelhead and resident rainbow trout that had been introduced above a barrier falls. However, there also is evidence that a small amount of gene flow occurs between the two life history types. The impact of the hatchery program on natural population productivity and longer-term effects on genetic variability will require the termination of hatchery releases and continued monitoring of the supplemented and control populations through the F_2 generation.

Effects of hatchery rearing on behavioral diversity—some tests with rockfish and steelhead

Jonathan Lee and Barry Berejikian
Northwest Fisheries Science Center
Manchester Research Station
Resource Enhancement and Utilization Technologies Division
Manchester, Washington
jon.lee@noaa.gov

The effects of hatchery rearing on fish behavior have attracted attention because behavioral differences between wild and hatchery fish can have negative impacts on wild fish. However while most studies focus on differences in behavioral means, differences in behavioral variation may also be important. Behavioral variation could be adaptively maintained in nature through heterogeneous selective environments or negative frequency dependence. Behavioral variation could also be maladaptive—resulting, for example, from relaxed selection in hatcheries. Hatchery rearing may alter behavior through differential survival of different behavioral phenotypes, and through environmental effects on behavioral development. We tested the former mechanism in juvenile brown rockfish (*Sebastes auriculatus*) and the latter mechanism in juvenile steelhead (*Oncorhynchus mykiss*). Brown rockfish showed considerable individual variation in feeding and predator inspection behaviors. However, after four months of hatchery rearing, there was no detectable relationship between behavioral phenotype and growth or survival. This may be due to the fact that behavioral profiles of individuals were unstable through time. For example, a fish could show high predator inspection rates at time x, but then low rates when tested several days later. We discuss a possible relationship between behavioral stability and selection, suggesting that intra-individual plasticity in behavior through time may have reduced the opportunity for selection. Next we will shift to an experiment with steelhead that examined the effect of alternative rearing environments on behavioral development. Compared to fish reared in structured environments, fish reared in barren environments exhibited greater variation in later feeding behavior, and appeared to exhibit greater variation in exploratory behavior. While most previous studies have focused on relaxed selection in captivity that leads to increased behavioral variation over multiple generations, these steelhead data provide another mechanism—changes in behavior through development, within a single generation.

Multiple facets of a salmon bacterial disease

Linda Rhodes, Mark Strom, Alison Coady, Sudheesh Ponnerassery, Lee Harrell, Shelly Nance, Colleen Durkin, Steviebrooke Wallis, and Surafel Gezahegne
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington
linda.rhodes@noaa.gov

Rebecca Deinhard, Cindra Rathbone, and William Fairgrieve
Pacific States Marine Fisheries Commission
Portland, Oregon

Casey Rice
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington

Gregory Wiens
U.S. Department of Agriculture-ARS
National Center for Cool and Cold Water Aquaculture
Leetown, West Virginia

Daniel Rockey
Oregon State University
College of Veterinary Medicine
Corvallis, Oregon

Anthony Murray and Diane Elliott
Western Fisheries Research Center
Fish Health Program
Seattle, Washington

As salmon populations decline in the Pacific Northwest, factors such as chronic diseases can become more important. Bacterial kidney disease (BKD) has been known as both a chronic and acute disease among salmon biologists for more than 60 years. Historically, progress in understanding the disease and its etiological agent, *Renibacterium salmoninarum*, had been slow, due to difficulty in culturing and manipulating the bacterium and to the often cryptic nature of infection and disease progression. Over the past 10 years, our studies of BKD and *R. salmoninarum* have ranged from genomics to clinical therapies to epidemiology. Through reverse genetics, we characterized the importance of a protein required for pathogenicity by *R. salmoninarum*, identifying it as a target for vaccine development. Complete genome sequencing of *R. salmoninarum* has provided information in formulating strategies for novel therapeutants. Clinical studies of BKD have evaluated antibiotic treatments and the risk of the emergence of drug-resistant strains of *R. salmoninarum*, and the genome sequence is offering insights into possible mechanisms of resistance. Efforts to develop a vaccine against BKD produced a

moderate efficacy reagent with both prophylatic and therapeutic value. Research on the host side of the disease equation is characterizing how fish can effectively fight infection and yielding markers that can be used in laboratory studies. Field surveys have identified factors that may be important contributors to infection among free-ranging salmon. These insights into BKD are providing critical focus and knowledge to our future investigations of a problematic, but fascinating, salmon disease.

Molecular approaches for understanding oil spill impacts on nearshore spawning fish

Heather Day, Tracy Collier, Nat Scholz, and John Incardona
Northwest Fisheries Science Center
Ecotoxicology Program, Environmental Conservation Division
Seattle, Washington
heather.day@noaa.gov

Mark Carls
Alaska Fisheries Science Center
Auke Bay Lab
Juneau, Alaska

Coastal oil spills can have severe ecological effects on intertidal and shallow subtidal zones, which provide critical spawning and nursery habitat for numerous species. In particular, several important northeast Pacific forage fish species deposit demersal, adherent eggs in these zones where they subsequently undergo development, including Pacific herring (*Clupea pallasi*), sand lance (*Ammodytes hexapterus*), surf smelt (*Hypomesus pretiosus*) and capelin (*Mallotus villosus*). The sensitivity of teleost embryos to toxic effects of crude oil was demonstrated by the Exxon Valdez oil spill, which coincided with the spawning of Pacific herring in Prince William Sound. Studies following the spill showed that embryonic exposure to polycyclic aromatic hydrocarbons (PAHs) in crude oil induces a common suite of developmental defects including pericardial and yolk sac edema, craniofacial and body axis defects. Moreover, significant sublethal effects result in the absence of obvious malformations. For example, pink salmon exposed to weathered crude oil as embryos and released as normal-looking smolts showed significantly lower rates of adult returns. Our basic studies of PAH toxicity using the zebrafish model identified the developing heart as the primary target of the tricyclic compounds most abundant in crude oil. Using the sophisticated genetic and molecular tools associated with the zebrafish model, such as gene knockdown techniques and DNA microarrays, are working to identify potential candidate genes expressed in the developing heart that are misregulated in response to PAH exposure. Toward the ultimate goal of developing physiologically relevant biomarkers for PAH exposure, we are ground truthing these findings in Pacific herring embryos.

Assessing Alaskan killer whale feeding ecology using chemical tracers

Douglas Burrows, David Herman, and Margaret Krahn
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
douglas.g.burrows@noaa.gov

Top predators in the marine environment integrate chemical signals acquired from their prey that reflect both the species and source region. These chemical tracers—stable isotope ratios of carbon and nitrogen; persistent organic pollutants (POPs); and fatty acid profiles—were measured in blubber/skin biopsy samples from a large number of eastern North Pacific (ENP) killer whales and were used to provide insight into their diet. Among the three recognized ecotypes in the ENP – offshore, residents and transients – only the fish-eating residents and mammal-eating transients can be unequivocally distinguished from one another by all three chemical tracers. The offshore killer whales can be separated from the other two ecotypes only by their fatty acid and POPs profiles. The high ΣDDTs/ΣPCBs ratios present in the blubber of the Alaskan offshore killer whales indicated that they also feed at the lower latitudes and this is supported by the photo-identification of these whales in California in winter months. Also, different chemical patterns are observed among differing regional groups of the same ecotype presumably due to differing diets and contaminant sources. For example, resident killer whale populations showed a gradient from west (central Aleutians) to east (Gulf of Alaska) in both stable isotope and specific POP contaminant ratios which may in part reflect a shift from off-shelf to continental shelf-based prey and the spread of pollutants of Asian origin into the ENP, respectively.

A history of telemetry technology development in the Columbia River Basin and its use in evaluating fish passage behavior and survival

Eric E. Hockersmith, Gordon A. Axel, Darren A. Ogden, and Douglas B. Dey
Northwest Fisheries Science Center
Fish Ecology Division
Seattle, Washington

The Columbia River Basin (CRB) including the Snake River and its tributaries has historically produced some of the largest runs of Pacific salmon *Oncorhynchus* spp. and steelhead *O. mykiss* in the world (Netboy 1980). More recently, however, some stocks have decreased to levels that warrant listing under the U.S. Endangered Species Act (ESA) of 1973 (NMFS 1991, 1992, 1998, 1999). Anthropogenic factors that have contributed to the decline and loss of some salmonid stocks include overfishing, hatchery practices, logging, mining, agricultural practices, and dam construction and operation (Nehlsen et al. 1991). A primary focus of recovery efforts for depressed stocks has been assessing and improving fish passage conditions at dams (Williams et al. 2001).

Over the past 50 years, the Fish Ecology Division (formerly the Coastal Zone and Estuarine Studies Division) of the Northwest Fisheries Science Center (NWFSC) has pioneered new fisheries research applications and technological developments in telemetry. The Fish Ecology Division uses telemetry to provide critical information and insights regarding migrational behavior, fish passage, and survival that inform fisheries and hydrosystem management decisions in the CRB.

Material and Methods

We reviewed and summarized the historical use of telemetry in the CRB by the NWFSC from 50 years of contract reports, technical memorandums, and peer reviewed publications. Other government and non-governmental organizations (NGOs) conducting telemetry studies in the CRB which were not included in this review include Washington Department of Fish and Wildlife (WDFW), the University of Idaho, Oregon State University, the Biological Resources Discipline of United States Geological Survey (USGS), and various consulting firms contracted by the mid-Columbia Public Utility Districts (PUDs).

Results and Discussion

More than 90,000 fish have been tagged with either acoustic or radio transmitters to evaluate fish passage issues in the Federal Columbia River Power System (FCRPS) over the past 50 years (Table 1). Most of the focus has been on passage for Chinook salmon or steelhead. Trefethen (1956) reported the first use of telemetry to study fish in 1956. This study used acoustic telemetry to examine the passage of adult Chinook salmon at Bonneville Dam. Until this development, fish behavior and movement could only be studied by direct observation. Telemetry brought two new advantages to research: the ability to identify individual animals and

to locate each animal when desired. We divided the history of telemetry use in the CRB into three periods: 1956 to 1969, 1970 to 1989, and 1989 to the present.

Table 1. Numbers and life stage by species of fish tagged with either radio or acoustic transmitters over the past 50 years to evaluate fish passage in the CRB.

Species	Adults	Juveniles	Total
Chinook salmon (stream type)	12,003	27,055	39,058
Chinook salmon (ocean type)	9,593	20,867	30,460
Steelhead	10,987	6,027	17,014
Pacific lamprey	3,320		3,320
Sockeye salmon	704		704
Coho salmon	179		179
White sturgeon	148		148
Rainbow trout	52		52
Chum salmon	9		9
Pink salmon	6		6
Total	37,001	53,949	90,950

During the 14 years from 1956 through 1969, over 3,500 acoustic transmitters were used to study adult salmon and steelhead migration, behavior, and passage at dams in the CRB. Innovations in acoustic telemetry developed by the Bureau of Commercial Fisheries (now NOAA Fisheries Service) during this period included improvements in tagging techniques (Stuehrenberg et al. 1978), fixed-site acoustic telemetry systems (Johnson 1964) and advances in tag life (Novotny and Esterberg 1962; Stuehrenberg et al. 1978). Studies assessing the impacts of the FCRPS from 1956 through 1969 were limited to relatively short duration (due to tag life) and adult life-history stage (due to the large size of the transmitters). In addition, acoustic telemetry worked poorly in turbulent areas such as those downstream of dams especially during periods of spill and required the receiving unit to be submerged in water which limited the ability to track highly mobile species over great distances. Because of these limitations, National Marine Fisheries Service (NMFS) began developing an extended range radio transmitter for use in the CRB in 1970 (Monan and Liscom 1971).

Radio telemetry had been used to study wildlife since 1956 (LeMuyan et al. 1959); however, it was not used to study fish until 1968 (Lonsdale and Baxter 1968). The detectability of radio transmitters in the air was a major advantage over acoustic telemetry for studying salmon and steelhead migrations in the large river systems. During the next 19 years (1970-1988) over 1,500 radio transmitters were used to study salmon and steelhead migration, behavior, and passage at dams in the CRB.

Innovations in radio telemetry developed and applied by the NWFSC from 1970 to 1988 included development of the first long-range radio transmitter for studying fish (Monan and Liscom 1971), development of the first automatic scanning receiver (Liscom and Monan 1976), development of the first coded radio transmitters for studying fish (Liscom and Stuehrenberg 1983), and the first smolt passage telemetry study in the CRB (Faurot et al. 1982). Studies

assessing the impacts of the FCRPS from 1970 through 1988 were limited to relatively short duration (due to tag life) and to large juvenile fish (due to the size of the smolt transmitters).

Over the past 17 years (1989-2006) advances in electronics combined with the listing of some stocks under the ESA has resulted in substantial increases in telemetry studies in the CRB in both scope and size. Over 85,000 transmitters have been used to study salmon and steelhead migration, behavior, and passage at dams in the CRB during this recent period.

Innovations in telemetry developed and applied by the NWFSC over the last 17 years have included techniques to conduct aerial tracking via GPS (Hockersmith and Peterson 1997), application of survival models to assess smolt survival (Hockersmith et al. 1999), reductions in transmitter size for smolts (from 2.9 g to 0.6 g), increases in the numbers of unique radio transmitters that can be used at the same time (from 630 to 4,545), improvements in receiver design, polling software for automatic downloading of receivers, and development of a juvenile salmonid acoustic telemetry system (JSATS) (McComas et al. 2005) to measure system survival for individuals through the entire hydropower system (eight dams and over a distance of more than 695 km). Studies assessing the impacts of the FCRPS from 1989 through 2006 have been transitioning from adult passage to smolt passage evaluations. In addition, ongoing passage studies in the FCRPS for Pacific lamprey (Moser and Close 2003) and white sturgeon have also benefited from the advances in telemetry technology.

Telemetry development and use in the CRB at the NWFSC has been a collaborative partnership among electronics engineers, electronics technicians, and fishery biologists to improve passage and survival within the FCRPS. These are the largest telemetry studies in the world with more than 90,000 fish tagged with transmitters over the past 50 years. These studies have resulted in structural and operational improvements for fish passage in the CRB and these techniques and technologies are being used to help solve fish passage issues worldwide.

The recent shift from adult passage and survival issues to those of juvenile salmonids – and in particular, the juveniles of ESA-listed stocks – has led to more than 15,000 smolts tagged annually in the CRB. With this shift new challenges have emerged, including increased sample sizes, the need for smaller tags, isolating specific passage routes at dams, collecting and processing massive amounts of data, developing analytical models, and providing virtually real time analysis.

Plans for 2007 include 900 radio transmitters to evaluate hydropower system passage of adult salmon and steelhead, 13,645 radio transmitters to evaluate smolt passage and survival at Lower Monumental and Ice Harbor Dams on the Snake River, and 25,000 JSATS transmitters to evaluate hydropower system and estuary survival for smolts. With continued technological innovation, future applications of telemetry will likely provide insights that are currently unavailable such as the development of a life-cycle tag which would allow evaluations to use the same individuals to assess passage behavior during both the juvenile and adult life-history stages.

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Using parasites to study marine populations and ecosystems: An old tool brought to the future

Kym Corporon Jacobson
Northwest Fisheries Science Center
Hatfield Marine Science Center
Fish Ecology Division
Newport, Oregon
kym.jacobson@noaa.gov

Rebecca Baldwin and Mary Bhuthimethee
Oregon State University
Cooperative Institute for Marine Resources Studies
Newport, Oregon

The integration of parasitology into marine ecology has a long tradition. As early as the 1930s parasites were being used as biological tags for marine populations. Parasites have been, and continue to be studied worldwide to understand marine fish populations and their environments by providing information on fish diet, migration, habitat use, and habitat health. To better understand migration and stock structure of marine fish, population studies have used single parasite species, parasite communities, and most recently genetic analysis of parasites. Parasites can also be used to examine trophic structure and history, as many parasites use transfer through predator-prey interactions to complete their complex life cycles. Because parasites can remain in hosts for months to years, their presence provides an extended history of trophic interactions beyond that provided by fish stomach contents. In the Estuarine and Ocean Ecology Program of the Fish Ecology Division, we have studied parasites to increase our knowledge of the trophic interactions of juvenile salmonids and associated marine nekton. We are currently studying parasites in juvenile salmon in the Columbia River estuary and during early marine residence as indicators of habitat residence and habitat differentiation. Recently we began a study using parasites as biological tags for delineation of expanding Pacific sardine (*Sardinops sagax*) stocks. This project will combine information from parasite community structure with the genetic structure of parasites; a recent tool showing promise for the future of fisheries research.

Using molecular genetic techniques to address key questions for Southern Resident killer whale conservation

Brad Hanson, Candice Emmons, and Jennifer Hempelmann
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington
brad.hanson@noaa.gov

Robin Baird and Greg Schorr
Cascadia Research Collective
Olympia, Washington

Don Van Doornik
Northwest Fisheries Science Center
Manchester Research Station
Conservation Biology Division
Manchester, Washington

A key risk factor identified as a possible cause of the decline of the ESA listed Southern Resident killer whale population was reduced prey availability. In order to evaluate the influence of this risk factor on this whale population a contemporary sample of prey preferences was needed. A prey selection study of this whale population was undertaken in the San Juan Island portion of their summer range from 2004 to 2006. By following the whales in a small boat, fish scales and tissue remains from predation events were collected using a fine mesh net. Fecal material was also collected in a similar manner. Fish scale analysis provided important information on species and age of the fish consumed. However, the application of genetic species identification techniques to fish tissue and fecal material has increased the sample size of identified prey. More importantly, for the fish scales and tissue, genetic stock identification (GSI) has identified prey items to stock. Based on a combination of these techniques, Chinook were identified as the primary species consumed and most fish originated from the Fraser River. This information will be of significant value in guiding management actions to recover the Southern Resident killer whale population. The fecal material also yielded killer whale DNA, which in many cases was from known individual whales. Samples have been collected from a substantial portion of the population and will allow determination of paternity patterns which will be of significant importance in evaluating the potential for inbreeding in this small population.

Beyond birth and death: The potential value of behavioral ecology to ecosystem management

Phillip Levin, Nick Tolimieri, Kelly Andrews, and Greg Williams
Northwest Fisheries Science Center
Fisheries Resource Analysis and Monitoring Division
Seattle, Washington
phil.levin@noaa.gov

Steve Katz
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington

Mary Moser
Northwest Fisheries Science Center
Fish Ecology Division
Seattle, Washington

Debbie Farrer and Greg Bargman
Washington Department of Fish and Wildlife
Olympia, Washington

While patterns of density are ultimately produced by the interaction of births, deaths and movement, far more attention as been paid to the processes of births and deaths than to movement. However, the manner in which organisms move through their environment is crucial to the success of individuals. Furthermore, individual patterns of movement can generate observed spatial patterns of the population. We have been examining patterns of movement in six gill sharks, seven gill sharks, copper rockfish, quillback rockfish and lingcod as a means to better predict the consequences of environmental change and/or human perturbation on these species. We will illustrate the use of three different systems to quantify movement patterns in this diverse group of fishes. Acoustic monitoring allows us to track coarse-scale movement of large, mobile species over long periods of time. Manual active tracking is used to quantify movement tracks over short (36 hour) time spans and provides us with a detailed understanding of how fish use their habitat. Remotely operated acoustic arrays allow us to track the fine-scale movements of species with small home ranges over long periods of time. Together, these tools are beginning to help us understand habitat requirements, the minimum area necessary for marine reserves, trade-offs in habitat preferences and the utility of census techniques for this group of species.

Poster Presentations

The nearshore development of a toxigenic *Pseudo-nitzschia* bloom and subsequent domoic acid contamination of intertidal bivalves

Nicolaus G. Adams
Northwest Fisheries Science Center
Environmental Conservation Division
Marine Biotoxin Program
Seattle, Washington
and
University of Washington
School of Aquatic and Fishery Sciences
Seattle, Washington
nicolaus.adams@noaa.gov

Vera L. Trainer
Northwest Fisheries Science Center
Environmental Conservation Division
Marine Biotoxin Program
Seattle, Washington

Amoreena MacFadyen and Barbara M. Hickey
University of Washington
School of Oceanography
Seattle, Washington

Along the coast of Washington State, periods of storm winds intensify in late summer and may advect domoic acid-producing *Pseudo-nitzschia* to the coast where they impact the coastal razor clam fishery, rendering the clams toxic to humans. To test this hypothesis, during the late summer and early fall of 2002 repeated measurements of *Pseudo-nitzschia* species, particulate domoic acid, temperature, and salinity were made in nearshore waters and at Kalaloch beach on the central Washington coast. A shift in the composition of the *Pseudo-nitzschia* population to *P. australis* cells was observed following a storm event during September 8-10. Following a second storm event that occurred September 16-18, *Pseudo-nitzschia* cell numbers multiplied and a concurrent increase in levels of particulate domoic acid occurred in nearshore waters. Data from moored instruments show the presence of Columbia River plume water on the inner shelf for several weeks beginning on September 8, persisting for approximately eight days after the second storm and effectively inhibiting upwelling near the Washington coast. Domoic acid in intertidal razor clams accumulated to levels that exceeded the regulatory safety limit 18 days following the shift to *P. australis* cells. These data indicate that the strategic placement of a mooring in nearshore waters with appropriate sensors for the detection of cells, toxins and

environmental data may provide coastal managers with an early warning of impending toxification of coastal shellfish.

Where's the point? Details of a large-scale inter-agency salmon hatchery release mapping project in the Pacific Northwest

Andrew M. Albaugh
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington

Jeffrey Cowen
Northwest Fisheries Science Center
Scientific Data Management
Seattle, Washington

To aid in the recovery of threatened and endangered Pacific Northwest salmon populations, the NMFS Northwest Fisheries Science Center has developed a geo-spatial database to house a wide array of salmon related data. One key component of the database, developed in cooperation with several state, federal, and tribal agencies, is a module to capture and display agency data on artificial propagation operations (hatcheries). One challenge of this endeavor was standardizing the agencies' diverse methods of documenting hatchery release locations. There were roughly 40,000 records submitted spanning years 1990 to 2002, with about 11,000 unique locations provided as narrative text or a stream name. Mapping these locations from this limited information required the development of tools for the ArcGIS software environment. The result is a comparatively accurate and highly useful display of hatchery release points and spawning events presented in a spatially rigorous form. A major benefit from our approach is that it will grant all agencies access to a central location, through a web-based interface, where they can download salmon related data. We foresee this mapping effort, coordinated with our database development as a whole, becoming a vital tool for policy and decision makers with regards to Endangered Species Act status updates for these salmon populations.

Objective

Previous to the development of this database there was no way for NWFSC and regional scientists to query and view salmonid hatchery data that spatially distinguished individual hatchery release events in once centralized location. In order to provide scientists with a database that actually displayed these points (Figure 1), as opposed to an entire river segment, the original datasets had to be first cleaned and standardized.

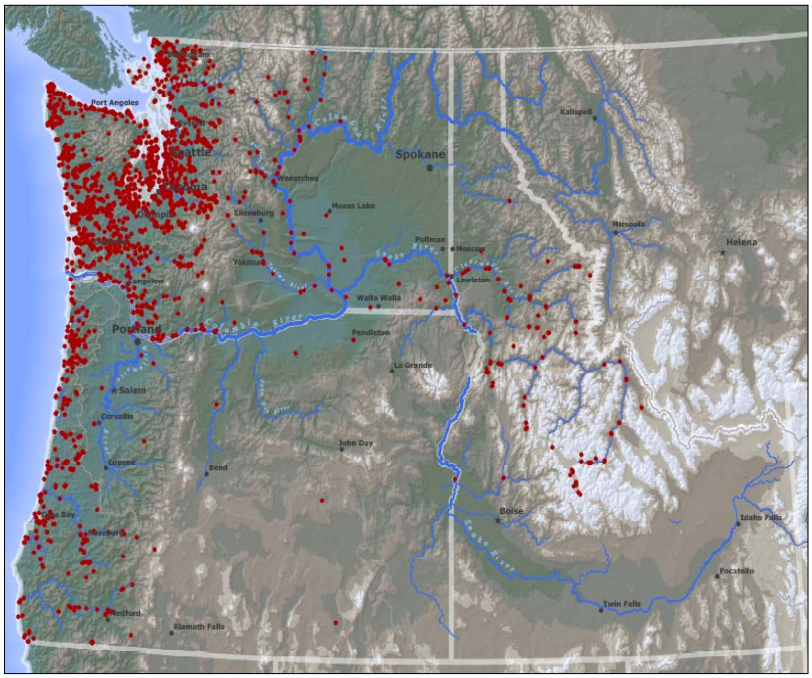

Figure 1. A map of WA, OR, and ID showing all release events. There may be many, many release events represented by a single point.

The dataflow consists of several steps and has a number of chances to suffer from human error. The data begins in the hands of the hatchery field technicians and hatchery operators. They then get passed onto the respective agency. The data may then move onto a secondary agency. Finally through a specific data request the data is given to the NWFSC in various forms. The hope for the future is that we will be able to make arrangements with the agencies to collect data in a uniform matter that will require a minimal amount of re-formatting.

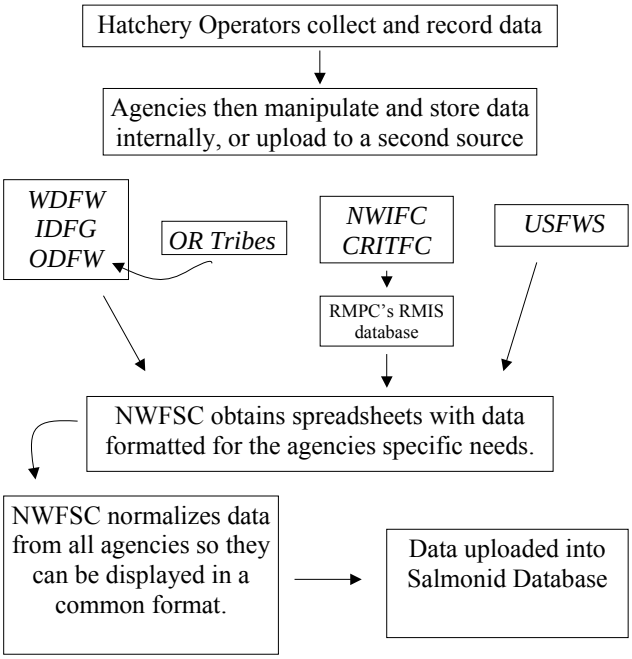

Figure 2. The data flow from hatcheries, to agencies, and finally to the NWFSC.

Figure 3 is an example of the varied forms in which the data is received. Each agency tends to gather the same type of data differently. In fact, even the same agency can vary in format from year to year. The result is a very disorganized conglomeration of hatchery data in need of a thorough cleaning.

ODFW

HATCHERY	SPECIES	DATE	WATERNAME	REMARKS	PLANTING LOCATION
COLE RIVERS	WINTER STEELHEAD	3/30/2000	EMIGRANT RES	R13 EXCESS RUNTS. WATER MURKY + VERY WINDY SPLIT LOAD WITH LIB#111634	WATERSLIDE BOAT RAMP
CEDAR CREEK	WINTER STEELHEAD	4/4/2000	THREE R/RHOADES ACCLIMATION PD	THREE R/RHOADES ACCLIMATION PD	GAULDY BRIDGE PD #15
ELK RIVER	FALL CHINOOK	10/22/2001	ELK R	SCREENS PULLED AT 4 PM P18-P24	HATCHERY OUTLET

WDFW

Facility	Year	Species	Release site	River Mile	Memo
REITER PONDS	1996	STEELHEAD	SKYKOMISH RIVEROMISH R 07.0012	28	FISH HAULD INTO CONDITIONING POND ON BARR CR., ON 4/1/96 & 4/2/96 & RELEASE ABOUT 3 WEEKS LATER.
PUGET POWER SPAWNING	1999	COHO	BAKER LAKE (03)		@ SEAPLANE LANCH (WEST PASS DIKE).FB(^) ON THE LEFT SIDE.
LK ABERDEEN HATCHERY	1998	CUTTHROAT	WYNOOCHEE PD(GRAY)	16	WYNOOCHEE ROAD RIVER MILE 16 POND.

IDFG

DATE_PLANT	SPECIES_NAME	STREAM_NAM	HATCHERY_NAME	COMMENTS
7/20/2001	SUMMER CHINOOK	S F SALMON R	McCALL	1st Load Stolle Acclimation Pond - Supplementation
7/31/2002	SPRING CHINOOK	WHITE SANDS CR	CLEARWATER	released at Colt Killed Creek cabin, done 8/8/02, 3 trucks
3/21/2001	SPRING CHINOOK	LOLO CR	CLEARWATER	released by NPT, finished on 3/28/2001

Figure 3. Each agency reports hatchery release data in varying ways. This is only a small subset of the columns of data that require cleaning and formatting.

The data that were loaded and mapped ranged from 1990 to 2004. There were an approximate total of 40,000 individual records with about 11,000 unique locations needing to be mapped. Figure 4 depicts the distribution of these points 6 major categories. The 'Named Feature' category proved to be the most difficult to decipher, it included records that gave nothing more than an obscure reference to a feature such as a boat ramp or a bridge. When only a 'River Name' was given with no other info a specific investigation was performed for that record determining where to place the point, if no logical place could be determined it was located at the respective hatchery.

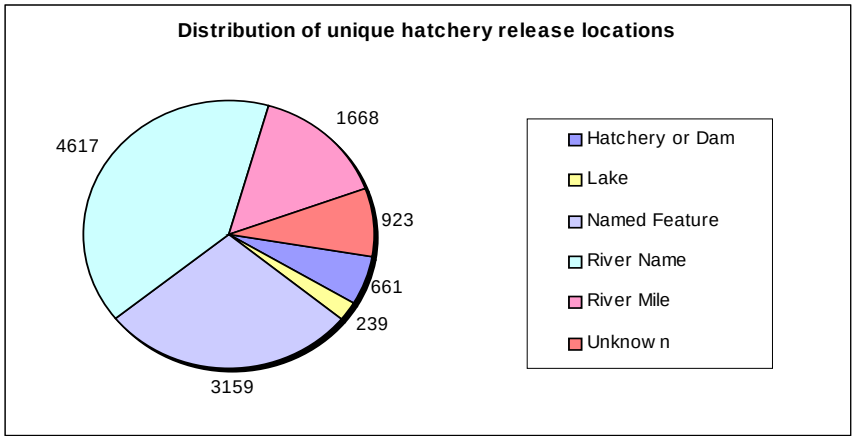

Figure 4. The types of release locations were broken down into 6 major categories, all containing data with wide degrees of accuracy.

It is interesting to note the distribution of hatchery release events amongst agencies (Figure 5). Of the nearly 40,000 records mapped, half of them were from WDFW. It is important to note that a single hatchery release event equals one record, i.e., a hatchery that released fish at a single site 10 times over the course of a summer would contribute 10 records to the database.

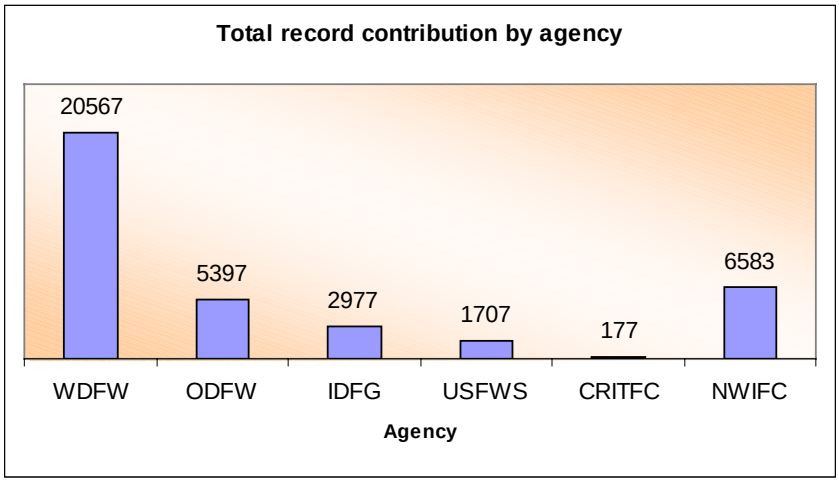

Figure 5. The total number of release events for each agency during the years of data provided.

Management Implications

It is envisioned that this hatchery release portion of the database, as well as the database as a whole, will play an important role in the salmon recovery decision making process. With continued efforts the database in the near future will be able to display a wide array of salmon data including artificial propagation, natural abundance, harvest, and hatchery fraction. Furthermore, the mapping application will be able to view different data-types for the same spatial location. For example viewing both natural abundance and hatchery release data of a specific basin on a single map. This tool will also enable those in the salmon restoration field an opportunity to thoroughly and quickly analyze geographic areas outside their specific area of interest, resulting in the ability to form decisions and pose questions regarding the interaction of geographic areas as a whole, as opposed to a narrow-scoped view.

Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos

David H. Baldwin and Nathaniel L. Scholz
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington

Jason F. Sandahl and Jeffrey J. Jenkins
Oregon State University
Department of Molecular and Environmental Toxicology
Corvallis, Oregon

Abstract

Chlorpyrifos is a common organophosphate insecticide that has been widely detected in surface waters that provide habitat for Pacific salmon in the western United States. Although chlorpyrifos is known to inhibit acetylcholinesterase (AChE) in the brain and muscle of salmonids, the relationship between sublethal AChE inhibition and more integrative indicators of neurobehavioral impairment is poorly understood. This is particularly true for exposures that reflect the typical range of pesticide concentrations in the aquatic environment. To directly compare the effects of chlorpyrifos on AChE activity and salmon behavior, we exposed juvenile coho salmon (*Oncorhynchus kisutch*) to chlorpyrifos (0–2.5 ppb) for 96 hours. A computer-assisted, three-dimensional video imaging system was used to measure spontaneous swimming and feeding behaviors in control and chlorpyrifos-exposed fish. Following the behavioral trials, brain and muscle tissues were collected and analyzed for AChE activity. Chlorpyrifos inhibited tissue AChE activity and all behaviors in a dose-dependent manner. Benchmark concentrations for sublethal neurotoxicity (statistical departure values) were < 0.5 ppb and were similar for both neurochemical and behavioral endpoints. Moreover, brain AChE inhibition and reductions in spontaneous swimming and feeding activity were significantly correlated. Collectively, these results indicate a close relationship between brain AChE inhibition and behavioral impairment in juvenile coho exposed to chlorpyrifos at environmentally realistic concentrations.

Introduction

Numerous studies have shown that exposure to organophosphates or carbamates results in a concentration-dependent inhibition of acetylcholinesterase (AChE) in the nervous system or muscle of various fish species. Accordingly, AChE inhibition is now generally accepted as a biomarker of exposure for pesticides that share the anticholinesterase mechanism of action. However, the extent to which AChE inhibition is a reliable or meaningful biomarker of effect is considerably less clear. AChE is an enzyme that regulates cholinergic signaling by hydrolyzing the transmitter acetylcholine at central and peripheral synapses in the vertebrate nervous system. The relationship between AChE inhibition and physiological or behavioral impairment has not been widely investigated, and studies exploring the effects of pesticides at ecologically relevant concentrations (i.e., at concentrations that have actually been detected in freshwater or estuarine

habitats) have been rare. Given the wide range over which AChE inhibition can be considered sublethal, and the lack of data relating degrees of inhibition to specific toxicological outcomes, caution has been urged in the use of AChE inhibition alone as a biomarker of effect.

A recent study by Sandahl and Jenkins (2002) found that a short-term (96 hour) exposure to the organophosphate chlorpyrifos significantly inhibited the brain AChE activity of juvenile steelhead (*Oncorhynchus mykiss*) at concentrations below one part per billion. This threshold is within or near the range of chlorpyrifos detections in many watersheds that provide freshwater rearing habitat for threatened or endangered salmonid populations in the western United States. In the present study we measured the effects of chlorpyrifos exposures on both the behavior and AChE activity of juvenile coho salmon (*O. kisutch*). A major aim of this study was to ascertain whether AChE inhibition could be linked to behavioral impairments by performing the following:

- Determine the effect thresholds for chlorpyrifos exposure on the swimming and feeding behavior of juvenile coho salmon.
- In the same fish, determine the effect thresholds for chlorpyrifos exposure on the AChE activity of brain and muscle tissue of juvenile coho salmon.
- Directly compare and correlate the behavioral impairments with the AChE inhibition.

Material and Methods

Animals. Coho salmon eggs were obtained from the University of Washington hatchery (Seattle, WA, USA) at the eyed egg stage and raised at the Northwest Fisheries Science Center‘s hatchery facility under natural photoperiod conditions. Fish were raised on standard commercial salmon pellets, until one month prior to experiments, at which point the diet was changed to frozen brine shrimp. Fish were age 4–5 months with an average size (± SD) of 4.5 ± 0.3 cm, and 0.7 ± 0.2 g, during the course of the study.

Chlorpyrifos Exposure

Analytical grade chlorpyrifos [99.3% purity; O,O-diethyl-O-(3,5,6-trichloro-2-pyridinol)-phosphorothionate] stocks were prepared in ethanol, and added in 100 mL volumes to 25 L water in glass aquaria (30 L) to produce nominal chlorpyrifos concentrations of 0, 0.6, 1.2, 1.8, and 2.5 µg/L (or ppb). The final ethanol concentration in exposure tanks was 0.004% of the total volume. Fish were exposed for 96 h, using a static-renewal (12 h) regimen. Fish were treated in groups of 5-6 individuals, and each exposure concentration was replicated in triplicate (n = 15-17 total fish per concentration). Water samples from each chlorpyrifos exposure group were analyzed to compare nominal and actual values, and to determine if chlorpyrifos concentrations decreased over the course of the static exposure interval. Nominal chlorpyrifos concentrations are reported.

Behavioral Analysis

Behavioral trials were conducted in a 30 L glass aquarium (Figure 1) filled with 25 L hatchery water. Following exposures to chlorpyrifos, individual fish were transferred to the observation tank and allowed to acclimate for 30 min. After acclimation, the spontaneous swimming rate of

the fish was measured over a 3 min interval. Subsequently, 30 adult brine shrimp (previously frozen) were injected into the circulation system (t = 0 s). The swimming speed of fish following the introduction of food continued to be measured for 3 min. A DV camcorder was used to monitor feeding strikes. Spatial movements of the fish were monitored by two orthogonally positioned Firewire digital cameras connected to a computer. A custom software program recorded simultaneously-acquired frames from the cameras every 2 seconds and continuously recorded keyboard input. Semi-automated computer video analysis of each pair of frames used the known geometry to triangulate the three dimensional location of the fish every 2 seconds. The time of brine shrimp introduction to the tank and subsequent strikes at the food items were indicated by keystrokes on the laptop.

Acetylcholinesterase Analysis

Fish were sacrificed following behavioral trials. Brain and muscle tissues were collected and immediately frozen at -80°C. AChE activity was analyzed within two weeks of collection, using the colormetric method of Ellman et al. (1961) as modified by Sandahl and Jenkins (2002).

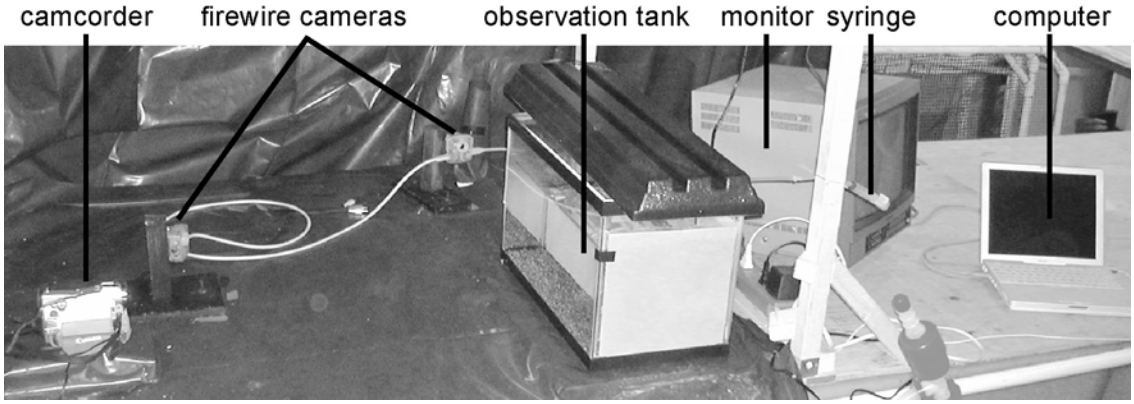

Figure 1. Photograph of the equipment used to perform the behavioral trials. Black plastic screening has been removed for the photograph.

Results

Juvenile coho salmon exposed to chorpyrifos for 96-hr showed dose-dependent reductions in both swimming and feeding behavior (Figure 2). While the background swimming rate (before the addition of food) of exposed fish was reduced, chlorpyrifos exposed fish showed a consistent, brief increase in swimming rate upon the addition of brine shrimp that could reach the background rate (albeit transiently) of the control fish. In addition to the decrease in feeding, fish exposed to the highest chlorpyrifos concentration (2.5 .µg/L) also showed an increase in the time of their first food strike. Figure 3 shows the dose-response relationship between chlorpyrifos concentration and the two behaviors. Both behaviors were significantly negatively correlated with chorpyrifos exposure concentration. Also, the effects thresholds for the two behaviors were very similar and both well under 1.0 µg/L.

Data from the same juvenile coho also showed dose-dependent reductions in the AChE activity of both brain and muscle tissue (Figure 4). The dose-response relationships were significant and, like those of the behaviors, had effects thresholds below 1.0 µg/L. Direct comparisons of the

effects of chlorpyrifos on each behavior and brain AChE activity are shown in Figure 5. Across the treatment groups, both the reduction in swimming rate and food strikes were significantly correlated with a reduction in AChE activity.

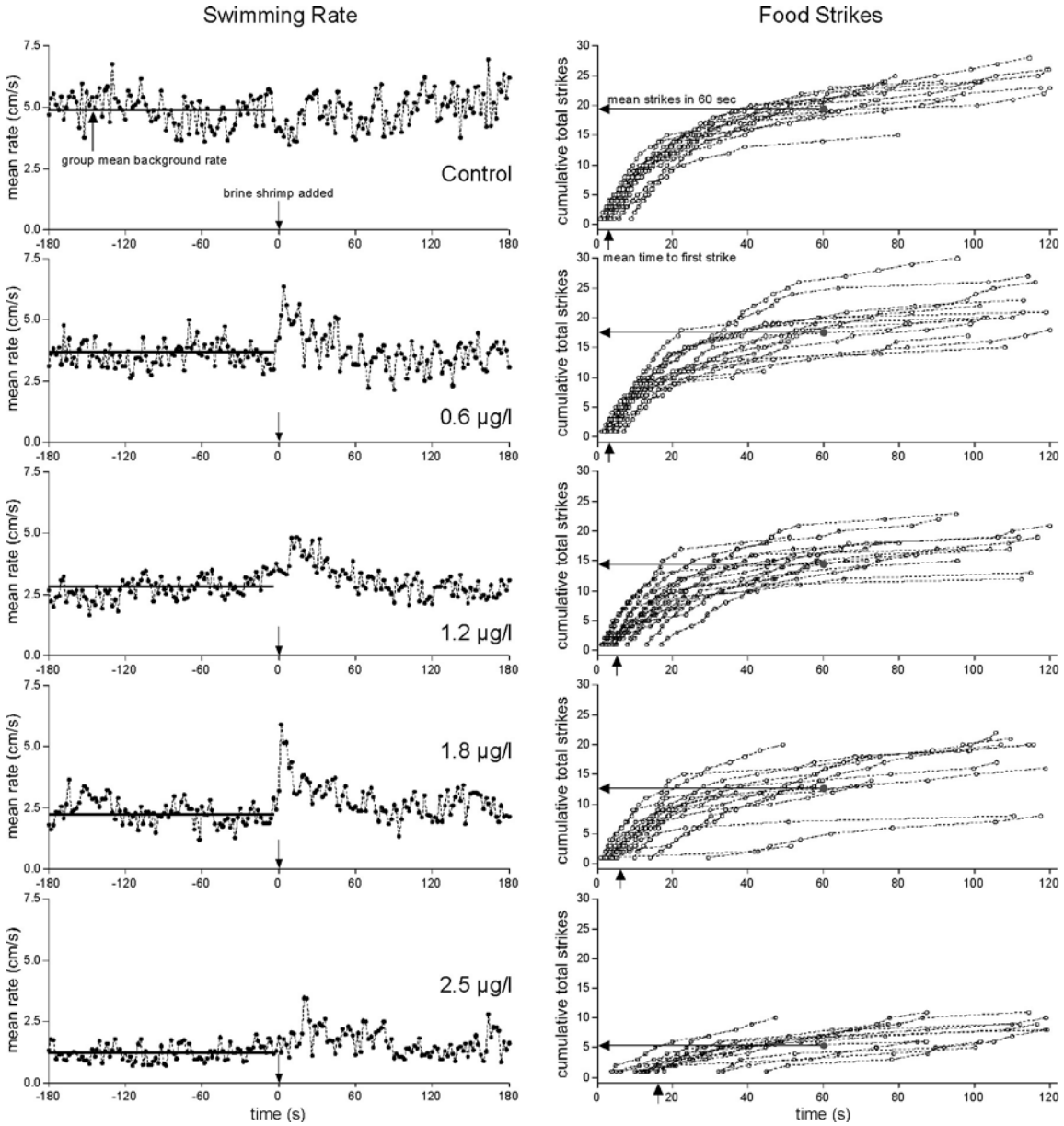

Figure 2. Behavioral activity of pooled data for each treatment group. Plots on the left show the swimming rate computed from the distance traveled in three-dimensions between each frame. Arrows indicate the introduction of 30 brine shrimp (time 0). A horizontal line indicates the mean swimming rate before the introduction of brine shrimp (background swimming). Plots on the right show cumulative food strikes based on input from the keyboard that time-stamped each strike. Arrows indicate the time of the first strike and the total number of strikes in the first 60 seconds.

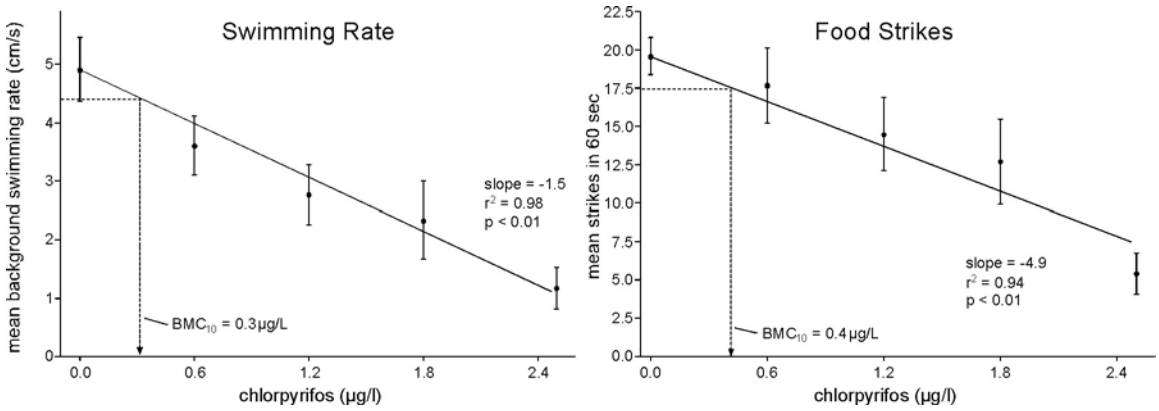

Figure 3. Both swimming rate and food strikes showed statistically significant negative correlations with chlorpyrifos concentration. Benchmark concentration (BMC) response curves for the inhibitory effects of chlorpyrifos were calculated from the data shown in Figure 2 using a linear model. Both plots show the mean and 95% confidence intervals for each treatment group. Vertical arrows indicate behavioral reductions of 10% (BMC_{10}).

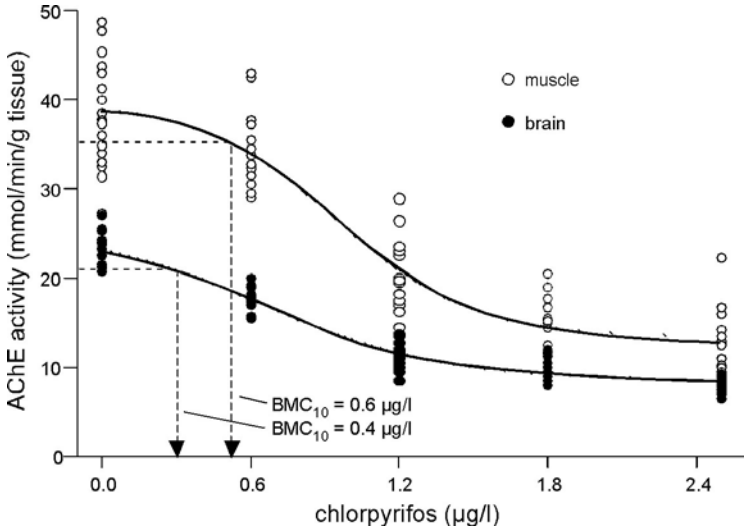

Figure 4. Data from the same fish shown in Figures 2&3 show a dose-dependent reduction in AChE activity caused by chlorpyrifos exposure. Each point represents the data from a single fish. BMC_{10} values (shown with vertical arrows) were calculated using a sigmoid model to account for the asymptotes. The values do not change significantly with a linear model. Brain AChE activity was significantly correlated with muscle AChE activity.

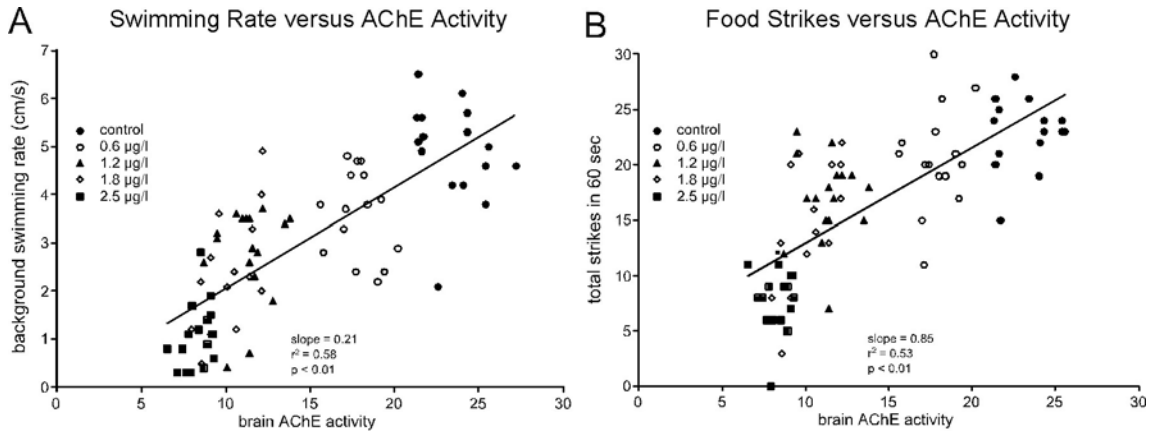

Figure 5. Data from each fish show a significant correlation between inhibition of swimming (A) and feeding (B) behaviors and AChE.

Discussion

Consistent with the use of AChE inhibition as a biomarker of exposure to anticholinesterase pesticides, we found that exposure to chlorpyrifos inhibited the AChE activity of juvenile coho salmon with thresholds below 1 µg/L. In addition, the chlorpyrifos exposure also inhibited two potentially ecologically relevant behaviors (swimming and feeding) with similar thresholds. More importantly, we found a significant correlation between the behavioral and biochemical inhibition. Levels of chlorpyrifos exposure that produce detectable AChE inhibition could, therefore, impair the behavior of coho salmon. For juvenile coho exposed to chlorpyrifos, AChE inhibition and these behavioral impairments may represent useful biomarkers of effect. Future experiments are needed to link these results to higher scales of biological organization (e.g., populations), for example by testing for impacts on the survival and growth of Pacific salmon.

Acknowledgments

We thank Jana Labenia and Barb French for invaluable technical assistance and Brad Gadberry and Tom Scott for facility support at the NWFSC's hatchery. This research was supported by a grant from the National Institute of Environmental Health Sciences to J.F.S. This work has been published as Sandahl et al. (2005).

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Freshwater habitat restoration actions in the Pacific Northwest: A decade's investment in habitat improvement

Katie Barnas and Steve Katz
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington

Ryan Hicks and Jeff Cowen
Northwest Fisheries Science Center
Scientific Data Management
Seattle, Washington

Jeanne M. Rumps, Mark D. Morehead, Stephen R. Clayton, and Peter Goodwin
University of Idaho
Center for Ecohydraulics Research
Boise, Idaho

Robin Jenkinson
Department of Fish, Wildlife and Range Resources
University of Idaho
Moscow, Idaho

Across the Pacific Northwest, both public and private agents are working to improve freshwater habitat for a variety of reasons, including improving conditions for threatened and endangered salmon. Projects are initiated with little or no knowledge of specific linkages between restoration actions and responses of target species. Effectiveness monitoring of these actions is required to redress this lack of mechanistic understanding, but such monitoring depends on detailed restoration information, i.e., implementation monitoring. Our goal in this project was to help inform the placement of future projects and the design of project effectiveness monitoring by creating a database of a Pacific Northwest freshwater restoration (PNW database) to catalog projects that directly or indirectly alter salmon habitat. In addition to documenting the distribution of restoration projects in the Pacific Northwest we examine spatial and temporal patterns of restoration and identify potential improvements in regional restoration project tracking. Even given the restoration database, the lack of existing monitoring design and the paucity of pre-treatment monitoring of restoration projects necessitates alternative methods to assess project success. Project managers may offer the next best available knowledge regarding project implementation and success. To assess the success of restoration projects in the PNW, we surveyed 47 project sponsors and contacts, a sub-sample of completed projects in the PNW Database.

Methods

We assembled a database of spatially referenced, project-level data on over 23,000 restoration actions initiated at over 35,000 locations in Washington, Oregon, Idaho and Montana over the last 15 years and asked the following questions of data providers: Where are the projects

located? When did the projects begin/end? What are the types of restoration actions? Who is sponsoring the projects? (Katz et al. in press)

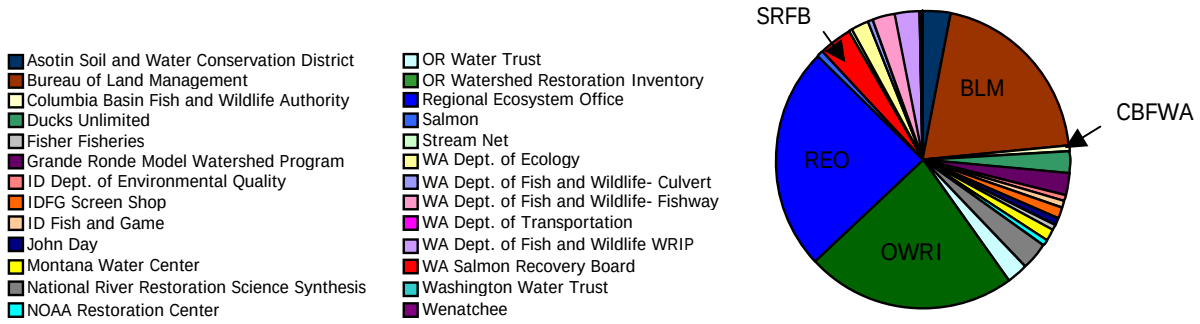

Figure 1. The relative contribution of the 26 project data providers.

Data sources included Federal, State, local, NGO, and tribal contributors (Figure 1). All projects were assigned a project type and subtype. The eleven project types we found in examining the full spectrum of project records were: Barrier Removal, Diversion Screens, Nutrient Enrichment, Other (if project information was too lacking to assign a type but the project had the potential to impact habitat), Restore Instream Flow, Restore Riparian Function, Restore Stream Complexity, Sediment Reduction, Upland Management, and Water Quality Improvement (Katz et al. in press). Combing disparate data sources created challenges ranging from data standardization (what defines a project or location) to data validation. Despite these difficulties, the database allowed us to select a subset of projects (47) for interviews with individuals directly involved in restoration (Rumps et al., in press). Projects were candidates for interviews if they: a) were implemented or completed between 1996-2002; b) had project contact information; and c) listed at least one of four selected project goals (riparian management, water quality management, in-stream habitat improvement or channel reconfiguration (Bernhardt et al. in press).

Results

Database

The spatial distribution of projects is not uniform across the landscape (Figure 2). We found higher project densities and a greater diversity of project types in the western portions of Oregon (~50% of total), and Washington (~30% of total).

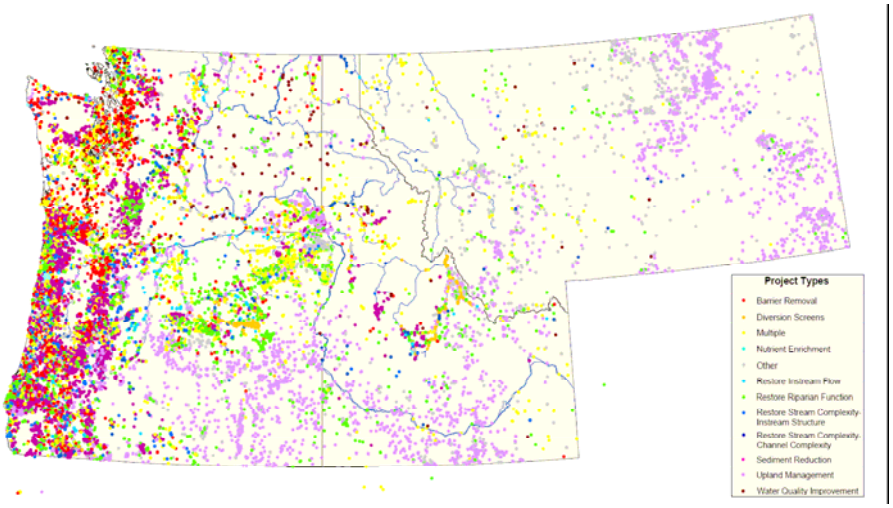

Figure 2. Spatial distribution of 23,126 restoration project records having 35,696 work sites.

In total, 80% (27,798) of project locations occurred in watersheds with anadromous fish, even though those watersheds were less than half the total area without anadromous fish (320,863 km^2 vs. 683,018 km^2) (Fig. 3). Further, over two-thirds of all locations are in the far western portion of Oregon and Washington, an area that correlates spatially with both the Northwest Forest Plan (created to respond to the threatened status of spotted owls) and part of the anadromous zone. Greater than 75% of all projects in each type category occur in anadromous areas with the exception of Upland Management and Other types. Areas without anadromous fish had no nutrient enrichment projects and only 1 diversion screen project.

In answer to the question 'When did the project start/ end?' eighty-five percent of the projects reported project completion year. The number of projects completed per year increases from the 1980's to 1998 then levels off until 2003 (Fig. 4). It seems likely that the trend of 2000 to 3000 projects completed per year continues beyond 2003. Information is insufficient to conclude that the decrease in 2003 and 2004 is a real change in restoration activity, rather than a product of a time lag in reporting. This suggests that restoration and habitat improvement projects may be increasing in number over the entire 10 to 15 year time period. We collected data prior to 1992 opportunistically; consequently, sparser records for that period may represent lesser restoration activity, lower effort in data collection, or a combination of the two.

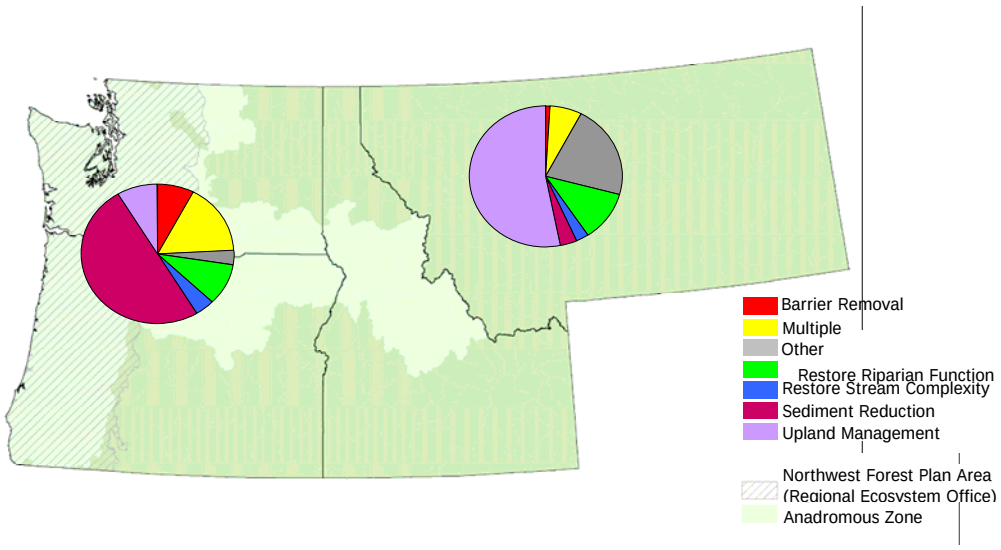

Figure 3. The relative abundance of project types inside and outside areas with ESA listed species.

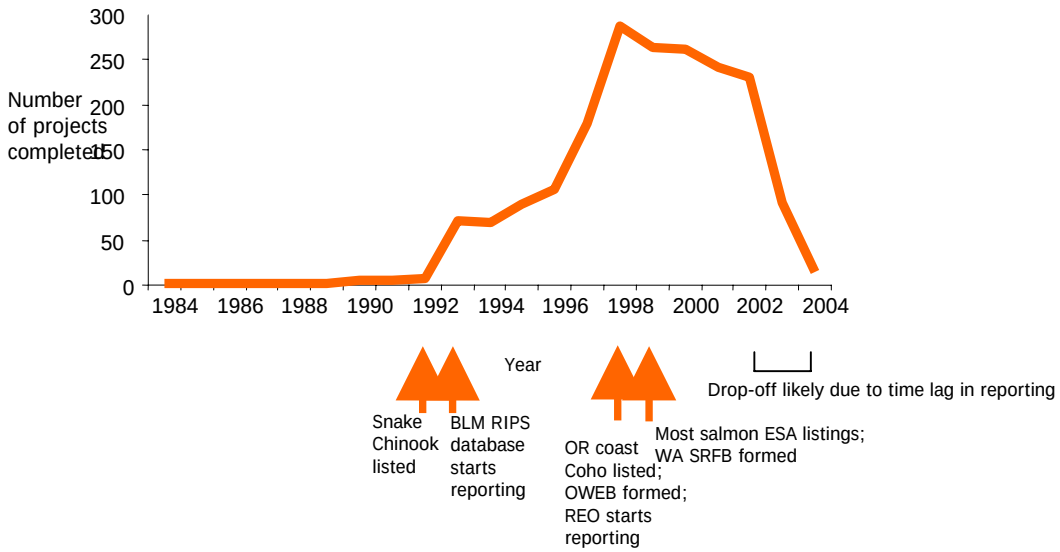

Figure 4. Time series of completion dates for PNW database restoration projects.

Examining more closely the total number of projects completed in a given year, we find events such as salmon ESA listings and the creation state level restoration funding boards and federal restoration databases coincident with the inflection points in Fig. 4. It is impossible to tease apart if the fluctuations in data represent increased effort in implementing habitat restoration, increased effort in reporting of projects or both (Fig. 4).

Of all project records, only 1,569, or 6.7% reported any type of monitoring. We did not request monitoring data from data donors so these numbers only represent where data donors volunteered it. However, this result should not surprise given that two of the largest contributors to the database, REO (24% of total) and BLM (20% of total) do not record any monitoring information.

Interviews

Half of the 47 individuals interviewed represented federal agencies (48%) the other half consisted of state (9%) and local government agencies (19%), tribes (2%), NGOs (13%), and timber industry representatives (9%).

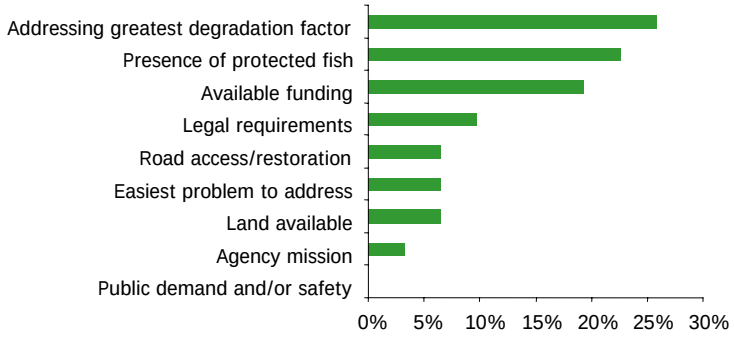

Figure 5. Distribution of factors that motivated the restoration project.

The greatest motivating factors for restoration were addressing the greatest degradation factor, protected fish, and funding availability (Fig. 5). Further, 66% of respondents anticipate a need for on-going project maintenance, but less than half have maintenance funds available.

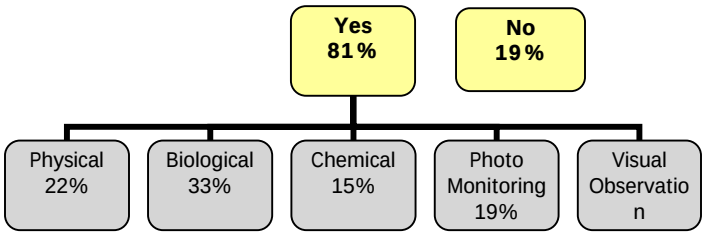

Figure 6. Distribution of answers to questions pertaining to monitoring; specifically, "did you monitor", and if yes, "what was monitored?"

Absent a regional standard for what constitutes "implementation monitoring" or "effectiveness monitoring", answers to the question 'Did you monitor' reflect a diversity of definitions in use. Among those that monitored, 70% monitored physical, biological, or chemical (water quality) data (Figure 6). One-third of the projects surveyed did not conduct sufficient monitoring to evaluate effectiveness (measurement of pre- and post- physical, biological, or chemical/water quality parameters). When asked about the duration of the monitoring, 18% of respondents answered a single observation or "one-shot deal." i.e., monitoring for implementation only (Fig. 7). This suggests that almost 1 out of 5 respondents do not discriminate between effectiveness and implementation monitoring.

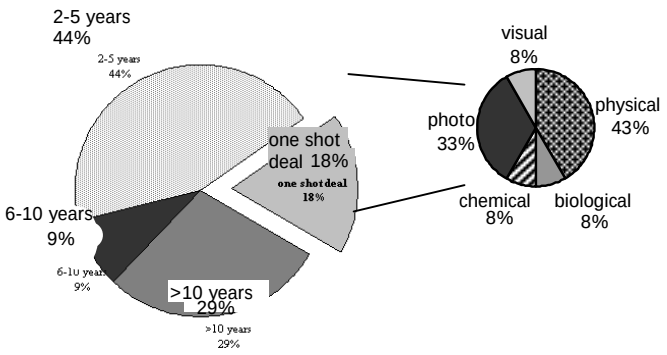

Figure 7. Distribution of interviewee answers regarding the duration of monitoring

While a large majority (70%) of respondents reported successful projects, 43% either had no success criteria or were unaware of any criteria for their project. These findings suggest that establishing a connection between project implementation and effectiveness is currently not a component of project design.

Discussion

The Driving Role of the Endangered Species Act

The role of ESA is evident in the timing of projects coincident with salmon listings, the initiation of restoration programs, the spatial distribution of projects, and the factors motivating restoration as provided by the interviewees. Habitat restoration increased coincident in time with Endangered Species Act (ESA) listings of salmonid fishes (Fig. 4). Moreover, the concentration of project records in the western portion of the region clearly surpasses that of the eastern portion suggesting that ESA listings (both salmon and spotted owl) also influenced the location of projects (Fig. 3). Though the impetus for restoration provided by the ESA is not surprising, the geographic concentration and diversity of projects in anadromous areas provides a measure of impact in more tangible terms than previously available.

The Need for Effectiveness Monitoring

Currently there is no regional format for reporting restoration with numerous consequences for regional assessments. Project reporting lags eighteen months from completion to data availability (Fig. 4), even without the additional time required to migrate the data into a single format. Additionally, in the database effort we found little documentation of monitoring activity, but interviews indicated that more monitoring may be occurring. The interviews allowed us to elicit the views of those who worked closely on projects, but their subjective expressions of success do not replace rigorous effectiveness monitoring. Overall, interviewees were very positive about the outcomes of their activities even though less than half of those interviewed had used any pre-implementation performance criteria. Despite this, most of the monitoring described in the interviews was not sufficient to evaluate effectiveness. If monitoring is used to identify which types of actions work (or do not work) and under what circumstances planners can expect success in future restoration, then key elements of restoration design can be maintained through the prioritization, implementation, monitoring, and assessment phases of projects. Our survey demonstrates that in the PNW there is still room for this maturation.

Further, acceptance of a regional standard for project data, and a single database host could motivate, or at least empower, more timely project implementation monitoring—a prerequisite for effectiveness monitoring of project success. We hope that lessons learned from this project will speed development of a single project tracking system for the Pacific Northwest.

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Estimates of specific toxicity in several *Pseudo-nitzschia* species from the Washington coast, based on culture and field studies

Keri Baugh, Jeannie Bush, Brian Bill, Kathi Lefebvre, and Vera Trainer
Northwest Fisheries Science Center
Environmental Conservation Division
Marine Biotoxin Program
Seattle, Washington
keri.baugh@noaa.gov

To gain an understanding of the species of *Pseudo-nitzschia* that are the major toxin producers in the Pacific Northwest, representative isolates from the Ecology and Oceanography of Harmful Algal Blooms in the Pacific Northwest (ECOHAB-PNW) study site were grown as clonal cultures. Both particulate and dissolved domoic acid (DA) were measured at various stages of growth using a receptor-binding assay and an enzyme-linked immunosorbent assay respectively, and compared to toxin data obtained during a cruise in September 2003. A clonal *P. multiseries* culture isolated from a northern beach site contained the highest particulate DA (pDA) level of 70.4 nmol l^{-1} and released the highest amount of dissolved DA (dDA, >5 nmol l^{-1}), illustrating the potential for this species to cause harm. A clonal *P. australis* isolate also produced relatively high levels of pDA (3.2 nmol l^{-1}) and dDA (4.3 nmol l^{-1}), whereas *P. delicatissima, P.* cf. *pseudodelicatissima*, *P. pungens* and *P. fraudulenta* produced low or undetectable levels of pDA and dDA. These culture studies suggest that the dDA levels of up to 17.6 nmol l^{-1} measured during September 2003 were owing to toxin produced and released primarily by *P. australis* cells. It is estimated that the maximum specific toxicity reached by *P. australis* at a toxic 'hot spot' off the Washington coast in mid-September 2003 was 94.4 pg $cell^{-1}$.

The first closure of shellfish harvesting due to elevated levels of domoic acid in Puget Sound, Washington

Brian D. Bill, Vera L. Trainer, and Shelly Nance
Northwest Fisheries Science Center
Environmental Conservation Division,
Marine Biotoxin Program
Seattle, Washington
brian.d.bill@noaa.gov

Frank H. Cox and Jerry A. Borchert
Washington State Department of Health
Food Safety and Shellfish Programs
Olympia Washington

Rita A. Horner
University of Washington
School of Oceanography
Seattle, Washington

The first domoic (DA)-related closure of shellfish harvesting in Puget Sound, Washington, occurred in September 2003. Subsequent beach and shipboard sampling in the area revealed a near mono-specific bloom of the diatom *Pseudo-nitzschia australis*. DA was detected in shellfish, including blue mussels, littleneck, geoduck, and manila clams, and Pacific oysters, over an 80 km^2 area from September 2 through October 22. Cell concentrations as high as 2.9×10^6 cells $liter^{-1}$ and particulate DA as high as 14.7 nM were detected. Since the early 1990s, *Pseudo-nitzschia* has usually been present in Puget Sound as multiple species populations and low levels of toxicity have been detected in shellfish, but such highly toxigenic, nearly mono-specific blooms have not been observed previously. We speculate that a more toxic oceanic strain may have been advected from the Pacific Ocean or that local environmental conditions at that time may have been more conducive to toxin production.

Macroparasite communities as an indicator of juvenile Chinook salmon life histories and habitat use in the Columbia River estuary

Mary Bhuthimethee
Oregon State University
Cooperative Institute for Marine Resources Studies
Newport, Oregon
mary.bhtuhimethee@oregonstate.edu

Kym C. Jacobson
Northwest Fisheries Science Center
Hatfield Marine Science Center
Fish Ecology Division
Newport, Oregon

Juvenile Pacific salmon utilize the Columbia River and its estuary as an important transition habitat to acquire resources for survival in the ocean. However, little is known about juvenile salmon life histories and habitat use in this important estuary. Because the river is also used as a shipping channel and to generate hydroelectric power, it is imperative to understand how salmon rearing habitats are affected by dredging and management of river flows. In this study we examined the macroparasite communities of subyearling Chinook salmon at different habitats in the Columbia River estuary to provide insights into migrating juvenile salmon life histories, residence time, and habitat use. Monthly sampling showed a change in the salmon parasite community at the Lower Elochoman Slough site (LES, upper estuary) in 2002 and 2003, suggesting a turnover in salmon life history types at that location. Salmon appeared to move into the estuary in late winter-early spring with similar parasite communities, or were resident at LES long enough to acquire similar parasites. An abrupt absence of acanthocephalans in June from salmon intestines suggests that fish were at LES for no more than 3–4 months and left as a group. These parasite communities may distinguish differences in diet and habitat utilization between hatchery-reared and naturally-produced salmon.

Preliminary findings from a study of juvenile rockfish (genus *Sebastes*) feeding habits off the Oregon Coast

Keith L. Bosley
Northwest Fisheries Science Center
Fishery Resource Analysis and Monitoring Division
Newport, Oregon

Ahna Van Gaest
Victorian Marine Science Consortium
Victoria, Australia

Richard D. Brodeur
Northwest Fisheries Science Center
Victorian Marine Science Consortium
Fish Ecology Division
Newport, Oregon

Chris Harvey
Northwest Fisheries Science Center
Fishery Resource Analysis and Monitoring Division
Seattle, Washington

Early life history stages of marine fish suffer high mortality rates making this period a critical determinant of year-class strength. Despite this importance, we know very little about the feeding habits of young-of-the-year rockfish (*Sebastes* spp.)—information crucial to understanding how these fish may be influenced by bottom-up processes. We conducted a study of the feeding habits of juvenile rockfish collected in 2006 off the Oregon coast by examining stomach contents and carbon and nitrogen stable isotope ratios of white muscle tissue.. Our objectives were: to determine whether there is significant interannual variation in the feeding ecology of juvenile rockfishes; to determine whether there are variations in the feeding ecology of rockfishes on either broad or localized scales, and to determine whether there is significant overlap in the feeding ecology of co-occurring rockfishes or of co-occurring rockfishes and other micronektonic fishes. It was hoped that these results may serve as a basis for continued studies during yearly surveys to see how feeding ecology may change with changes in fish population abundance and oceanographic conditions

Material and Methods

Pelagic juvenile rockfish were collected along two transects, off Willapa Bay (Wash.) and the Columbia River (Ore.), during the 2006 Northwest Fisheries Science Center's GLOBEC Predator Surveys in July (Figure 1). Rockfish were collected using a Nordic-264 rope trawl (30-m wide by 18-m deep) towed for 30 minutes. Upon collection, rockfish were immediately frozen on ship (-20ºC) and later taken to the laboratory for processing.

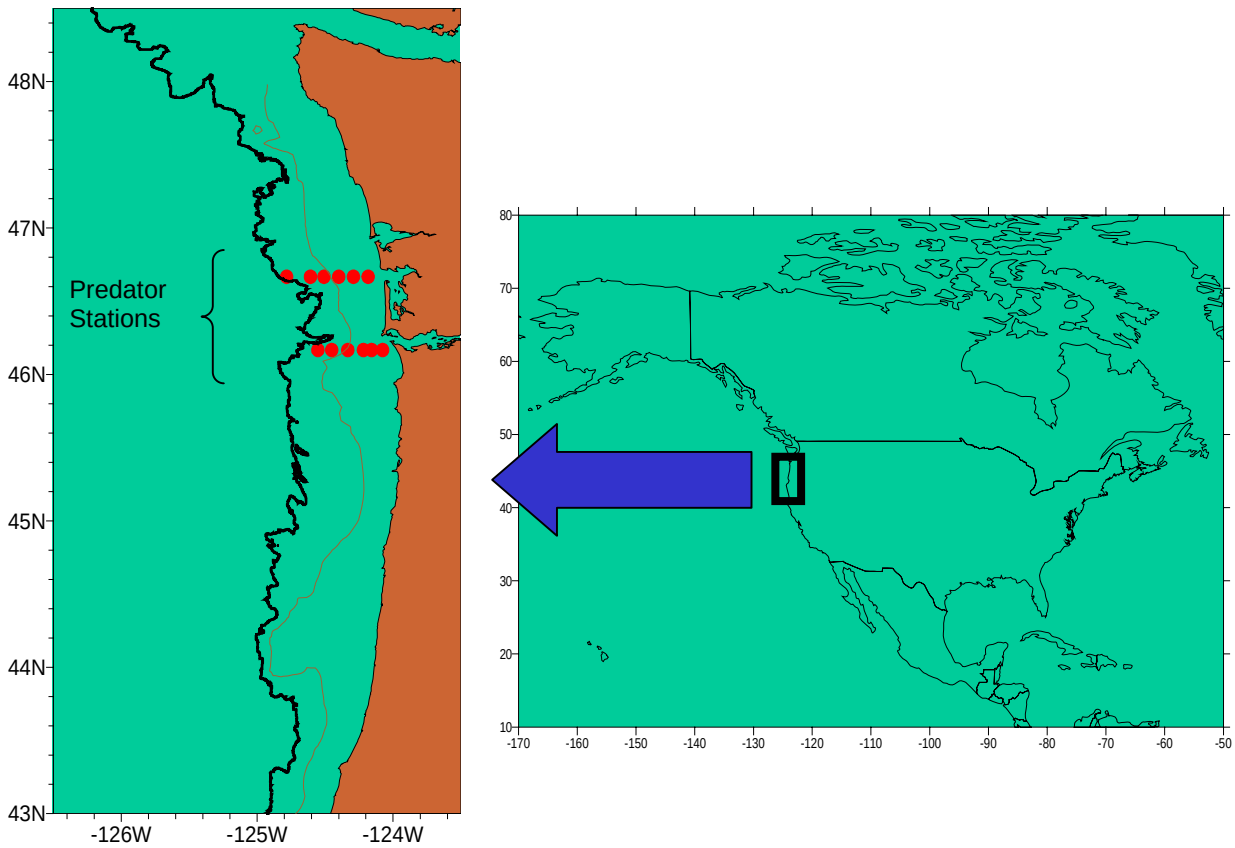

Figure 1. The location of transects sampled during the 2006 GLOBEC Predator Cruises.

Rockfishes were tentatively identified in the laboratory using morphometrics and meristics. For fish that were too difficult to identify from visual characteristics alone, a fin clip was stored in pure ethanol and sent to the NWFSC Conservation Biology Division genetics laboratory (Linda Park) for positive identification. Genetic species identification (performed by Anna Elz) was based on DNA sequence analysis of the cyt-b mitochondrial region (Rocha-Olivares et al. 1999) and the nuclear S7 ribosomal protein intron 2 (Chow and Hazama 1998). Laboratory processing of rockfishes involved measurement of individuals, dissection of white muscle tissue from the left side, just below the dorsal spines, for later isotope analysis, and extraction of stomachs for diet analysis. Lengths of fish were measured (±1.0 mm) using standard length (SL). A total of 157 rockfish stomachs were examined. Stomachs were extracted and immediately placed in 70% ethanol. Diet analysis was performed by assessing fullness, digestive condition, and identification and quantification of prey taxa in each stomach. Fullness was assessed using a scale of 0-5, with 0 being empty, 4 full, and 5 distended. Digestive condition of individual prey was assessed using a 0-4 scale, with 0 being unrecognizable and 4 being fresh. Prey was identified to lowest possible taxon, enumerated, and weighed (±0.0001g). White muscle tissue samples were dried and ground to a fine powder, and subsamples (~0.40 mg) were loaded into tin capsules for analysis at the NWFSC stable isotope facility in Seattle, Wash.

Results

The predominant species collected in 2006 were darkblotched (*S. crameri*), canary (*S. pinniger*), yellowtail (*S. flavidus*), widow (*S. entomelas*) and black (*S. melanops*) rockfish. By percent number (Figure 2a): canary, yellowtail, and widow have similar diets (unidentified copepods and euphausiid eggs). Darkblotched rockfish feed primarily on the larger life history stages of euphausiids, with a surprisingly large proportion of *Euphausia pacifica* adults (do they avoid *Thysanoessa spinifera*?), and a smaller proportion of copepods than the other three species presented. Hyperiid amphipods are the third most abundant taxa found in the diet of darkblotched, while barely registering in the diets of the other three species. By percent wet weight (Figure 2b), there are similar patterns: euphausiid eggs make up a smaller relative proportion of the diets of canary, yellowtail and widows, which would be expected as euphausiid eggs are the smallest significant prey item counted. Euphausiids (unidentified, *E. pacifica*, and *T. spinifera*) make up a larger proportion of all rockfish diets than when examined by % number because of their large size.

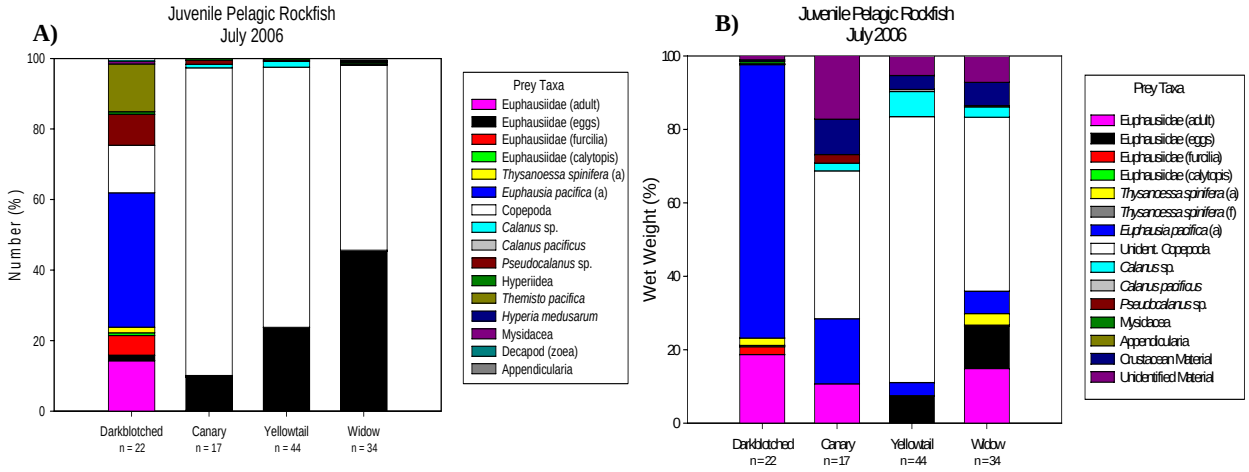

Figure 2. Comparison of juvenile pelagic rockfish diets as percent (%) number (A) and % wet weight (B) for the four dominant species collected in July 2006. Life history stages of prey are noted in parenthesis.

There was significant overlap in the diets of canary and widow, canary and yellowtail, and yellowtail and widows as a function of percent number (Table 1). Diet overlap between darkblotched and the other three species was low. There was considerable overlap between canary and widow, and moderate overlap between canary and yellowtail and yellowtail and widow rockfish as a function of percent wet weight as well (Table 2). The diet overlap between these three species is most likely a response to the large proportion of unidentified copepods in each diet. The diet overlap between darkblotched and the other three species of rockfish was low to medium when considered by wet weight. Stable isotope results confirm that all were feeding at the same trophic level (as indicated by nitrogen) and darkblotched had the most enriched carbon signal, perhaps reflecting their more varied diet (Figure 3).

Table 1. Percent Similarity Index (PSI) of juvenile rockfish diets generated from % number of each prey category. PSI values >60% (in bold) designate diet overlap considered significant.

Species	Darkblotched	Canary	Yellowtail	Widow
Darkblotched	X			
Canary	17.5	X		
Yellowtail	15.7	**84.6**	X	
Widow	17.4	**62.1**	**76.4**	X

Table 2. Percent Similarity Index (PSI) of juvenile rockfish diets generated from % wet weight of each prey category. PSI values >60% (in bold) designate diet overlap considered significant.

Species	Darkblotched	Canary	Yellowtail	Widow
Darkblotched	X			
Canary	30.0	X		
Yellowtail	5.1	55.1	X	
Widow	44.4	**71.7**	56.0	X

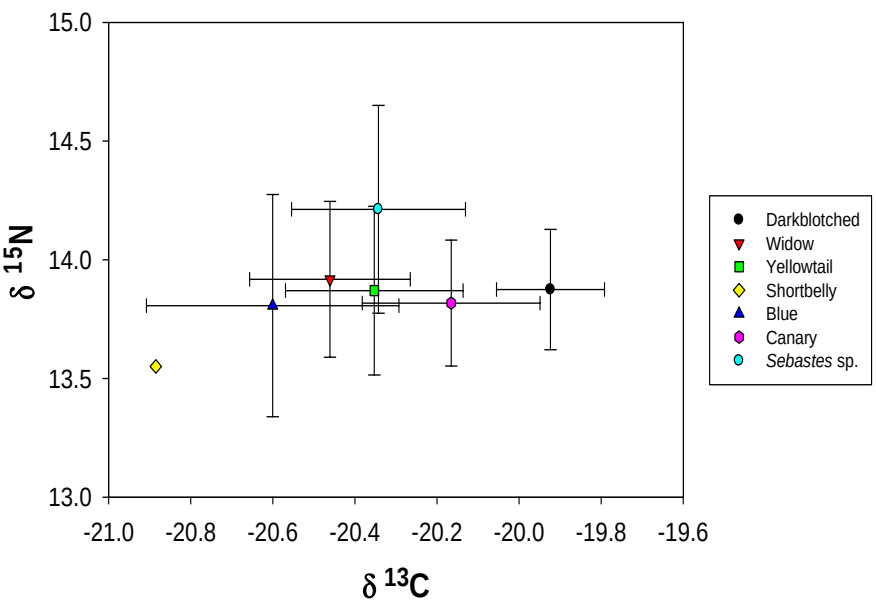

Figure 3. Mean δ13C vs. δ15N of rockfishes collected during the July 2006 GLOBEC Predator Cruises. Error bars are ±1 standard deviation.

Discussion

Darkblotched tend to eat lower numbers of larger prey (Euphausiid adults & furcilia), while canary, widow, and yellowtail rockfish tend to eat high numbers of smaller prey. The darkblotched rockfish collected on the Predator cruises tended to be larger (mean SL = 54.6 ± 3.1mm) than the other species, both in length and depth (depth was observed but not measured), however there is some size overlap between darkblotched and widow. Canary rockfish were the

smallest of any species (mean SL = 38.6 ± 2.8mm) and had the least number of prey taxa in their diets. Samples of zooplankton collected on a separate cruise from the same area around the time of rockfish collections are being processed to provide stable isotope data of potential prey items. Results will be combined with data from similar samples collected along the Oregon Coast during 2000 and 2002 (Miller 2007) to look for temporal and spatial variations in feeding ecology as outlined in the objectives. Gut content and stable isotope data are being collected from co-occurring mesopelagic fishes to provide a more complete picture of the pelagic foodweb.

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Toxic nurseries: Do English sole from urban nurseries recruit to the adult population?

Paul M. Chittaro, Rachel J. Petrik-Finley, and Phillip S. Levin
Northwest Fisheries Science Center
Fishery Resource Analysis and Monitoring Division
Science for Ecosystem-based Management Initiative
Seattle, Washington
paul.chittaro@noaa.gov

Determining where fish live during early life history stages and the extent to which different areas contribute individuals to adult populations is critical for the management and conservation of a species. To establish where subadult English sole reside as juveniles, we used otolith chemistry of juvenile English sole to produce a chemical map of Puget Sound against which subadult otolith chemistry was compared. Examination of the otolith chemistry in subadult fish provided evidence that northern Puget Sound sites and non-urban areas are important for the maintenance of adult populations of English sole.

Guaranteeing seafood safety after Hurricane Katrina

Tracy Collier, Margaret Krahn, Donald Brown, Catherine Sloan, and Gina Ylitalo
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
gina.ylitalo@noaa.gov

Mark Strom, Rohinee Paranjype, and William Nilsson
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington

Usha Varanasi
Northwest Fisheries Science Center
Science Director's Office
Seattle, Washington

The de-watering of New Orleans following Hurricane Katrina created an unprecedented situation regarding the public perception of seafood safety. The floodwaters pumped into nearshore areas of the Gulf of Mexico contained oils, metals, and a wide range of pathogens and enteric bacteria. The Northwest Fisheries Science Center was asked to mount an immediate response to this situation, both to collect samples of fish and shrimp from the potentially affected area, as well as to provide a wide range of chemical and microbiological analyses. We conducted several sampling efforts from a variety of research vessels and chartered fishing boats, and focused on Atlantic croaker (*Micropogonias undulatus*) and white shrimp (*Litopenaeus setiferus*) as the primary target species. Sampling began on 13 September 2005, within one week after floodwater pumping began, and continued into December 2005. Samples were analyzed for a suite of organic contaminants, including organochlorines, PBDEs, and PAHs. Samples were also tested for bacterial contamination, including potentially pathogenic Vibrio species and fecal contaminants such as *E. coli*. While a range of both chemicals and microbiological substances were detected, none were present in edible tissues at levels that appeared to pose any appreciable risk to human consumers of seafood products from the Gulf, assuming normal seafood preparation practices were followed. We found no evidence of increased levels of contaminants immediately following the hurricane, however the lack of baseline data hampered that determination. Sampling resumed in April 2006 to determine if this trend persists.

Evaluating the intrinsic potential of steelhead spawning within interior Columbia Basin streams

Tom Cooney and Damon Holzer
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington
tom.cooney@noaa.gov

Within the Interior Columbia Basin (ICB), steelhead (*Oncorhynchus mykiss*) spawn and rear in streams flowing through a broad spectrum of natural environments. Even with the modern spatial constriction of available habitat, spawning occurs in landscapes ranging from low elevation semi-arid basins to moisture rich alpine cirques, often in the same watershed. This ecological kaleidoscope of potential occupancy has made habitat modeling a challenging task. However, certain common geomorphologies exist within favored spawning and rearing streams even among highly divergent ecotypes. In order to evaluate steelhead habitats within ICB populations, we developed a GIS based model for calculating intrinsic spawning potential. This metric has enabled us to quantify and qualify habitat based on how spawning preferences relate to local stream features and watershed characteristics. Of primary interest were datasets describing spawning distribution, instream geomorphology, and adjoining landforms, from which we established model parameters by comparing mapped steelhead distribution to stream physiography. In general, we utilized spawning surveys and stream-transect juvenile sampling data to describe relative densities of steelhead in geospatially specific stream reaches. Mapped distributions were then evaluated against stream attributes calculated from common spatial data themes. These included Digital Elevation Models (DEM), the National Hydrography Dataset (NHD), climatic data from the National Climatic Data Center (NCDC), and soil features from the Natural Resource Conservation Service (NRCS). Using the results from this analysis, we were able to identify a set of significant relationships between observed spawning use and landscape attributes. These correlations formed the rationale for how our model was calibrated.

Life stage and odorant-induced changes in odorant receptor expression and olfactory sensitivity in coho salmon (*Oncorhynchus kisutch*)

Andrew Dittman and Jaime Athos
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Physiology Program
Seattle, Washington
andy.dittman@noaa.gov

Darran May
University of Washington
School of Aquatic and Fisheries Sciences
Seattle, Washington

David Baldwin and Nat Scholz
Northwest Fisheries Science Center
Environmental Conservation Division
Ecotoxicology Program
Seattle, Washington

Over the lifetime of an organism, the sensitivity of the olfactory system to specific odors may change in response to developmental changes, hormones, environmental stimuli, and odorant exposure. Salmon provide an excellent model for studying such changes because almost every aspect of their lives is influenced by olfaction and they experience dramatic developmental and environmental transitions (smolting, maturation, freshwater vs. oceanic rearing). Furthermore, the homing migrations of salmon are governed by olfactory discrimination of home stream odors that juvenile salmon learn (imprint to) prior to their seaward migrations. Our previous studies demonstrated that salmon imprinted to the odorant phenylethyl alcohol developed a long-term sensitization of peripheral olfactory neurons to this odorant. To further examine the mechanism of peripheral sensitization during imprinting, we exposed juvenile coho salmon to L-Arginine during smolting, the presumptive sensitive period for imprinting. Arginine is a potent salmon odorant for which a candidate odorant receptor has been identified. To assess life stage and olfactory imprinting associated changes in the sensitivity of the olfactory system to arginine, we recorded electrical field potentials (electro-olfactograms) generated in response to arginine and measured mRNA expressions levels of the candidate arginine receptor at several life stages. Our results suggest that olfactory sensitivity and odorant receptor expression changes over the lifetime of the salmon and that previous odor exposure can influence olfactory responses.
Funded by the BPA, the NWFSC, and the HSRG.

Can hatchery supplementation promote recolonization of underutilized spawning habitat? Homing patterns of hatchery-reared and wild Yakima River spring Chinook salmon

Andrew Dittman and Donald Larsen
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Physiology Program
Seattle, Washington

Darran May
University of Washington
School of Aquatic and Fisheries Sciences
Seattle, Washington
darran.may@noaa.gov

Mary Moser
Northwest Fisheries Science Center
Fish Ecology Division
Migrational Behavior Program
Seattle, Washington

Mark Johnston and David Fast
Yakama Nation
Toppenish, Washington

A number of conservation and supplementation hatchery programs utilize satellite acclimation facilities to "seed" or repopulate underutilized rivers or streams. The effectiveness of releases from satellite facilities for ensuring successful imprinting, minimizing straying and contributing to salmon recovery has not been demonstrated. The goal of this project was to describe the spatial and temporal patterns of homing and spawning by wild salmon and salmon released from acclimation facilities in the upper Yakima River, Washington. Specifically, we examined the spatial patterns of homing and spawning of spring Chinook salmon released as part of the Yakima/Klickitat Fisheries Project (YKFP) supplementation program relative to a central hatchery, 3 acclimation facilities, wild spawning fish and available habitat. Over four years (2002-2005), we comprehensively surveyed the spawning area of the Upper Yakima River spring Chinook population and GPS mapped and sampled (length, sex, origin) every carcass recovered (n=7391; 28.9% of total run). Results from this study indicated that juveniles released from different acclimation sites had significantly different adult spawning distributions within the sub-basin but homing patterns confirmed tradeoffs between homing and habitat selection. Ultimately, these studies will shed light on the efficacy of supplementation to re-establish natural spawning in underutilized habitat. Funded by the NWFSC and NOAA Fisheries.

Application of a polyclonal antibody in the development of methods for detecting domoic acid

Bich-Thuy Eberhart, Sylvester Spencer, John Wekell, and Vera Trainer
Northwest Fisheries Science Center
Environmental Conservation Division
Marine Biotoxins Program
Seattle, Washington
bich-thuy.le.eberhart@noaa.gov

Rick Stevens, Scott Solberg, and Clem Furlong
University of Washington
Department of Genome Sciences and Medicine
Division of Medical Genetics
Seattle, Washington

Polyclonal antiserum raised in rabbits against ovalbumin (OVA)-domoic acid conjugate was employed in the development of methods to detect domoic acid (DA), a neurotoxin produced by diatoms of the genus *Pseudo-nitzschia*. The DA molecule was coupled to the carrier protein (OVA) through one of its three carboxyl groups using a carbodiimide reaction. This immunogen produced an anti-DA serum that is sensitive and specific to free DA. We have successfully used the resulting antibody in an indirect competitive enzyme-linked immunosorbent assay (cELISA) to determine DA levels in contaminated shellfish. This antibody is also being tested in a new surface plasmon resonance (SPR) detector system developed at the University of Washington, which uses minute changes in surface refractive index to detect analytes. Recent advances in SPR sensor technology make it possible to use our anti-DA antibody to develop a small, compact, battery-operated system for real-time monitoring of DA in the field. The SPR detector system is a competition-based assay where a low concentration of antibody is exposed to the immobilized DA conjugate attached on the gold sensor surface. A mixture of antibody plus sample or standard is then added to the flow cell over the sensor. Rates of antibody binding to the domoate immobilized on the sensor surface are determined in the absence and presence of varying concentrations of DA in the sample or standard. Since very dilute concentrations of antibody are needed for the competition assay, many assays may be carried out with small amount of antibody. We are presently testing the prototype unit for establishing DA standard curves, examining matrix effects and determining detection limits.

Confirming sex determination in longspine (*Sebastelobus altivelis*) and shortspine (*S. alascanus*) thornyheads

Erica Fruh and Victor Simon
Northwest Fisheries Science Center
Hatfield Marine Science Center
Fishery Resource Analysis and Monitoring Division
Newport, Oregon
erica.fruh@noaa.gov

Jessica Trantham
The Wildlife Conservation Society
New York, New York

Determining the sex of thornyheads (*Sebastelobus alascanus* and *S. altivelis*) can be difficult at certain times of the year under field conditions. When in the resting stage, thornyhead gonads atrophy, making macroscopic identification difficult and inaccurate. The purpose of this study was to examine thornyhead gonads both macroscopically and microscopically, to test sex determinations and obtain an error rate for field classifications. A total of 2330 longspine and shortspine thornyheads were retained from the 2003 Northwest Fisheries Science Center (NWFSC) West Coast Groundfish survey. Specimens were collected throughout the latitudinal range (32º34.2'-48º26.4' N) at depths of 88-1,280 m from June to October. Fork length ranged from 7 to 35 cm for longspine and 7 to 72 cm for shortspine thornyhead. In the laboratory, frozen specimens were thawed, dissected and an initial determination of sex made macroscopically (following a procedure similar to that used in the field on unfrozen samples). Subsequently, a portion of the gonadal material from each fish was placed on a slide and stained with aceto-carmine. The stain's differential absorption by gonadal tissue allowed oocytes to be easily identified using a microscope. An error rate of 13% was found, with the majority of the incorrectly sexed fish being small, less than 25 cm in length. Throughout the sampling period, the highest frequency of misidentification occurred in fish collected in the months of June and October. There was also a higher incidence of misidentification in fish caught above 40º N latitude.

Out of sight, out of mind: Derelict fishing gear and its impacts on the marine fauna of the Puget Sound-Georgia Straits Basin

Thomas P. Good
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington
tom.good@noaa.gov

Jeffrey A. June
Natural Resource Consultants
Seattle, Washington

Ginny Broadhurst
Northwest Straits Commission
Mt. Vernon, Washington

Introduction

Derelict fishing gear, recreational or commercial fishing gear that is lost or abandoned in the environment impacts marine ecosystems worldwide. It is a significant threat to marine fauna (U.S. Commission on Ocean Policy 2004) and is degrading the Pacific region's economic and ecological resources (Asia-Pacific Economic Cooperation 2004). Modern synthetic fishing gear can take from decades to centuries to decompose and can impact marine ecosystems by killing invertebrates, fish, birds, and mammals as well as degrading marine ecosystems and habitats.

The Puget Sound gillnet fleet may have lost 9,000 full-size nets (1,800' x 100') over the past 30 years; 117,000 derelict nets and pots weighing ca. 2.6 million pounds may lie beneath the waters of Puget Sound/Hood Canal (WDFW, unpubl. data). Inventory and removal operations have estimated that 8–10% of the Dungeness crab fishery is lost to derelict pots in some areas (Natural Resources Consultants 2006). Removing derelict gear is thus an immediate priority action for a healthy Puget Sound by 2020 (Puget Sound Partnership 2006).

Materials and Methods

Reporting occurs from a variety of local sources, with the Washington Department of Fish and Wildlife maintaining a reporting database. Locating the derelict gear is accomplished via a GPS-referenced data linked to onboard Nobeltec™ navigation software. Derelict gear is then recovered using boats with a hydraulic winch and divers with surface-supplied air & two-way radio communications, float bags, and cutting tools. During recovery, data is collected on gear location, type, length and width, legality, and owner ID; all entangled fauna is identified during recovery or later in the laboratory via examination of tissue and skeletal elements.

Results

Although the area surveyed represents < 1% of the normal pot and net fishing grounds in the Puget Sound/Northwest Straits region, over 3900 derelict fishing nets and pots have been

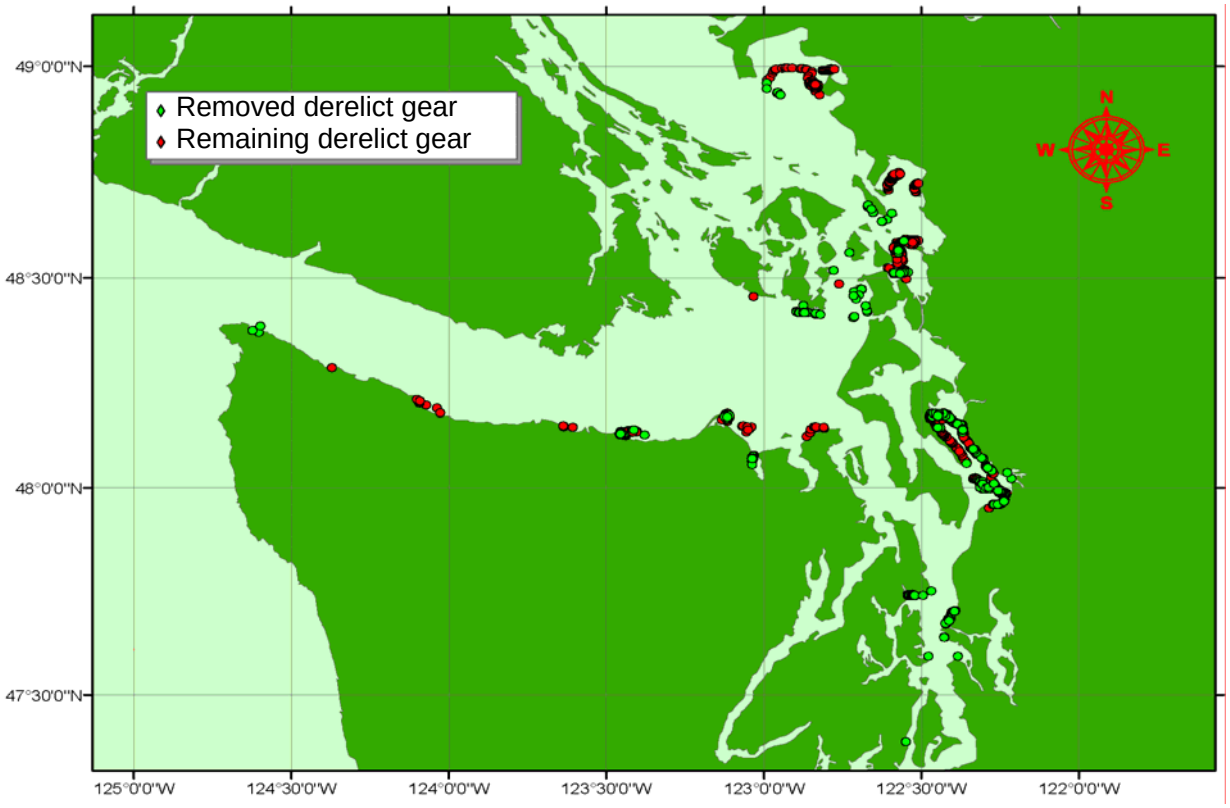

Figure 1. Map of the Puget Sound and Northwest Straits showing locations of gear removed (n=1323) and remaining (n=2581) in the (as of 12/2005).

documented in the Northwest Straits since 2002 (Fig. 1).

Of the derelict gillnets recovered from the Puget Sound-Northwest Straits region, 76% were <15,000 ft^2 in area; a few (n=7) were 55,000 - 150,000 ft2 in area (Fig. 2).

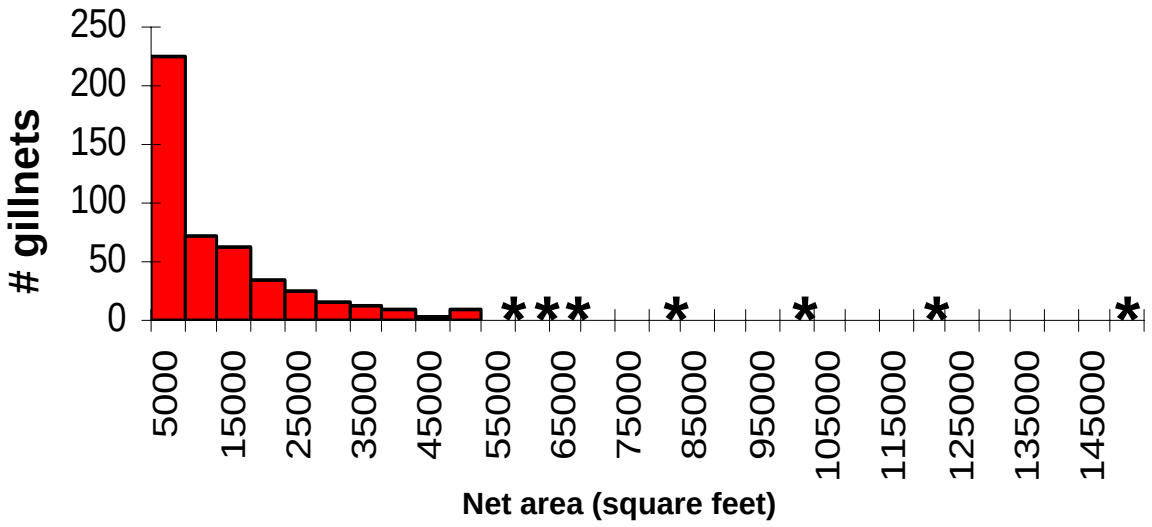

Figure 2. Size distribution of 473 derelict gillnets recovered from Washington's inland marine waters (* = 1 net).

Patterns of lethality were influenced by derelict gear type, net age and varied among marine taxa. Newer nets were much more likely to be lethal (301/305) than older nets (81/168; $\chi 2=177$, df=1; $p<0.001$). Lethality (% organisms found dead) was greater in derelict nets (n=473 nets; Fig. 3a) than in derelict pots (n=980 pots; Fig. 3b). For derelict nets, lethality (% organisms dead) was high for marine invertebrates (69%; n=6374) and fishes (93%; n=851) but was complete for birds (100%; n=120) and mammals (100%; n=12; Fig. 3a). For derelict pots, lethality (% organisms dead) was greater for fishes (33%; n=12) than invertebrates (17% n=2062; Fig. 3b).

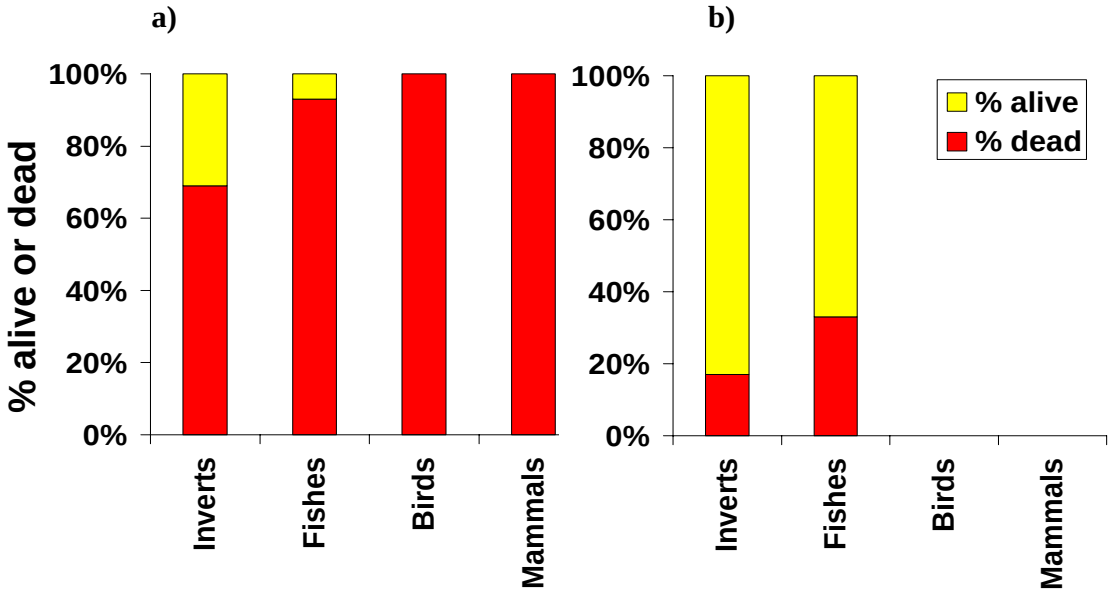

Figure 3. Mortality of marine taxa in a) derelict pots and b) derelict nets removed from the waters of Puget Sound and the Northwest Straits from 6/2002 – 10/2006.

Derelict nets and pots captured and killed at least 27 species of marine invertebrates, 25 species of marine fishes, 10 species of marine birds and 3 species of marine mammals (Tables 1 - 4).

Table 1. Marine invertebrate species identified in derelict gear (nets & pots) recovered from the inland marine waters of Washington.

	Alive	Dead
Giant Barnacle	982	2447
Butter Clam	0	766
Dungeness Crab	1748	558
Red Rock Crab	594	417
Smooth Pink Scallop	15	263
Oregon Triton	96	136
Kelp Crab	55	45
Longhorn Dec. Crab	44	31
Pacific Littleneck Clam	0	27
Puget Sound King Crab	4	14
Leafy Hornmouth	43	12
Lyre Crab	35	12
Nuttall's Cockle	0	8
Starfish spp.	28	7
Heart Crab	2	4
Northern Kelp Crab	5	2
Sunflower Star	10	1
Golfball Crab	7	1
Brown Box Crab	0	1
Granular Claw Crab	0	1
Tanner Crab	0	1
Spiny Pink Star	4	0
Green Sea Urchin	2	0
Helmet Crab	2	0
Giant Pacific Chiton	1	0
Red Fur Crab	1	0
Pacific Octopus	1	0

Table 3. Marine mammal species identified in derelict gear (nets) recovered from the inland marine waters of Washington.

	Alive	Dead
Harbor Seal	0	8
California Sea Lion	0	2
Harbor Porpoise	0	1
Marine mammal (unid.)	0	1

Table 2. Marine fish, bird, and mammal species identified in derelict gear (nets & pots) recovered from the inland marine waters of Washington

	Alive	Dead
Salmon (unid.)	0	154
Spotted Ratfish	0	111
Flatfish (unid.)	0	92
Fish (unid.)	0	78
Lingcod	2	73
Kelp Greenling	5	57
Spiny Dogfish Shark	1	52
Black Rockfish	0	51
Rockfish (unid.)	7	46
Sockeye Salmon	0	25
Peamouth Chub	0	15
Cabezon	8	14
Sculpin (unid.)	3	6
Red Irish Lord	5	5
Chinook Salmon	0	5
Quillback Rockfish	3	2
Great Sculpin	1	2
Starry Flounder	0	2
English Sole	0	1
Greenling (unid.)	0	1
Puget Sound Rockfish	2	1
Six-gill Shark	0	1
Prickleback (unid.)	31	0
Striped Surf Perch	1	0

Table 4. Marine bird species identified in derelict gear (nets) recovered from the inland marine waters of Washington.

	Alive	Dead
Seabird (unid.)	0	58
Cormorant (unid.)	0	26
Brandt's Cormorant	0	14
Pelagic Cormorant	0	7
Double-crested Cormorant	0	6
Grebe (unid.)	0	4
Gull Unid.	0	2
Common Loon	0	1
Merganser	0	1
Shorebird (unid.)	0	1

Conclusions

- Derelict gear poses a continuous hazard to marine fauna in Washington's inland waters; of derelict gear reported during surveys (< 1% of the fishing grounds), 1/3 of it has been removed.
- 95 acres of critical marine habitat have been recovered by recovering derelict gear; however, gear removal needs to continue, especially for newer, more lethal nets.
- Derelict gillnets are especially lethal for marine fishes, birds, and mammals, a number of which are of commercial and conservation concern.
- Derelict pots form--the majority of the gear in the Puget Sound area--also capture and often kill commercially important species, even though lethality is lower than derelict nets.
- Derelict gillnets capture a diverse assemblage of marine invertebrates, fishes, birds, and mammals.
- Parameterizing survival times of captured animals, deterioration and self-baiting rates, and seasonal animal densities are the necessary next steps to estimating impacts of derelict fishing gear on marine fauna in the Puget Sound and surrounding marine waters.

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Acknowledgments

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Project partners include NOAA, WDFW, Washington Department of Natural Resources, Washington Department of Ecology, Washington Sea Grant, Puget Sound Action Team, Salmon Recovery Funding Board, the Stillaguamish Tribe, Tulalip Tribes, U.S. Navy, U.S. Army Corps of Engineers, U.S. Coast Guard, Hood Canal Salmon Enhancement Group, commercial fishing and diving companies, sport dive clubs, and private foundations.

We thank Dr. Mike Etnier for identifying bone specimens.

Do invasive brook trout impact Chinook salmon feeding habits?

Adam Goodwin, Kate Macneale, and Beth Sanderson
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington

The destructive consequences of invasive species are well known and undisputed, yet the implications of one such invasion in the 1800s have not been examined. A recent study has shown the presence of non-native brook trout (*Salvelinus fontinalis*) has an inverse relationship with juvenile Chinook salmon *(Oncorhynchus tshawytscha*) survival; however the direct impact that brook trout have on this threatened species is uncertain.

In 2004, we examined the stomach contents of >100 fish in four streams in Central Idaho: South Fork Salmon, Curtis, Bear Valley, and Elk Creek (Fig.1), two of which had shown decreased survival correlated to the presence of brook trout. Also, we examined fish densities and aquatic invertebrate drift to determine whether the diet and prey selection of juvenile salmon and steelhead differs with increasing brook trout density.

Brook trout diets did not significantly overlap with that of Chinook salmon, but the diet of native juvenile steelhead trout (*Oncorhynchus mykiss*) and brook trout overlap considerably (48 - 75%) (Fig. 2). In streams with lower brook trout densities, Chinook salmon have a weak preference for mayflies, however this trend does not provide strong evidence for a shift in prey (Fig. 3). The amount of food within Chinook and Steelhead stomachs did not differ significantly across the brook trout density gradient.

Chinook primarily feed from the drift and that trend did not change considerably with greater brook trout densities. Brook trout exhibit more diet overlap with Steelhead than Chinook. Brook trout do not show strong evidence of food competition with Chinook. Our results suggest that predation or a behavioral division of habitat, rather than direct competition for prey, might be the cause of the Chinook's decreased survival rate.

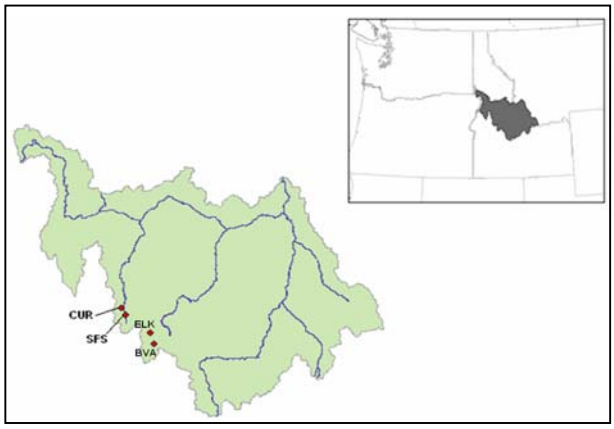

Figure 1. Study sites in the Salmon River watershed. Curtis (CUR), South Fork Salmon (SFS), Elk, Bear

Brook trout have little overlap with Chinook salmon

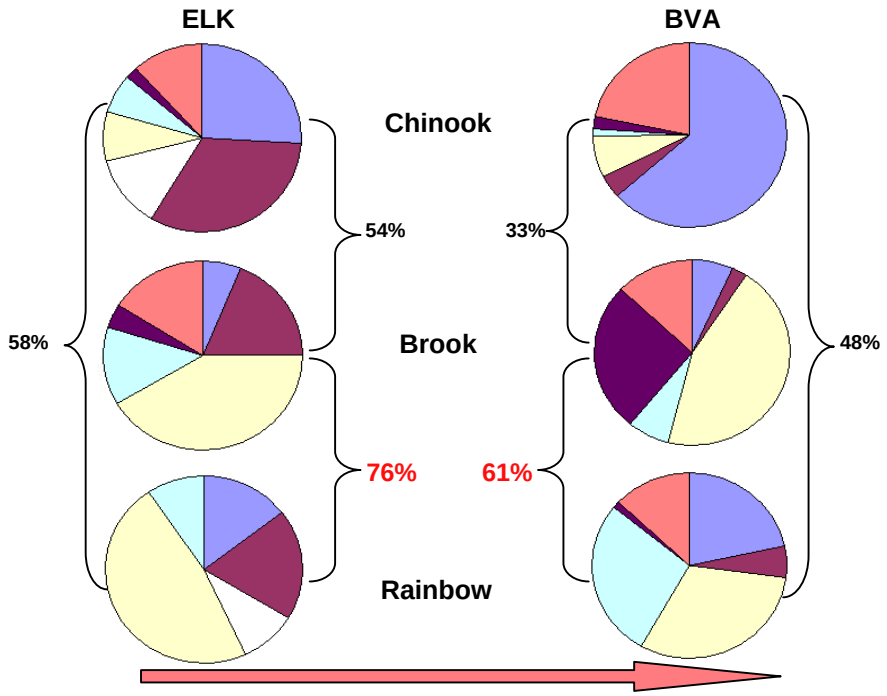

Figure 2: Percentage of overlap that each fish species experiences with other species. Horizontal arrow indicates rising ratio of BK:CH.

Diet selectivity across species and streams

	South Fork Salmon			Curtis		Bear Valley		
	CH	RB	BK	CH	RB	CH	RB	BK
Aquatic Diptera	-0.11	-0.10	-0.31	0.16	-0.25	0.14	-0.27	-0.42
Ephemeroptera	0.14	-0.14	-0.04	-0.17	-0.13	-0.12	-0.10	-0.13
Trichoptera	-0.04	-0.02	0.07	-0.10	0.02	-0.03	0.20	0.34
Other Aquatic Insects	-0.02	0.06	-0.02	-0.01	0.00	-0.06	0.20	-0.01
Aquatic Non-Insects	-0.06	-0.10	0.25	-0.05	0.06	-0.07	-0.08	0.17
Terrestrial	0.09	0.31	0.05	0.15	0.31	0.15	0.06	0.06

Figure 3: Selectivity exhibited for each food item by each species of fish in each stream. Food item's percentage of the diet subtracted by that same item's percentage of the drift. Larger, more positive numbers indicate more selection for this food item. More negative numbers indicate more selection against this food item. Horizontal arrow indicates rising ratio of BK:CH.

Methods and preliminary results from a hook and line survey for shelf rockfish in the Southern California Bight

John Harms
Northwest Fisheries Science Center
Fisheries Resource Analysis and Monitoring Division
Seattle, Washington
john.harms@noaa.gov

Jim Benante and Matt Barnhart
Pacific States Marine Fisheries Commission
Seattle, Washington

Historically, there has been limited information on the abundance of structure-associated rockfish species important to the sport/recreational community within the Southern California Bight (SCB). This region contains a considerable amount of rocky bottom which is the preferred habitat of some important species of rockfish, but it is difficult to sample with the annual trawl survey, so a different method of surveying these habitats needed to be developed. In 2003, the NWFSC, in cooperation with PSMFC and the local fishing industry, initiated a hook and line survey for shelf rockfish within the SCB. Fishermen have provided considerable input into the survey in areas including gear configuration, site selection, and sampling operations. The goals of the 2003 pilot project included assessing the feasibility of a habitat-specific survey, using hook and line gear to sample those habitats, and collecting CPUE and biological information to develop an index of relative abundance for shelf rockfish in the region. In 2004, site selection was modified to target specific GPS locations, and effort was partitioned between fixed and randomly-selected points. In 2006, the survey moved to a wholly fixed site design. Some preliminary data that will be presented include catch rate comparisons for bocaccio and vermilion rockfish between 2004 and 2005, identification of areas of high and low abundances for those species, and a summary of their length frequency distributions during the sampling period.

Advancements in fisheries acoustics: From splitbeam to multibeam

Larry Hufnagle, Nick Tolimieri, Steve de Blois, Rebecca Thomas, Kelly Andrews, and Greg Williams
Northwest Fisheries Science Center
Fisheries Resource Analysis and Monitoring Division
Seattle, Washington
lawrence.c.hufnagle@noaa.gov

The purpose of this project is to develop and test a new acoustic method of surveying rockfishes (*Sebastes*) by using water-column multibeam sonar. During this pilot study, data collected by the multibeam sonar will be compared to data collected by splitbeam echo sounders and diver observations. The long-term aim is to improve NOAA's ability to assess rockfish stocks in areas where traditional methods (e.g., trawling) are unable to sample. Current trawl surveys provide poor information on population status of rockfishes since many (if not most) species primarily inhabit untrawlable, rocky habitat. A number of economically important species including widow (*Sebastes entomelas*), yellowtail (*S. flavidus*), black (*S. melanops*) and dusky rockfish (*S. ciliatus*) show semi-pelagic behavior by inhabiting the water column over rocky structures and kelp—thus making them ideal candidates for acoustic enumeration.

Toxic contaminants in outmigrant juvenile salmon from the Columbia River estuary

Lyndal Johnson, Bernadita Anulacion, Dan Lomax, O. Paul Olson, Julann Spromberg, Catherine Sloan, Sean Sol, Maryjean Willis, Gina Ylitalo, Mary Arkoosh, and Joseph Dietrich
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
lyndal.l.johnson@noaa.gov

Paul Moran
Northwest Fisheries Science Center
Conservation Biology Division,
Seattle, Washington

Frank Loge
University of California, Davis
Department of Civil and Environmental Engineering
Davis, California

Jennifer Morace
U.S. Geological Survey
Oregon Water Science Center
Portland, Oregon

Jill Leary
Lower Columbia River Estuary Partnership
Portland, Oregon

In a collaborative project with USGS and the Lower Columbia Estuary Partnership, NWFSC scientists are monitoring concentrations of contaminants in the Lower Columbia Estuary environment and in outmigrant juvenile salmon to evaluate their potential risk to the productivity of ESA-listed Columbia River salmon stocks. Contaminant levels have been determined in juvenile Chinook salmon, water, and sediment samples from six sites in the Lower Columbia River and Estuary, from Bonneville to the estuary mouth, as well as in juvenile Chinook salmon and food samples from six hatcheries along the Columbia. Juvenile Chinook are exposed to PCBs, DDTs, PBDEs and PAHs via their diet, with especially high concentrations of contaminants in stomach contents of fish from sites in the Portland/Vancouver area. Contaminant levels in bodies and stomach contents of some fish are above thresholds for effects on salmon health, such as delayed mortality, poor growth, and reduced disease resistance. Salmon from the Portland sites also show signs of exposure to estrogenic compounds. Food and bodies of juvenile Chinook collected from Columbia River hatcheries contain low to moderate concentrations of PCBs, DDTs, and low molecular weight PAHs, which likely contribute to background levels of DDTs and PCBs found in outmigrant salmon. Moreover, concentrations of copper and organophosphate pesticides in the water column were at levels that could interfere with olfaction in salmon at some sites. Field data are being used in bioaccumulation and

population models to better understand pathways of exposure for salmon, and potential impacts on stock recovery.

Scientific data management at the NWFSC

Richard Kang, Jeff Cowen, Rod Davidson, Vathsala Desilva, Bob Gref, Priya Jhangiani, Robert Larson, Robert Marsicek, Adam Mouton, Vimal Nair, Martin Park, Abdul Sait, Yazan Suleiman, and Brendan Sylvander
Northwest Fisheries Science Center
Scientific Data Management Team
Seattle, Washington
richard.kang@noaa.gov

The Scientific Data Management (SDM) Team at the Northwest Fisheries Science Center (NWFSC) develops and maintains a coordinated approach to managing data and integrating their use in scientific research related to endangered species recovery and marine species management in the Pacific Northwest. To this end, SDM provides expertise in database design, application development, and Geographic Information Systems (GIS), for desktop clients and over the web. SDM works with NWFSC and NWR researchers and staff to collect, synthesize, organize and report diverse sets of data, including restoration and recovery project data (PCSRF, PNSHP); monitoring and evaluation data (ISEMP, habitat, water quality); and biological and physical data (salmonid abundance, artificial propagation, genetics, bird predation, oceanographic). Additionally, we offer tools and applications to facilitate analysis and collaboration among Center staff and with external partners (SWAM tool for ArcView, ArcSDE database project space, Matlab/STAR-P modeling, Oracle Collaboration Suite).

An ecosystem model of the California Current: Incorporating biological indicators in fisheries stock assessment and decision rules

Isaac C. Kaplan, Chris J. Harvey, Ian J. Stewart, and Phillip S. Levin
Northwest Fisheries Science Center
Fishery Resource Analysis and Monitoring Division
Seattle, Washington
isaac.kaplan.@noaa.gov

Fish populations off the U.S. west coast experience dramatic changes in reproduction and growth due to the dynamic oceanography of the region, including El Niño-Southern Oscillation and the Pacific Decadal Oscillation. Fisheries managers need a modeling framework to test management strategies that incorporate this environmental variability. To address this need, we built a spatially explicit ecosystem model of the California Current System, extending from the US/Canada border to Point Conception, California, and out to the 1,200 meter isobath. The model structure (Atlantis) includes the trophic dynamics of 54 functional groups in the food web, including habitat-forming species like kelp, corals and sponges, as well as phytoplankton, zooplankton, vertebrates, benthos, and cephalopods. The model is forced with a high-resolution ROMS oceanographic model, and by fisheries catches for 1981–2004. Model outputs reproduce observed seasonal patterns of primary productivity, and are consistent with estimates of historical fish and mammal abundance. We use the model to identify indicator species of climate shifts (copepods and euphausiids), and to show how these biological indicators can be included in stock assessments and management decisions. Biological indicators can inform statistical estimates of reproduction, reducing the uncertainty in this key demographic parameter. The abundance of groups like copepods and euphausiids can also serve as warning flags to tell managers to adjust fish catches even before changes in productivity are detectable in fish stocks. Simulation tests show that incorporation of biological indicators in assessment and management decisions leads to improved short term forecasts of stock size, and higher and more constant catches of target species.

The NWFSC's West Coast groundfish trawl survey: Design, operations, methods, and results

Aimee Keller, Erica Fruh, Victor Simon, Beth Horness, Vanessa Tuttle, Keith Bosley, Dan Kamikawa and John Buchanan.
Northwest Fisheries Science Center
Fishery Resource Analysis and Monitoring Division
Seattle, Washington
aimee.keller@noaa.gov

The Northwest Fisheries Science Center's (NWFSC) Fishery Resource Analysis and Monitoring Division's (FRAM) survey team conducts field studies focused on the abundance, distribution and biological characteristics of managed groundfish species off the US West Coast. The West Coast groundfish fishery management plan includes 82 commercially fished stocks off Washington, Oregon and California. The goal of the groundfish survey is to provide fishery-independent data used in the assessments of the status and trends of commercially important species. Since 1998, the NWFSC bottom trawl surveys have been conducted annually; in 2003 depth and geographic coverage were extended to include all US West Coast waters from depths of 55 to 1,280 m. All survey operations are conducted in strict compliance with NOAA national protocols for bottom trawl surveys. Quality of catch and related information are initially reviewed at sea. This survey provides data on abundance, biomass, spatial distributions, sex ratios, size, and age structure of groundfish in trawlable habitats. The program also collects data on the life history of key groundfish species, genetics, fecundity and diet. New to the groundfish survey in 2006 were an inventory of West Coast corals and a stationary seabird survey. The primary objectives of the survey are: to describe distribution and abundance of commercially important groundfish species; to examine changes in species composition, size, and age with geographic area and depth; and to describe the physical environment of groundfish habitat (temperature, bottom type, structure).

Here we described the design, operations, and methods utilized during the groundfish survey and summarize data collected from 1998–2006 (preliminary). We examined trends in stations sampled, otoliths collected, individuals sexed and measured, taxa identified and stomach samples. During this period of increased data collection, the seasonal extent, geographical area and depth coverage expanded to the current levels. Corals and seabird inventories were added in 2006. Corals were encountered in 367 tows with 297 whole specimens retained and 348 samples preserved for DNA analysis. Seabird abundance was generally estimated at the start of each tow with data intended to advance the current understanding of the distribution and oceanic habitats of seabirds. We provided an example of catch analysis which revealed that total catch per haul (kg) and species richness are severely depressed in a deep portion (>475 m) of the Santa Barbara basin, commonly known to be hypoxic. Similarly low catches were observed in an hypoxic area off the OR coast in 2006 (preliminary data).

Catch data are typically converted to catch per unit effort (CPUE) during data processing for use by assessors with CPUE (kg/ha) = catch (kg) / area swept (ha). We presented an example of rockfish distribution based on catch per unit effort (CPUE, kg/ha) and abundance (number/ha) for a single species: canary rockfish from 2003–2005. The data revealed repeated high catch in

the same geographic areas over time and are suggestive of habitat selection by these older larger individuals. Preliminary data from 2006 also indicated high canary catch in the same areas. Similar figures reveal information about the distribution and abundance of other species.

In addition we examined age distributions for Dover sole and sablefish, commercially important species, based on otolith readings and presented an example of the change in size composition for sablefish with depth and geographic area. Like many other species, size increased with greater depth and along a south to north latitudinal gradient. CPUE, age and size are important elements in population models for groundfish. The goal of the NWFSC groundfish survey is to provide fishery-independent data (including catch, size and age) used in the assessment of commercially important groundfish species. Data are used to develop a variety of models to examine the status and trends of groundfish stocks. Periodic assessments, in turn, provide guidance for managing West Coast fisheries.

Synergistic toxicity in juvenile Coho salmon exposed to mixtures of organophosphate and carbamate insecticides

Cathy Laetz, David Baldwin, and Nathaniel Scholz
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
cathy.laetz@noaa.gov

Vincent Hebert
Washington State University
Food and Environmental Quality Laboratory
Richland, Washington

John Stark
Washington State University
Puyallup Research and Extension Center
Puyallup, Washington

Organophosphate and carbamate insecticides are commonly detected in surface waters that provide habitat for threatened and endangered species of Pacific salmon (*Oncorhynchus sp.*) on the west coast of the United States. These pesticides inhibit the activity of the enzyme acetylcholinesterase (AChE), thereby interfering with chemical signaling (neurotransmission) at nerve synapses. Mixtures of these pesticides are frequently detected in the environment, but the effects of these mixtures on the neurobiology and behavior of salmon and other fish are poorly understood. Previous work in our lab found that *in vitro* treatments with mixtures of organophosphate and carbamate pesticides produced dose-additive toxicity, as measured by AChE inhibition. To determine if *in vivo* exposure also produces additive toxicity, we exposed juvenile coho salmon (*Oncorhynchus kisutch*) to sublethal concentrations of the pesticides diazinon, malathion, chlorpyrifos, carbaryl and carbofuran both individually and as binary mixtures. Single pesticide exposures produced dose-dependent inhibition of brain AChE. However, AChE inhibition was greater than expected after exposure to mixtures, indicating synergistic (i.e., greater-than-additive) toxicity for many of the binary mixtures. Moreover, several mixtures containing organophosphates caused mortality at concentrations that were sublethal in single pesticide exposures. These results indicate dose-additive models of toxicity may underestimate the risks that mixtures of organophosphates and carbamates pose to salmon throughout the Pacific Northwest.

Genetic Stock Identification (GSI) technology transfer to fishery management

Peter Lawson, Paul Moran, and David Teel
Northwest Fisheries Science Center
Hatfield Marine Science Center
Conservation Biology Division
Newport, Oregon
peter.w.lawson@noaa.gov

Renee Bellinger and Michael Banks
Northwest Fisheries Science Center
Hatfield Marine Science Center
Coastal Oregon Marine Experiment Station
Newport, Oregon

Genetic markers using microsatellites can be used to identify Chinook salmon stocks at a level that is useful for fisheries management. The NWFSC has coordinated a 5-year project to build a standardized data base (GAPS) of Chinook salmon microsatellites that enables coastal genetics laboratories to identify individuals from 165 stocks from 42 reporting groups of Chinook salmon from the Pacific Northwest. These identifications can be made within 24 hours of collecting the sample. Ocean fisheries in 2006 were severely restricted to protect natural fall Chinook salmon escapement to the Klamath River. These fishery restrictions caused coastal communities to lose many millions of dollars in economic activity. Genetic Stock Identification (GSI) has been proposed as a way to identify Klamath fall Chinook caught in the ocean fisheries and to use this information to provide fishermen access to stronger runs while avoiding the weak Klamath stock. The Coastal Marine Experiment Station at Oregon State University, along with the Oregon Salmon Commission, Oregon Sea Grant, NMFS/NWFSC, and others have teamed up to develop techniques for using GSI in ocean fisheries. The project, called Cooperative Research on Oregon Ocean Salmon (CROOS), employed 80 fishermen to collect genetic samples along with geographical positioning system (GPS) coordinates, sea surface temperatures, depth of catch, and other information for each fish caught during the regular season openings off the Oregon coast in 2006. Using these data they have produced fine-scale maps of the stock composition and distribution of catch and effort. The project is developing coordinated sampling programs coast-wide and seeks to use the information gathered for fishery management, research into the ocean ecology of adult Chinook salmon, and marketing. The project is key to transferring GSI technology and the GAPS data base to practical applications. The NWFSC's role includes laboratory work, theoretical development of GSI theory, software and database development, management models, interface with fishery management, and collaborative project coordination.

Evaluating potential health impacts of polybrominated diphenyl ether flame retardants: Endocrine disrupting and developmental toxicity in two fish models

Sean C. Lema and Penny Swanson
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technology Division
Seattle, Washington
sean.lema@noaa.gov

Jon T. Dickey
University of Washington
School of Aquatic and Fishery Sciences
Seattle, Washington

Nathaniel L. Scholz and John Incardona
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington

Irvin B. Schulz
Battelle Marine Sciences Laboratory
Pacific Northwest Division
Sequim, Washington

Polybrominated diphenyl ethers (PBDEs) are flame retardant chemicals used in plastics, polyurethane foams, and textiles. Concerns about the health risks of PBDEs arose recently because PBDEs have become pervasive environmental contaminants and levels in animal tissues are increasing, yet little is known about their toxicity in any laboratory model animal. We are examining the potential biological effects of the PBDE congener 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) using two model fishes: fathead minnow and zebrafish. Breeding pairs of adult fathead minnows were given oral doses of PBDE-47 bioencapsulated in brine shrimp for 21 days. The effects of PBDE-47 exposure on plasma thyroid hormone (TH) levels and expression of genes for pituitary thyroid-stimulating hormone subunit (TSHβ) and TH receptors were assessed. Minnows treated with PBDE-47 had depressed plasma levels of thyroxine (T_4) and altered expression of genes for TSHβ in the pituitary and TH receptors in the brain. Early developmental effects of PBDEs were examined in zebrafish embryos exposed continuously to dissolved PBDE-47 beginning at 3-5 hrs post-fertilization (hpf). Larvae treated with PBDE-47 delayed hatching, were smaller in body size and developed a dorsally-curved tail that was first detectable at 72 hpf. Further examination showed that PBDE-47 induced an elevation in heart rate, which subsequently developed into a cardiac arrhythmia. This arrhythmia may have contributed to the elevated mortality seen in larvae exposed to high levels of PBDE-47. Taken together, our findings indicate that PBDE-47 can disrupt the thyroid system and cause developmental abnormalities in fish, underscoring concerns that rising PBDE contamination may impact animal populations and human health.

Genomic biomarkers for assessing fish reproductive health

J. Adam Luckenbach and Penny Swanson
Northwest Fisheries Science Center
Seattle, Washington
adam.luckenbach@noaa.gov

Graham Young
University of Washington
School of Aquatic & Fishery Sciences
Seattle, Washington

Dimitar B. Iliev and Frederick W. Goetz
University of Wisconsin
Great Lakes WATER Institute
Milwaukee, Wisconsin

Urbanization, coastal population growth, agricultural practices and climate change have greatly affected water quality throughout our aquatic ecosystems. Many chemicals contaminating fish and aquatic ecosystems are known to disrupt endocrine function, potentially leading to impaired development and reproduction of wildlife and humans. To evaluate relationships between environmental conditions/contaminants and fish reproductive health, researchers require modern, comprehensive bioassessment tools. Thus the aim of this research is to develop tools to measure large-scale gene expression in the gonad as a way to assess reproductive health. As a first step toward this goal, genes that respond to known environmental stressors must be identified. Using suppression subtractive hybridization (SSH) we are working to identify genes that are specifically up- or down-regulated in the coho salmon ovary during normal development and after exposure to environmental stressors, such as xenoestrogens and prolonged feed restriction. Our focus is on immature fish with gonads in early stages of gametogenesis, because it is during these stages development that salmon are migrating to sea through contaminated waterways and estuaries. Using SSH we have recently identified a number of differentially expressed genes during the normal progression of early stages of oogenesis in coho salmon. Ultimately as we continue this work, including future exposure studies, we plan to utilize the suite of genes identified by SSH and other important genes related to gametogenesis found in the fish EST databases, to construct a custom oligonucleotide microarray that will serve as a 'fingerprint' for assessing the reproductive health of salmon in their native habitat.

Effects of pesticides on salmonid food webs: Current knowledge and uncertainties, and implications for endangered salmon

Kate Macneale, Peter Kiffney, Julann Spromberg, David Baldwin, and Nat Scholz
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
kate.macneale@noaa.gov

The risk pesticides pose to anadromous salmonids in the Pacific Northwest is an emerging concern for researchers, managers and regulators. For salmonids listed as threatened or endangered under the U.S. Endangered Species Act, there is an urgent need to quantify the direct and indirect effects of pesticide exposure. Because pesticides are effective at killing the very invertebrates on which salmon depend, we are particularly interested in impacts that currently-used pesticides have on salmonid prey. We present conceptual models illustrating our current understanding of the spatial and temporal impacts pesticides have on freshwater salmonid food webs. For example, an hour-long exposure of a cocktail of pesticides at a concentration sublethal to fish may reduce benthic invertebrate densities, and consequently the density of invertebrates available to fish in the drift, for months following exposure and for significant distances downstream of the exposure. These indirect effects on prey are being incorporated into models analyzing direct impacts on fish behavior and population growth. In addition, we identify data gaps and present suggestions for research and monitoring that will help fill those gaps.

Impacts of stormwater runoff on coho salmon in restored urban streams

Sarah McCarthy, Julann Spromberg, John Incardona, Blake Feist, Jana Labenia, Mark Myers, Linda Rhodes, Gina Ylitalo, Tracy Collier, and Nathaniel Scholz
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
sarah.mccarthy@noaa.gov

Jenifer McIntyre
University of Washington
School of Aquatic and Fishery Sciences
Seattle, Washington

Laura Reed and Katherine Lynch
City of Seattle
Seattle Public Utilities
Seattle Washington

Jay Davis
U.S. Fish and Wildlife Service
Western Washington Fish and Wildlife Office
Lacey, Washington

Beginning in the late 1990s, several agencies in the greater Seattle area began conducting fall surveys for spawning salmon to evaluate the effectiveness of local stream restoration efforts. These surveys detected a surprisingly high rate of mortality among migratory coho females that were in good physical condition, but had not yet spawned. In addition, adult coho from several different streams showed a similar progression of symptoms (disorientation, lethargy, loss of equilibrium, gaping, fin splaying) that rapidly led to the death of the affected animals. In recent years, pre-spawn mortality (PSM) has been observed in many lowland urban streams, with overall rates ranging from ~ 25% to 90% of the fall runs. Continuous daily surveys of wild coho spawners in a forested reference stream in northwest Washington revealed < 1% PSM. Although the precise cause of PSM in urban streams is not yet known, conventional water quality parameters (i.e., temperature and dissolved oxygen) and disease do not appear to be causal. Rather, the weight of evidence suggests that adult coho, which enter small urban streams following fall storm events, are acutely sensitive to non-point source storm water runoff containing pollutants that originate from highly developed landscapes. These findings have important implications for restoration and conservation efforts in urban and urbanizing watersheds, respectively. This project was supported by the NOAA Coastal Services Center, the NOAA Coastal Storms Program, the U.S. Fish and Wildlife Service's National Contaminants Program, and the City of Seattle.

Chemosensory deprivation of juvenile coho salmon exposed to dissolved copper under varying water quality conditions

Jenifer McIntyre
University of Washington
School of Aquatic and Fishery Sciences
Seattle, Washington
jmcintyr@u.washington.edu

David Baldwin, James Meador, and Nathaniel Scholz
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington

Dissolved copper concentrations in storm water often reach levels known to impact olfaction and olfactory-mediated behaviors in salmon under laboratory conditions. A wealth of literature, integrated into the Biotic Ligand Model, shows that water quality affects the toxicity of copper at the fish gill. Does water quality affect the toxicity of copper at the olfactory epithelium? Is dissolved copper bioavailable to the salmon nose in western U.S. streams? We exposed juvenile coho salmon (*Oncorhynchus kisutch*) to artificial waters of variable hardness (0.2-1.6mM Ca), alkalinity (0.2-3.2mM HCO_3^-), DOC (0-6mg/L), and pH (7.6 vs 8.6). Electro-olfactograms were used to quantify the olfactory response before and after a 30-min exposure to 20 µg/L Cu. At the end of the copper exposure in the lowest-ion water, olfactory response was reduced by 82%. Olfactory response was independent of pH, weakly correlated with hardness and alkalinity, but correlated strongly with DOC concentration. Ionic copper measurements and copper speciation modeling showed that copper bound to carbonates were bioavailable to the salmon olfactory epithelium. Our results suggest that hardness, alkalinity, and pH in natural waters of the western U.S. are not protective against the neurotoxic effects of copper, whereas DOC may have significant protective effects if present at sufficiently high levels.

Improved flatfish health following remediation of a PAH-contaminated site in Eagle Harbor, Puget Sound, Washington

Mark Myers, Bernadita Anulacion, Barb French, William Reichert, Cathy Laetz, Jon Buzitis, Paul Olson, Sean Sol, and Tracy Collier
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle Washington
mark.s.myers@noaa.gov

Eagle Harbor became a Superfund site in 1987 due to high sediment concentrations of polycyclic aromatic hydrocarbons (PAHs) released chronically from a nearby creosoting facility, which operated from 1903-1988. Early studies here with English sole (1983-86) demonstrated high prevalences (up to ~80%) of toxicopathic liver lesions, including neoplasms, which have been consistently associated with PAH exposure in multiple field studies, and induced by injections of a PAH-rich fraction extracted from Eagle Harbor sediment. Before remediation, lesion prevalences and other biomarker values for PAH exposure and effect in English sole from Eagle Harbor were among the highest in Puget Sound. In 1993-1994 a clean sediment cap was placed over 54 acres of the most contaminated portions of Eagle Harbor, with a secondary 15-acre cap added between 2000–2002, to sequester PAH-contaminated sediments. Lesion prevalences and biomarker values just before initial capping were reduced, consistent with facility closure in 1988 and shore-based source controls. Data on lesion risk, hepatic cytochrome P4501A (CYP1A), and biliary fluorescent aromatic compounds (FACs) from fish collected just after and at regular intervals up to ~2 years after primary capping were highly variable relative to pre-capping values. However, over the entire monitoring period (142 months) since capping, but particularly after ~3 years, there was a significantly decreasing trend in hepatic lesion risk, and for biliary FACs and hepatic DNA adducts in English sole. Hepatic lesion risk has been consistently low (> 0.20) compared to risk at capping (1.0), from ~4 years after primary capping through April 2005. These results show that the sediment capping process has been effective in reducing PAH exposure and associated biological effects in resident flatfish, and that longer term monitoring of pollutant responses in biological resources, such as resident fish, is necessary and far superior to monitoring of only sediment contaminants, in order to demonstrate the efficacy of this type of contaminant remediation.

Materials and Methods

In September 1993, prior to initiation of primary sediment cap placement, English sole were collected by otter trawl in Eagle Harbor within an area just west of and adjacent to the cap placement area (Figure 1). Samples were taken of liver tissue and bile for analysis of biomarkers indicative of exposure to and effects of PAHs, including hepatic xenobiotic-DNA adducts (Reichert and French, 1994), toxicopathic hepatic lesions in histological sections (Myers et al., 1987), and fluorescent aromatic compounds (FACs) or metabolites of PAHs in bile, expressed as $FACs_{BaP}$, or FACs measured at benzo(a)pyrene (BaP) wavelengths (Krahn et al., 1984). Subsequent samplings of sole were carried out in March 1994, July 1994, Sept. 1994, March 1995, July 1995, Oct-Nov. 1995, Dec. 1995, Oct. 1996, Dec. 1997, May 1998, May 1999, April 2000, May 2001, May 2002, April 2003, April 2004, and May 2005 (histology samples only in

2005) to assess post-capping temporal trends in these biomarkers of PAH exposure and effect. A target sample size of at least 15 individuals was established for each of the 17 field samplings and this target was achieved in most cases, but at most of the annual samplings in Sept. 1993 and from 1998 on at least 60 sole were necropsied. Otoliths were also collected from all English sole to determine fish age, and gonads collected to confirm fish sex by histological examination. Over the course of the study 789 English sole were necropsied and sampled for histology, 553 were sampled for bile, and 255 hepatic xenobiotic-DNA adduct samples were collected and analyzed.

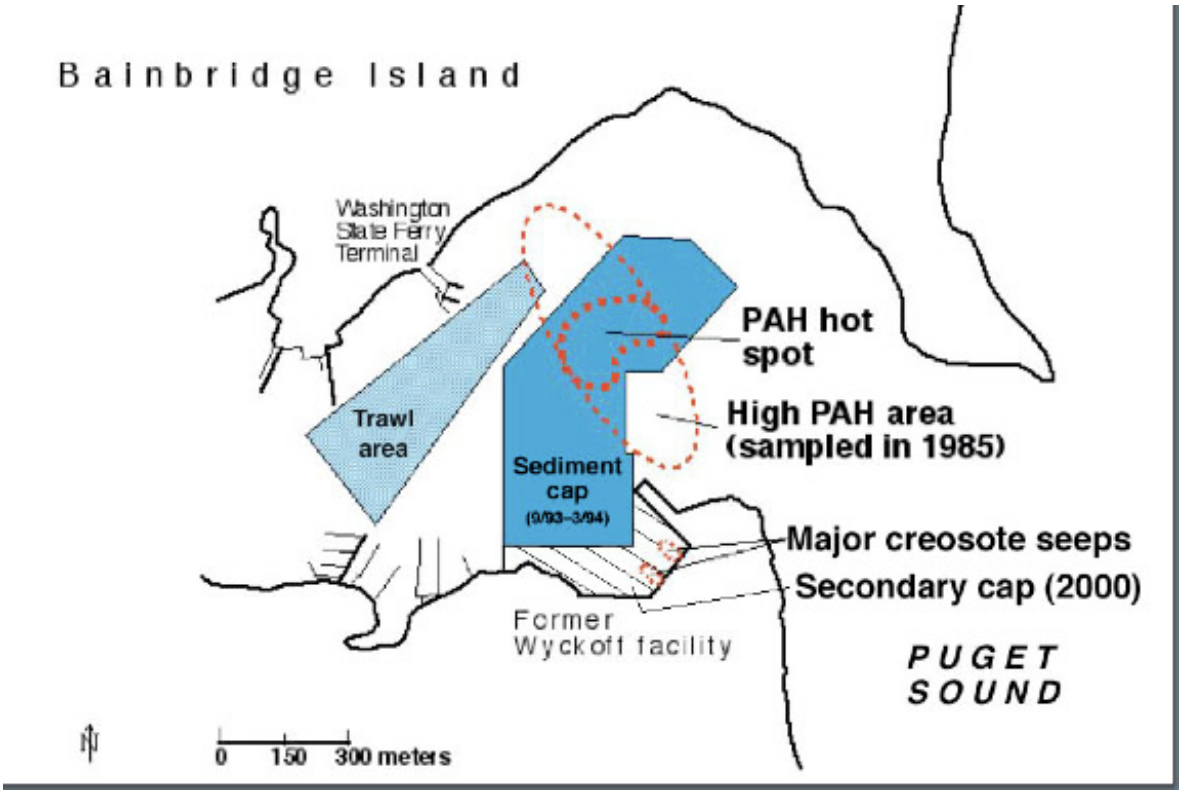

Figure 1. Map of area of west Eagle Harbor in which fish sampling operations were conducted, in relation to the past area of high sediment PAHs and the areas sequentially capped 1) in Sept. 1993 to March 1994 with sediments composed of dredge material from the Snohomish R. (polygonal shaded area including the PAH "hot spot"), covering 54 acres, and 2) in Nov. 2000-Feb. 2002 as a secondary cap of clean sand, joining the southern edge of the original cap with the northern shoreline of the former Wyckoff facility, adding another 15 acres of area capped with clean sediments.

Statistical analyses of the bile FACs and xenobiotic-DNA adduct data included linear regression and analysis of variance. Biliary FACs data were log-transformed prior to statistical treatment to reduce heteroscedasticity. Data for hepatic xenobiotic-DNA adducts were not log-transformed because the data were normally distributed. For clarity of presentation, all figures shown depict untransformed values for these respective biomarkers. Relationships among biomarker measures and fish age were determined by linear correlation; age was not correlated with any of these biomarker measures, and was therefore not considered in subsequent analyses. Possible sex differences in biomarker measures were tested by two-way analysis of variance, using sex and capture date as factors; there were no consistently significant differences between values for

male and female fish over the sampling periods for any of the above biomarker measure, so sexes were combined in subsequent treatment of the data. The critical level of statistical significance in all statistical tests was set at $p \leq 0.05$.

Liver lesion occurrence was statistically related to fish age and sex, and time elapsed since initiation of the first sediment capping process, using stepwise logistic regression techniques, as reported fully elsewhere (Myers *et al.*, 1994). This analysis determines the influence of the time elapsed since cap initiation on hepatic disease risk, while simultaneously accounting for the influence of fish age and sex, and is commonly used in epidemiological and epizootiological studies. Risk factors were considered significant at $p \leq 0.05$. Two types of analyses were performed. The first determined the odds ratio as an estimate of relative risk for lesions in individual fish in relation to sex and age, and time elapsed since initiation of the sediment capping process in September 1993. In this analysis the odds ratio represents the degree of association between a risk factor and lesion occurrence. Odds ratios for lesion categories at a particular sampling time point (months post-cap initiation) were calculated and interpreted relative to the risk of lesion occurrence at the September 1993 time point just prior to the beginning of placement of the primary sediment cap, defined as 1.0. Decreased probabilities of lesion occurrence at the respective post-capping sampling points were indicated by odds ratios less than 1.000, and the overall regression could be expressed as the influence of each successive month elapsed since cap initiation on the risk of lesion occurrence. Odds ratios for age were interpreted for the influence of each additional year in age on the risk of lesion occurrence. In no case was fish sex a significant risk factor for toxicopathic liver lesions, so both male and female sole were combined in subsequent analyses.

Results

Fluorescent Aromatic Compounds (FACs) in Bile

Mean concentrations of biliary $FACs_{BaP}$ at the pre-capping sampling point were far below the peak levels determined in Eagle Harbor English sole in 1984, and lower than in 1988 and slightly lower than in 1991 (Fig. 2). At the first post-capping sampling point (March '94), the mean concentration of $FACs_{BaP}$ in bile was significantly lower than pre-capping values, but by July '94 levels were significantly higher than the pre-capping values. In subsequent time points through October 1995, means were highly variable, ranging from ~250 to ~1,300 ng BaP equiv/mg bile protein, suggesting that English sole from Eagle Harbor were exposed to varying concentrations of high molecular weight PAHs for up to two years following cessation of sediment capping operations. However, subsequent to this time point, there was a sharp decline in $FACs_{BaP}$ in bile until December 1997 (51 months after primary cap initiation), with levels remaining relatively constant through April 2004 (128 months post-capping), with only a slight increase since May 1998. Over the entire study, there was a significant decline in $FACs_{BaP}$ in bile subsequent to the Sept. 1993 sampling as determined by linear regression ($p<0.0001$, $Y=2.717-.003 * X$; $R^2 = .107$, plot not shown). However, the relatively low mean $FACs_{BaP}$ concentrations in Eagle Harbor English sole subsequent to the Dec. 1997 sampling still remained slightly above the mean concentrations (162 ng BaP equiv./mg bile protein) measured in English sole during six separate samplings in 1987-88 at Polnell Pt., a Puget Sound reference site.

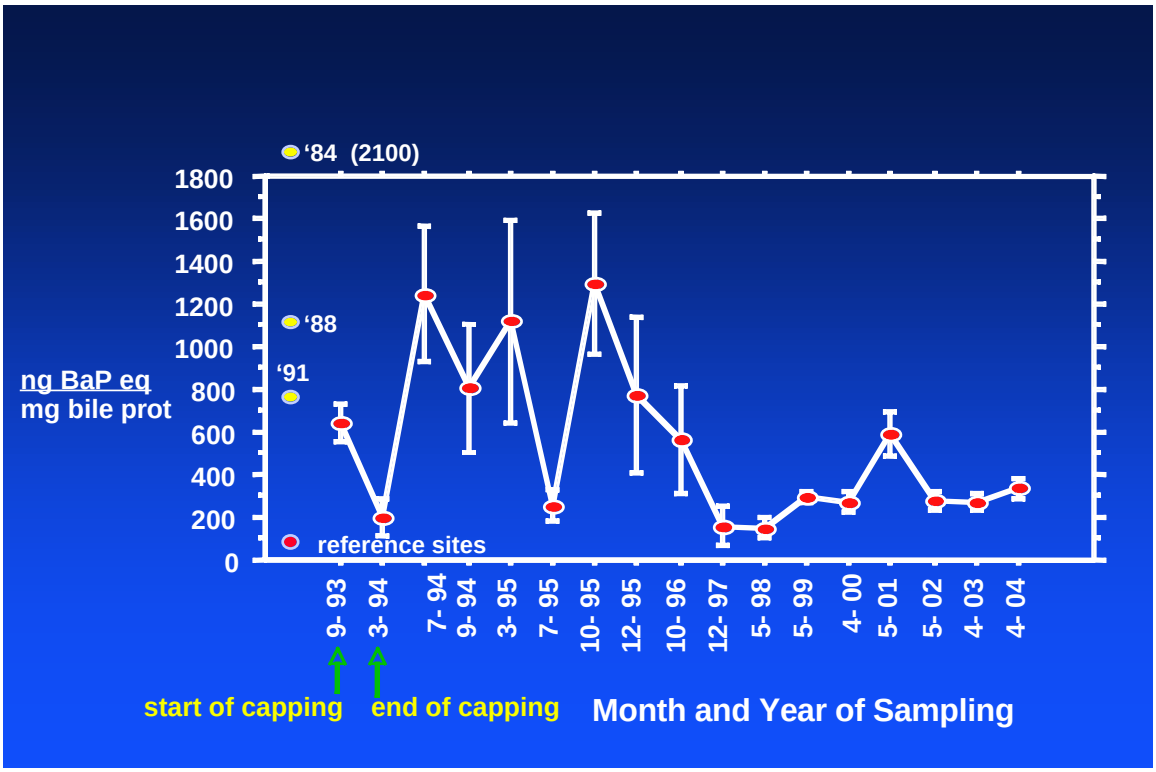

Figure 2. Mean concentrations (ng BaP equiv./mg bile protein) of fluorescent aromatic compounds measured at benzo(a)pyrene wavelengths ($FACs_{BaP}$) in bile of English sole sampled from Eagle Harbor between 9/93 and 4/04. Error bars indicate $\pm$ 95% confidence interval. Data points above the 9/93 sampling point represent mean values in English sole from Eagle Harbor in 1988 and 1991. Also shown are biliary $FACs_{BaP}$ values typical of English sole from reference sites in Puget Sound.

Hepatic Xenobiotic-DNA Adducts

Mean adduct concentrations at the pre-capping sampling in September 1993 (70 $\pm$ 8 nmol DNA adducts/mol DNA bases) were comparable to those measured in Eagle Harbor sole in 1992, but higher than those measured in 1988 (Figure 3). Because of cost considerations, hepatic xenobiotic-DNA adduct concentrations were determined only at eleven sampling points between Sept. '93 and April '04. There is a clear and progressive decrease in adduct concentrations over time, with significantly lower hepatic DNA adduct concentrations at all of the post-capping sampling points relative values at the pre-capping time point ($p \leq 0.05$), with values in the April 2004 sampling being indistinguishable from adduct levels in sole from reference areas such as in Tulalip Bay (7 nmol adducts/mol bases) and Useless Bay (4 nmol adducts/mol bases) in 1991. This generally decreasing trend was confirmed as highly significant by linear regression on log-transformed data as well as untransformed data ($p < 0.0001$; $Y = 54.313 - .444 * X$, $R2 = .322$, plot not shown).

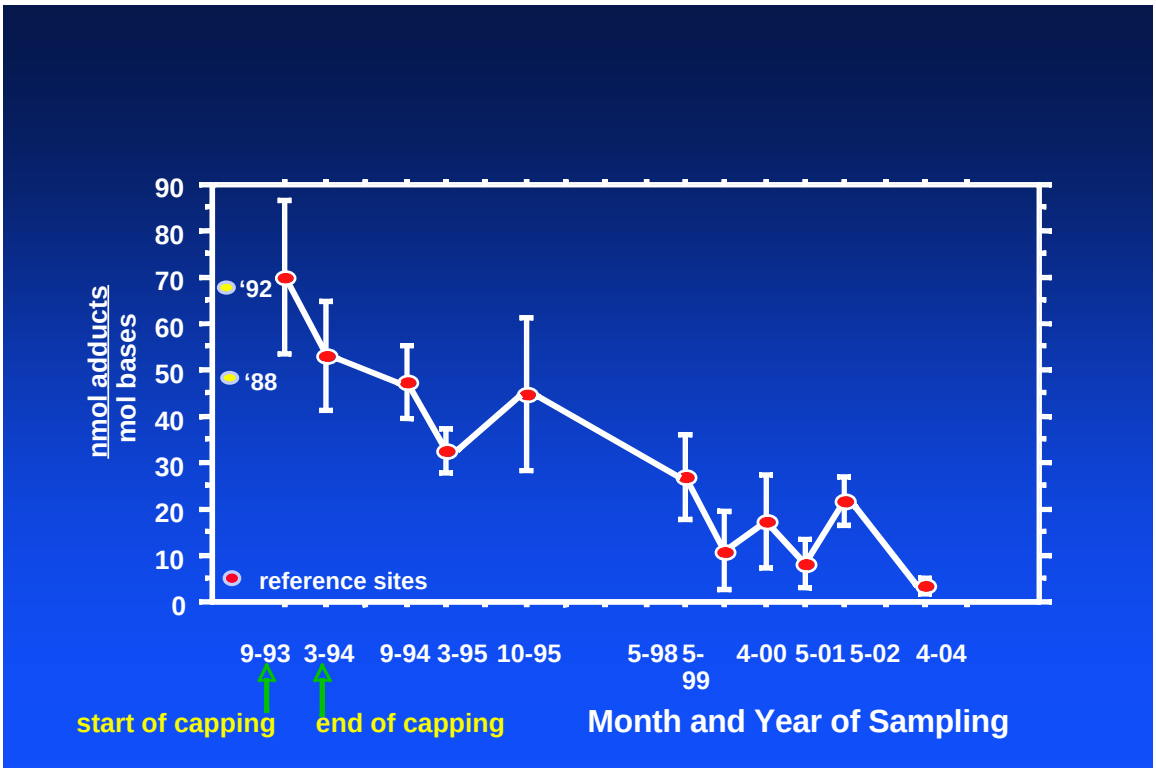

Figure 3. Mean concentrations (nmol adducts/mol bases) of bulky hydrophobic xenobiotic-DNA adducts in liver of English sole sampled from Eagle Harbor at various time points between 9/93 and 4/04. Error bars indicate ± 95% confidence interval. Data points below and to the left of the 9/93 sampling point represent mean values in English sole from Eagle Harbor in 1988, 1991, and 1992. Also shown are DNA adduct levels typical of English sole from reference areas in Puget Sound.

Toxicopathic Hepatic Lesions

Hepatic lesion prevalences detected at the September 1993 pre-capping sampling were substantially below those determined in adult English sole from Eagle Harbor in 1983-1986. This was especially true for neoplasms, which were detected in only 1% of the sole examined, as compared to 26% in the 1983-1986 samplings. The lesion most commonly detected in Sept. 1993 (as in 1983-1986) was hepatocellular megalocytosis/nuclear pleomorphism (16%), followed by nonneoplastic proliferative lesions (10%), preneoplastic focal lesions (5%), and neoplasms (1%), with prevalences for all of these categories substantially below the 1983-1985 values (Myers et al., 1987). Overall, the pattern of hepatic lesion prevalence showed a reduction between 1983-86 and cap initiation, followed by an approximate three-year period of highly variable lesion prevalences, and then a sharp decline in TOXLIV prevalences since Oct. 1996 to values consistently below 20%. These lower prevalences have remained consistent through May of 2005, and have typically been below 10%.

However, since age has been determined to be such a strong risk factor for most of the toxicopathic liver lesion types, especially preneoplastic lesions and neoplasms (reviewed in Myers et al., 2003), and since mean ages for English sole among the sampling periods ranged from 3.4 to 8.5 years, it was not possible to accurately compare raw lesion prevalences among the sampling periods. To account for these age differences and the strong influence of fish age

on risk of lesion occurrence, we utilized stepwise logistic regression to 1) determine the influence of fish age on lesion risk, followed by 2) determining the influence of the period of time since capping on risk of hepatic lesion occurrence. The net effect of this method is to be able to compare lesion risks in fish of equivalent ages among the different sampling periods.

In the analysis above that accounted for fish age differences among the temporal sampling points, the risk of occurrence of lesions in the inclusive TOXLIV category, relative to the pre-capping risk (referred to as relative risk), was highly variable for approximately 3 years following the beginning of capping, with relative risks ranging from 0.209 at 18 months post-cap initiation to 1.690 at 22 months post-capping (Figure 4). At the 36 month post-capping sampling point the relative risk was 0.793, with relative risks subsequent to this time point dropping to values consistently well below 0.500, and ranging between 0.0597 at 104 months (8.7 years) post-capping to 0.397 at 116 months (9.7 years) after cap initiation. In fact, all relative risks for TOXLIV occurrence subsequent to the 3-year post-capping sampling point (Oct. 1996) and up through the final May 2005 (11.8 years) sampling point have been significantly lower, by stepwise logistic regression ($p \leq 0.05$), than the Sept. 1993 baseline value of 1.000. Overall, there was a significantly declining trend in risk of toxicopathic liver lesion occurrence over the course of the study, with the risk of TOXLIV occurrence declining by 0.9813 times for each month after primary cap initiation (Figure 4).

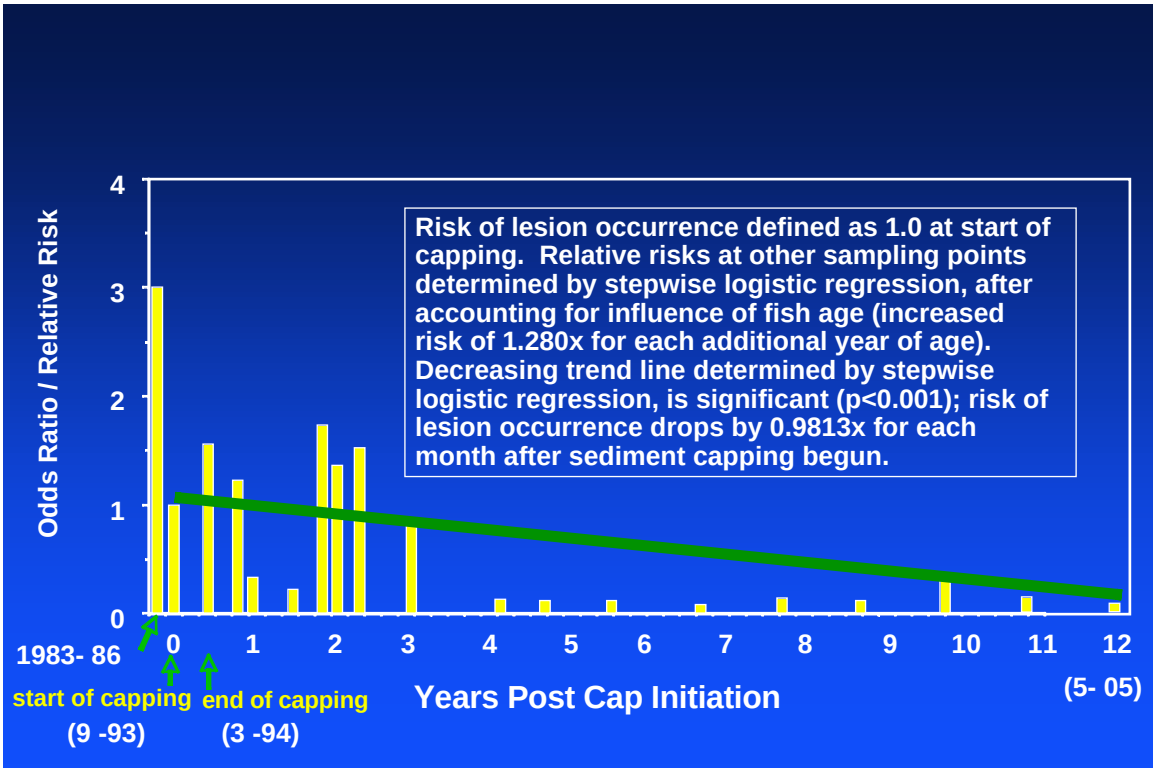

Figure 4. Estimated odds ratios or relative risks of toxicopathic hepatic lesion (TOXLIV) occurrence in English sole from Eagle Harbor over the period 1983 through May 2005. The risk of lesion occurrence is defined as 1.000 at the start of sediment capping activities in September 1993. Relative risks at other sampling points are determined by stepwise logistic regression, after first accounting for the influence of fish age on risk of lesion occurrence, which in this analysis showed a significantly increased risk of 1.280 times for each additional year of age. The relative risk of lesion occurence for the period 1983-1985

(~3.0) is estimated based on mean age and hepatic lesion prevalences for English sole at sampling points over that period. The decreasing temporal trend line (red line) is determined by stepwise logistic regression, and is highly significant ($p<0.001$), with risk of lesion occurrence decreasing by 0.9813 times for each month after initiation of sediment capping.

Discussion

This investigation demonstrated clearly that the sequential placement of two major sediment caps over a large portion of eastern Eagle Harbor contributed towards a subsequent overall reduction in PAH exposure of, and a consequent reduction in occurrence of toxicopathic hepatic lesions in, English sole captured within Eagle Harbor. However, factors other than exposure associated with the capped area may have also been important in determining total PAH exposure and associated biological effects in resident English sole. Generally, indicators of short-term exposure to PAHs (e.g., concentrations of FACs in bile) were considerably reduced in English sole from Eagle Harbor even before cap placement began, in comparison to historical values. This indicates that previous source control measures enacted between 1988 and September 1993, such as closure of the facility which pressure-treated wood pilings with creosote, and control of upland and groundwater PAH contamination via the on-site groundwater extraction and treatment system, may have contributed to reduced PAH exposure in epibenthic fish in Eagle Harbor, as represented by English sole.

However, the most dramatic reductions in biomarkers of PAH exposure and effect in English sole have been subsequent to the two major sediment caps placed over contaminated sediments in Eagle Harbor. Data on hepatic lesion risk, and biliary fluorescent aromatic compounds (FACs) from fish collected just after and at regular intervals up to ~2 years after primary capping were highly variable relative to pre-capping values. However, over the entire monitoring period (142 months) since capping, but particularly after ~3 years, there was a significantly decreasing trend in hepatic lesion risk, as well as parallel and significantly decreasing trends for biliary FACs and hepatic xenobiotic-DNA adducts. Hepatic lesion risk has been consistently low (> 0.20) compared to risk at capping (1.0), from ~4 years after primary capping through April 2005. These results show that the sediment capping process has been effective in reducing PAH exposure and associated biological effects in resident flatfish, and that longer term monitoring of pollutant responses in biological resources, such as resident fish, is necessary and far superior to monitoring of only sediment contaminants, in order to demonstrate the efficacy of this type of contaminant remediation.

A major lesson of this biomonitoring and biological resource recovery study is found in the fact that no such trends in decline of the measured PAH-exposure and effect parameters were apparent within an approximate time frame of three years following the beginning of placement of the primary sediment cap. However, longer term monitoring over at least a five-year period after sediment remediation was able to clearly demonstrate the efficacy of sediment remediation measures, as well as associated improvements in the health of a resident epibenthic fish species. Considering that the site remediation measures conducted just within the East Harbor Operable Unit of the Wyckoff/Eagle Harbor Superfund site have cost over $100 million, it is only prudent to spend a very small fraction of the total remediation costs on biological monitoring of the recovery of living marine resources within the same area, to adequately assess the efficacy of those remediation actions at the site in question, as well as for application to other similar sites

scheduled for remediation. We recommend that, as remedial actions are planned for other sites, they be accompanied by biological surveys of marine biota as well as biochemical assessment of fish and invertebrate populations prior to, during, and subsequent to these actions. As agencies take actions to redress past insults to marine resources resulting from chemical contamination, the timely use of a combination of assessment tools will allow for an intelligent and objective evaluation of the efficacy of such actions.

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A comparison of feed intake, growth, and nutrient and energy utilization between a domesticated strain of coho salmon (*Oncorhynchus kisutch*) and its parent stock

Kathleen Neely
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington
kathleen.neely@noaa.gov

Karl D. Shearer
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technology Division
Seattle, Washington

Feed intake, growth and nutrient and energy utilization of a domesticated strain (12 generations of selection for rapid growth) of coho salmon (*Oncorhynchus kisutch*) were compared to that of this strain's parent stock. Fish were reared under identical conditions from fertilization to 184 days post hatch. Fish were fed a commercial salmon feed, either to satiation or a predetermined ration. Fish were sampled fortnightly to determine weight gain and whole body composition. After the growth trial the satiation fish were fed feed containing Y2O3 to determine nutrient and energy digestibility. When fed to satiation, the domesticated strain ingested 53% more feed and grew at a higher rate (SGR) 5.09 vs. 3.81. When pair fed, the domesticated strain had higher FCE (gain/feed, 1.63 vs. 1.42) and PER (67.0 vs. 60.0%). Digestibility of macronutrients and energy did not differ significantly. At the end of the study the parent-stock fish had significantly higher levels of whole body lipid. The results of this study indicate that faster growth of the domestic strain was the result of the multiplicative affects of greater feed intake and the sparing, by lipid, of protein for growth. Our findings will be related to the findings of similar experiments.

Detection and quantification of *Vibrio parahaemolyticus* in Pacific Northwest estuarine and marine environments by real-time quantitative polymerase chain reaction (qPCR) analysis

William B. Nilsson, Anne E. Mataia, Raymond S. Febus, and Mark S. Strom
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technology Division
West Coast Center for Oceans and Human Health
Seattle, Washington
william.nilsson@noaa.gov

Vera L. Trainer
Northwest Fisheries Science Center
Environmental Conservation Division
West Coast Center for Oceans and Human Health
Seattle, Washington

Vibrio parahemolyticus (Vp), a bacterium that is a natural member of the estuarine and marine bacterial community, is a major cause of a seafood-related illness known as Vibriosis. This disease is usually associated with the consumption of raw or under-cooked shellfish and is typically characterized by self-limiting gastroenteritis. Many outbreaks of Vibriosis have occurred in the Pacific Northwest. Most recently, a large outbreak in the summer of 2006 was traced to shellfish cultured in the Puget Sound. Prevention of similar disease outbreaks in the future will be dependent upon development of more effective risk assessment tools. We have applied a real-time quantitative polymerase chain reaction (qPCR) assay for detection of Vp in environmental samples. Samples for qPCR analysis were collected in association with the 2005 and 2006 ECOHAB cruises from Puget Sound, the Strait of Juan de Fuca, the Juan de Fuca Eddy and most of the length of the Washington Coast. Near-shore samples in the area of commercial shellfish operations were also collected and analyzed by qPCR. The results of these efforts will be described and the implications of the findings will be related to what is known about the ecology of Vp. In addition, the potential value of qPCR as a risk assessment tool will be discussed.

Behavior of Southern Resident killer whales in the presence of vessels in San Juan Islands, Washington

Dawn P. Noren
Northwest Fisheries Science Center
Marine Mammal Program
Seattle, Washington

Introduction

Vessel disturbance has been identified as one of the potential risk factors to Southern Resident killer whales (Krahn et al. 2002). Since the late 1980s, these whales have experienced a significant increase in vessel-based whale watching (private and commercial) in the San Juan Islands (Otis and Osborne 2001).

Vessels may impact killer whales in several ways. Past studies have quantified impacts of vessels on Southern Resident killer whales by modeling the potential acoustic effects (Erbe 2002) or measuring short-term responses of individual whales to vessels (e.g., Kriete 2002). Northern Resident killer whales as well as other cetacean species can demonstrate horizontal and/or vertical avoidance behavior in response to boats (Nowacek et al. 2001, Williams et al. 2002, Lusseau 2003, Ng and Leung 2003). Agonistic behaviors, such as tail and pectoral fin slaps on the surface of the water, may also be displayed (Williams et al. 2002).

Although cetaceans tend to respond to boat traffic with stereotyped, short-term avoidance tactics, determining a link between short-term responses and a long-term effect is difficult. By collecting behavioral data that can be quantified in terms of energetic costs to individuals, we can begin to understand how short-term avoidance tactics and displays of agonistic surface active behaviors may have long lasting effects. The purpose of this study is to determine if adult Southern Resident killer whales demonstrate avoidance and/or agonistic behaviors in response to vessels off San Juan Island. These data will later be used to model the potential energetic impacts of vessel disturbance to Southern Resident killer whales.

Material and Methods

Research was conducted in inshore waters near San Juan Islands, USA and off the east cost of Vancouver Island and the southern Gulf Islands, CA from late August through mid-September in 2003, early June through mid-September in 2004 and 2005, and mid-May through early August in 2006. Data was collected from a 5.18-meter Bayliner power boat with a 90 hp two-stroke outboard motor in 2003 and 2004 and from a 7.92-meter Pacific aluminum skiff with a 225 hp four-stroke outboard motor in 2005 and 2006. The research vessel departed from the west side of San Juan Island each morning between 6:00 a.m. and 10:00 a.m., weather permitting. Data were collected in Beaufort Sea state ≤3 and while visibility conditions were adequate for locating and following killer whales. Southern Resident killer whales were located by searching areas they frequent and by monitoring the VHF radio and pager system used by commercial whale-watchers. When killer whales were sighted, the boat approached to within approximately 100 m

to allow for positive identification of individuals and then retreated to a working distance of >100 m for the collection of data.

Data were collected continuously from individual adult male and female Southern Resident killer whales using a focal follow approach. Data were recorded, using Event 3.0 software created by J. Ha on a Palm III*xe*, while the boat was motionless or traveled at a slow speed in parallel with the focal whale at a distance >100 m. During focal follows, one identified whale was followed for the collection of continuous behavioral and energetic data. For the duration of each focal follow, the occurrence of every surface active behavior (e.g., spy hop, breach, tail slap, pec slap, porpoise, etc.), initiation of each dive, termination of each dive, and each breath taken by the individual was recorded. Swim speed through the water was estimated by paralleling the animal at a distance of >100 m and matching the speed of the boat with that of the whale. At 10-minute intervals throughout the focal follow, GPS position, pod identification, focal group size, spatial (contact, tight, loose, spread) and formation (flank, linear, non-linear) arrangement of the focal group, and number of boats (characterized as private, commercial whale watch, or kayak) within approximately 1000 m of the whale were recorded. Distances between the whale and the nearest vessels (closest, second closest, and the research vessel) were measured using a laser range finder (Bushnell Yardage Pro 1000) at least every 10 minutes or more frequently if vessel placement changed dramatically. In 2006 the distance between the focal whale and nearest vessels (closest, second closest, and the research vessel) and the behavior of these vessels (idling stationary, shut-down stationary, moving under motor) were recorded every time the focal whale performed a surface active behavior.

Due to the large sexual dimorphism in Southern Resident killer whales and the documented difference in responses of male and female Northern Resident killer whales to vessels, data from males and females were analyzed separately. Data from focal follows that consisted of 15–80 minutes of continuous data collection without missing an event (e.g., dive, surface, breath, occurrence of surface active behaviors) were included in the analysis. Because data from this study are still being analyzed, only preliminary, less sophisticated statistical analyses are presented. General linear model analyses were used to assess the significance of relationships between whale behaviors (dive duration, post-dive surface duration, the ratio between post-dive surface duration and previous dive duration, number of surface active behaviors, swim speed, and respiration rates), pod size, and vessel parameters (number of vessels present and the closest distance measured between the focal individual and a vessel during the entire focal follow). Although interactions between several variables are potentially significant, these interactions were not addressed in these preliminary analyses.

Results

In 2003, a pilot study was conducted for 8 days, resulting in 7 days of data collection. In 2004, 65 days in the field resulted in 27 days of data collection. The use of a larger research vessel in 2005 and 2006, increased the number of days in the field to 72 and 55 days, resulting in 39 and 38 days of data collection, respectively. A summary of the data collected is presented in Table 1.

Table 1. Summary of focal follows ≥15 minutes in duration (2003-2006).

Year	Adult Female	Sprouting/Adult Male	Total Focal Follows
2003	#focal follows: 0 # individuals: N/A	# focal follows: 11 # individuals: 3	11
2004	# focal follows: 11 # individuals: 7	# focal follows: 33 # individuals: 5	44
2005	# focal follows: 25 # individuals: 19	# focal follows: 63 # individuals: 7	88
2005	# focal follows: 48 # individuals: 19	# focal follows: 80 # individuals: 11	128
TOTAL	**# focal follows: 84** **# individuals: 28**	**# focal follows: 187** **# individuals: 13**	**271**

Analyses of the data collected during continuous focal follows ≥15 minutes from 2003 and 2004 (2003: n=10 male follows; 2004: n=31 male follows and n=10 female follows) only are presented because data from focal follows conducted in 2005 and 2006 are still being prepared for analysis. However, results from the preliminary analysis of data collected in 2006 on the performance of surface active behaviors relative to vessel distance and vessel behavior are presented.

The preliminary results suggest that only a few of the recorded behaviors change in response to the number of vessels present. There were no significant relationships between the number of vessels within 1000 m and swim speed (ms^{-1}), respiration rate (breaths min^{-1}), or the total number and rate of surface active behaviors (all $P>0.05$). Furthermore, there were no relationships between the number of individuals in the focal group of killer whales and these behaviors (all $P>0.05$). However, the relationships between these behaviors and other characteristics of the focal group (e.g., pod identification and spatial and formation arrangement) still need to be assessed.

Relationships between the number of vessels present and diving parameters are more complex. The preliminary results suggest that these relationships may differ between males and females (Figures 1, 2, and 3). In addition, relationships may depend on whether there are few (0-15) or many (>15) vessels present (Figures 1 and 2). Specifically, in males, surface duration and the ratio between surface duration and the previous dive duration significantly decrease with increasing number of vessels present when the number of vessels within 1000 m are low (0-15), but when there are many (>15) vessels present, there is no significant relationship between these variables. Interestingly, there was no significant relationship between the number of vessels within 1000 m and dive duration for either males or females.

Although focal whales performed one or more surface active behaviors during only 25.2% of all focal follows conducted in 2006, the data suggest that the performance of surface active behaviors may be related to vessel operation practices. In general, the number of surface active behaviors performed in a bout and the number of bouts of surface active behaviors performed were greater when the nearest vessel was moving under power, rather than when the nearest

vessel was stationary (Figures 4 and 5). The peak in the number of bouts of surface active behaviors performed occurred when the nearest vessel transited within 100-225 m of the whales (Figure 5). The results demonstrating that only a few surface active behavior bouts were performed by whales when the nearest vessel was within 0-100 m and there were no transiting vessels within 100 m of the whales (Figure 5) are likely artifacts of the "Be Whale Wise" Guidelines which discourage vessels from approaching whales within 100 m.

Discussion

Focal follows can provide detailed information to assess behavioral responses to vessel disturbance in Southern Resident killer whales. Preliminary results suggest that vessels may cause short-term, minor changes in killer whale behavior. However, the relationships between the number of vessels present within 1,000 m and diving parameters are not simple and may differ between males and females. For the limited sample of data analyzed from females, there appear to be no relationships between the number of vessels present and diving parameters. In contrast, the number of vessels present within 1,000 m has a slight but statistically significant effect on surface duration and the ratio of surface duration to previous dive duration in males. Yet, these relationships are complex and appear to change when there are few (0-15) versus several (>15) vessels present. Additional more sophisticated analyses are needed to assess the effect of the number of vessels present on diving parameters when few and many vessels are present. Furthermore, analyses of additional data from females are needed to better assess these relationships in female killer whales. The performance of surface active behaviors do not appear to be related to the total number of vessels present, but the data suggest there are relationships between the performance of surface active behaviors and the distance to and behavior of the vessel closest to the whale. Specifically, when vessels under power approach whales closely (within 225 m), surface active behaviors such as tail and pectoral fin slaps may be performed.

Future investigations will focus on calculating the energetic costs of avoidance behaviors (e.g., modifications of diving parameters and swimming speeds) and agonistic behaviors (e.g., surface active behaviors) performed in response to vessels. These results will then be used to assess whether these short-term behavioral changes have the potential to increase energetic costs, and consequently daily caloric requirements, of adult Southern Resident killer whales.

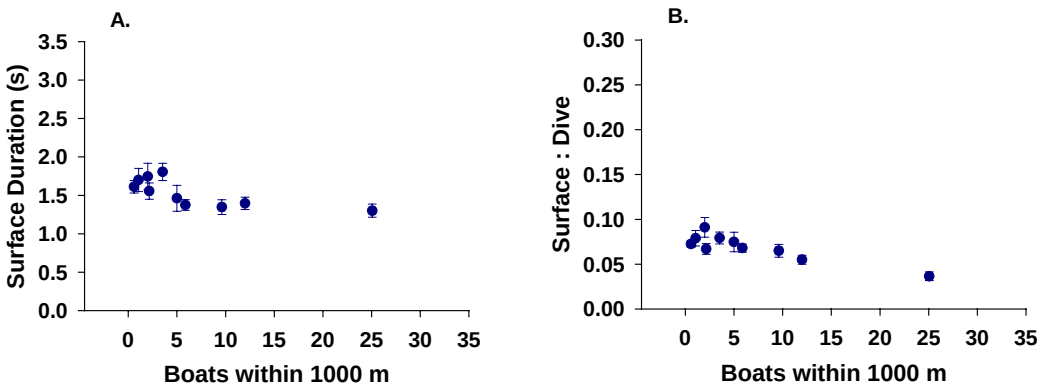

Figure 1. Relationship between the number of vessels within 1000 m of the focal whale and the A) surface duration and B) ratio of surface duration to previous dive duration during focal follows of individual adult male Southern Resident killer whales in 2003. Mean values for each focal follow (n=10) are presented with ± 1 SE bars.

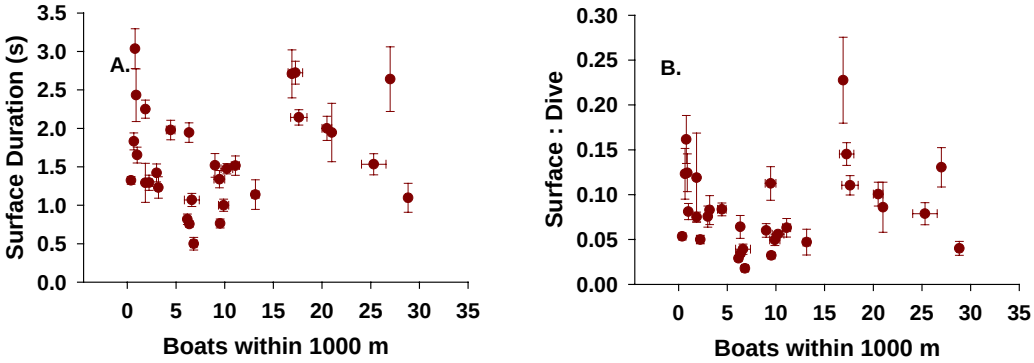

Figure 2. Relationship between the number of vessels within 1000 m of the focal whale and the A) surface duration and B) ratio of surface duration to previous dive duration during focal follows of individual adult male Southern Resident killer whales in 2004. Mean values for each focal follow (n=31) are presented with ± 1 SE bar.

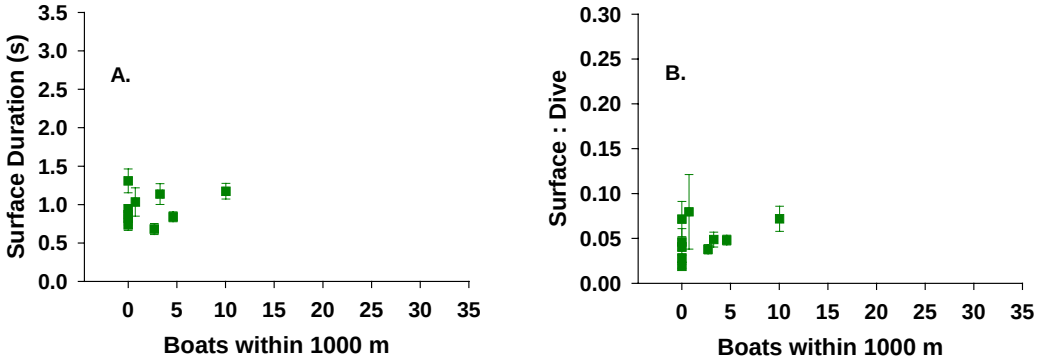

Figure 3. Relationship between the number of vessels within 1000 m of the focal whale and the A) surface duration and B) ratio of surface duration to previous dive duration during focal follows of individual adult female Southern Resident killer whales in 2004. Mean values for each focal follow (n=10) are presented with ± 1 SE bar.

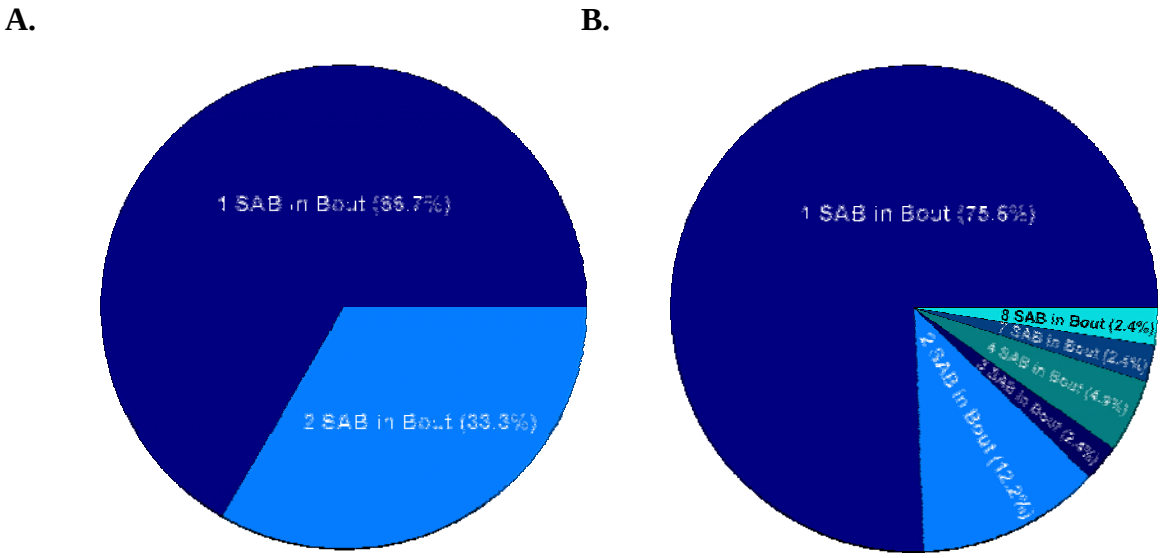

Fig. 4. Proportion of surface active behavior bouts that consisted of 1 to 8 successive surface active behaviors (SABs) in 2006 when A) the nearest vessel was stationary (includes idling) and B) the nearest vessel was moving under power. SABs often occurred in bouts. Bouts are presented here to correct for the inflated numbers that result from presenting individual occurrences of each SAB. Individual SABs were lumped into one single bout when the time that passed between the performance of each successive SAB was 1 minute or less.

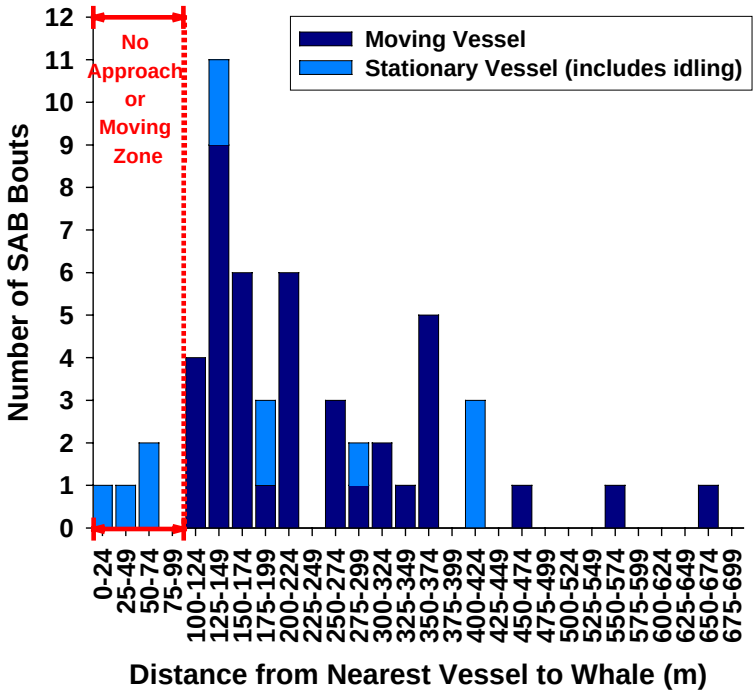

Fig. 5. Total number of surface active behavior (SAB) bouts for focal follows in 2006 in relation to the distance between the focal whale and the nearest vessel at the time the first SAB was performed. SABs often occurred in bouts. Bouts are presented here to correct for the inflated numbers that result from presenting individual occurrences of each SAB. Individual SABs were lumped into one single bout when the time that passed between the performance of each successive SAB was 1 minute or less. Distance data are presented in 25 m bins. Total number of SAB bouts is presented in stacked bars with moving

vessels designated by dark blue bars and stationary vessels designated by light blue bars. The distance from whales (0-100m) that is designated by "Be Whale Wise" Guidelines as an area that boats should not enter or move under power is shown in red.

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Health effects and potential evolutionary consequences of contaminant exposure in salmonids from Pacific Northwest rivers and estuaries

O. Paul Olson, Lyndal Johnson, Gina Ylitalo, and Mary R. Arkoosh
Northwest Fisheries Science Center
Environmental Conservation Division
Ecotoxicology Program
Seattle, Washington

Introduction

The Ecotoxicology group at the NWFSC has monitored a number of sites throughout the Pacific Northwest in recent years for the presence of chemical contaminants and their potential health effects on juvenile salmonids. These sites include restoration sites in Commencement Bay, Washington, various sites in Puget Sound, the Columbia River and it's estuary, and sites in river and estuary systems in Oregon. This poster compares some of the results of these studies and potential health effects due to chemical contaminant exposure in different salmon species and stocks, and possible evolutionary impacts on Pacific Northwest salmonids.

Life History Type and Exposure

Our results suggest that the likelihood of contaminant exposure varies with species and stock. Generally, we have found that contaminant concentrations are highest in species and stocks with an extended estuarine residence time, such as chum and fall Chinook salmon. Contaminant body burdens are typically lower in species and stocks with a stream-type life history, such as coho salmon.

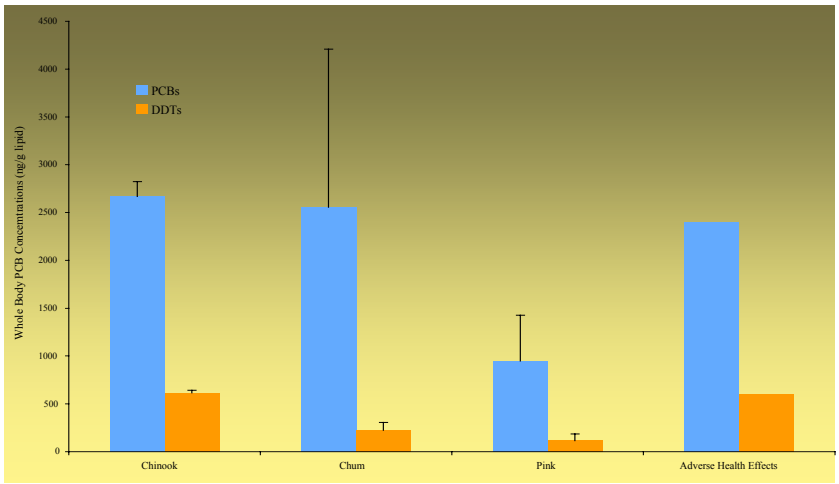


Figure 1. PCB and DDT concentrations in three species of juvenile salmonids from Tahoma Salt Marsh in Commencement Bay, WA.

Geographical Distribution of Contaminants and Salmon Stocks

Juvenile Chinook salmon, water, and sediment samples were collected by NOAA and USGS at six sites along the Lower Columbia River and Estuary (Warrendale, Morrison Street Bridge, Columbia/Willamette Confluence, Columbia City, Beaver Army Terminal, Point Adams, West Sand Island), from Bonneville to the estuary mouth. Juvenile Chinook salmon and food samples for chemical analysis were also obtained from six hatcheries along the Columbia.

Prespawn mortality: A special threat to urban coho stocks

Coho pre-spawn mortality

- Affects adult coho
- Symptoms are disorientation, lethargy, loss of equilibrium, gaping, death
- Rates of 20-90% in urban streams; rarely seen in non-urban streams
- Associated with storm events
- May be linked to non-point source pollutants in stormwater?

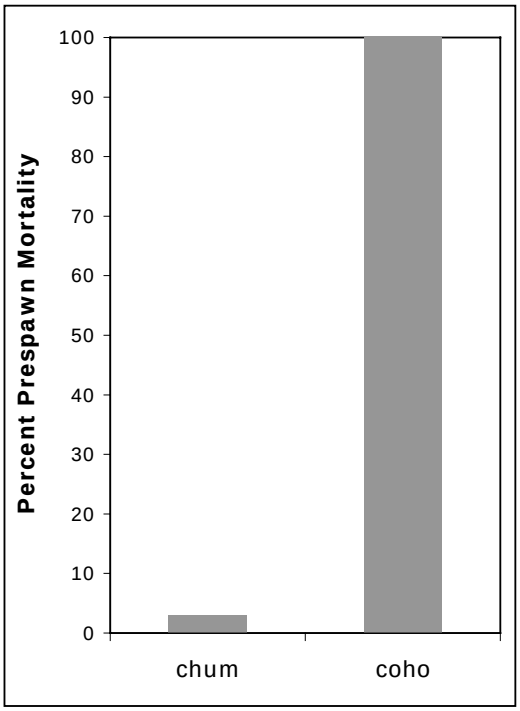


Figure 2. Percent pre-spawn mortality in chum and coho salmon.

Contaminants in Juvenile Salmon from Different Regions of the PNW

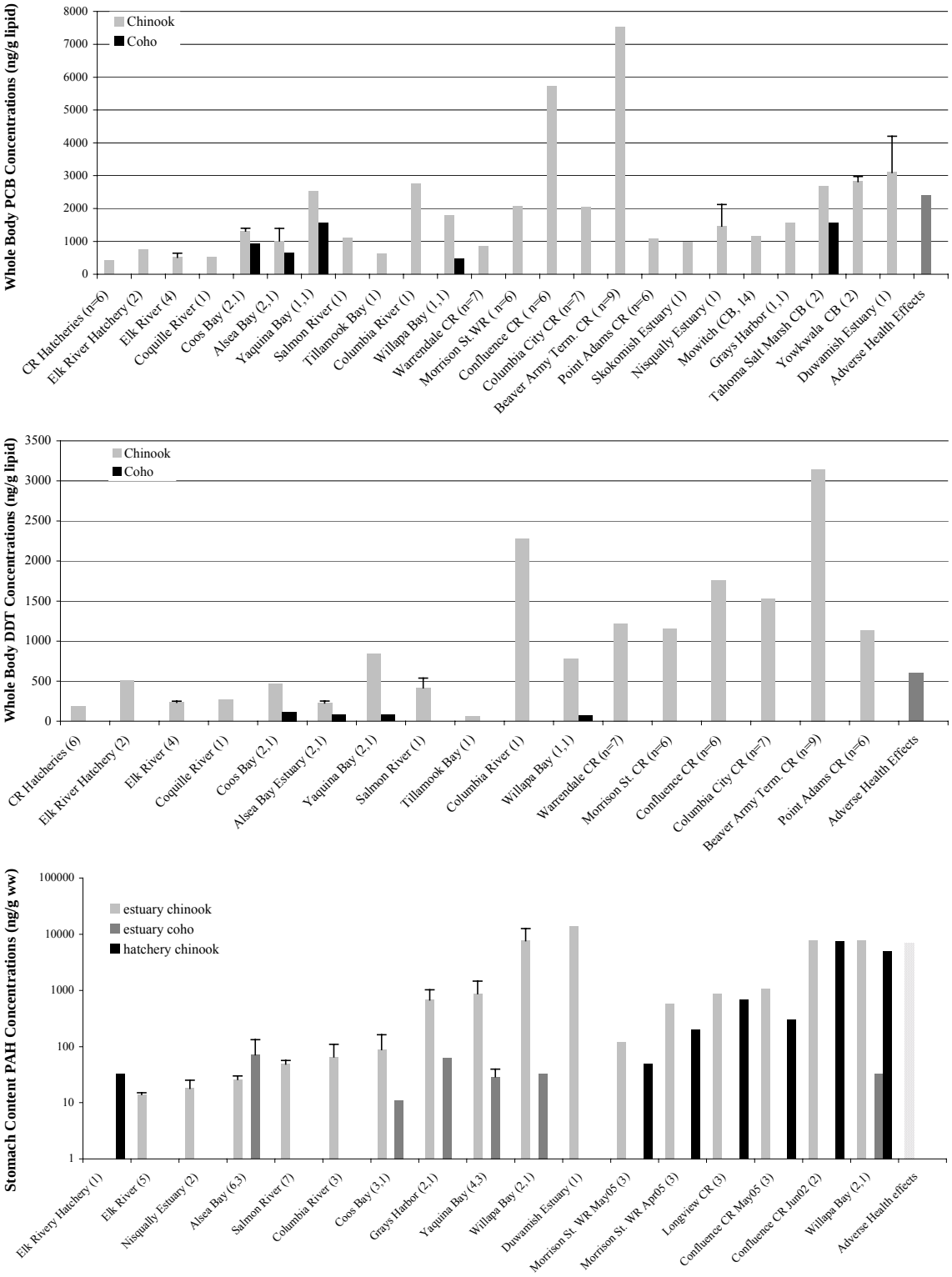
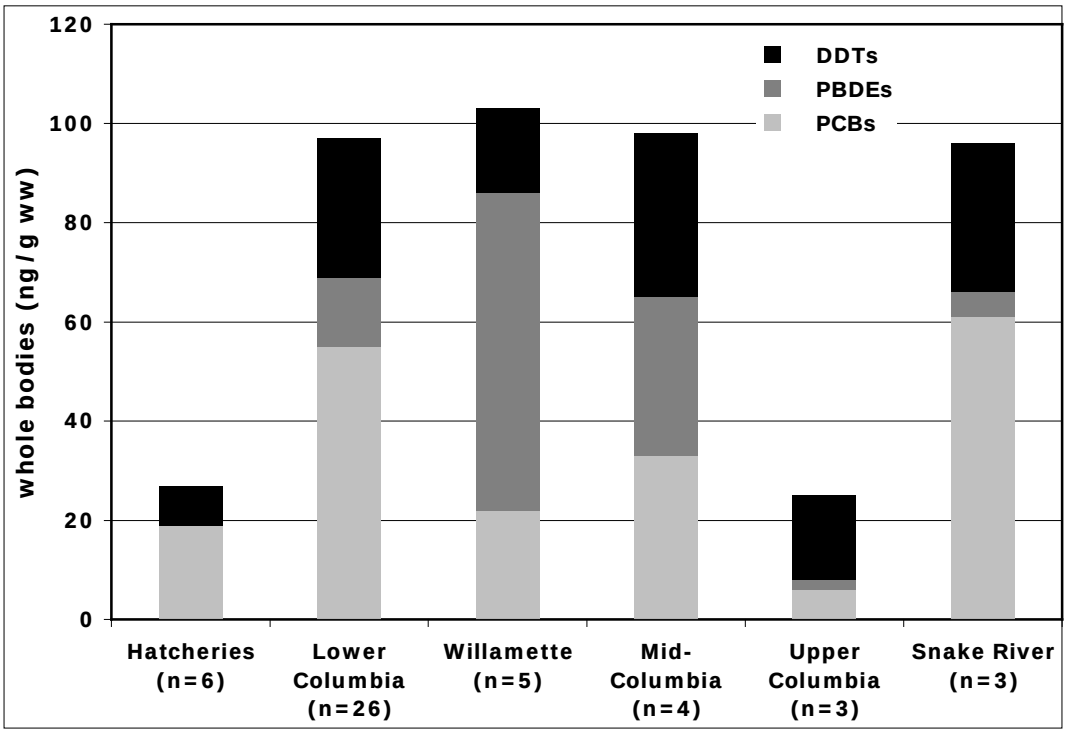


Figure 3. Whole body and stomach contents levels of PCB,s DDTs and PACs in juvenile Chinook and coho at Pacific NW sites (Columbia River (CR), Willamette River (WR), Commencement Bay (CB)).

Contaminant Uptake by ESU



We have also observed differences in contaminant body burdens in various stocks in the Pacific Northwest. For example, Willamette River Chinook generally have high body burden of PCBs, DDTs, and PBDEs, while Upper Columbia River fall Chinook do not.

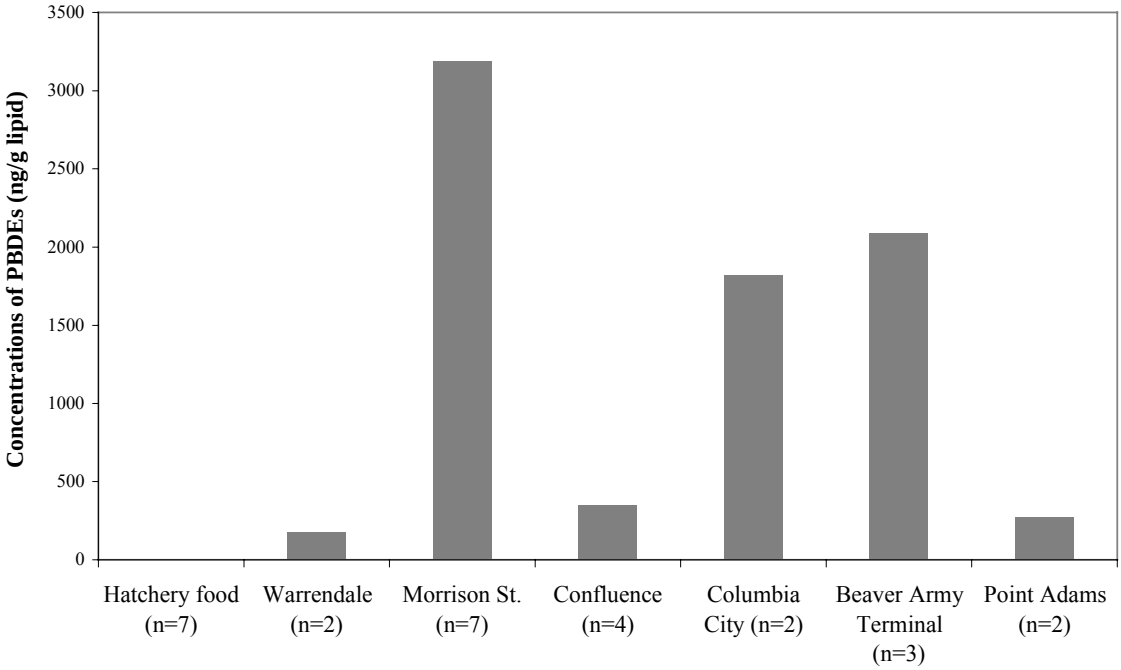


Figure 4. PBDE levels in whole bodies and hatchery food in juvenile Chinook from sites along the Columbia river.

Contaminant Impacts on Salmon

- Reduced growth and altered energy balanee (PCBs, PAHs)
- Increased disease susceptibility (PAHs, PCBs, PBDEs)
- Problems with neurological development and thyroid function (PBDEs)
- Endocrine disruption (wastewater compounds)
- Behavioral changes (reduced function, that may cause increased likelihood of predation, straying)
- Prespawn mortality of returning coho associated with urban environments

Summary and Conclusions

Our results suggest that the likelihood of contaminant exposure varies with species and stock. Generally, we have found that contaminant concentrations are highest in species and stocks with an extended estuarine residence time, such as chum and fall Chinook salmon. Contaminant body burdens are typically lower in species and stocks with a stream-type life history, such as coho salmon.

We have also observed differences in contaminant body burdens in various stocks in the Pacific Northwest. For example, Willamette River Chinook generally have high body burden of PCBs, DDTs, and PBDEs, while Upper Columbia River fall Chinook do not.

The persistent presence of chemical contaminants at Pacific Northwest sites may pose a threat to the survival of affected stocks. Concentrations of PACs, PCBs, and DDTs in sediment, prey, and fish at some sites are above concentrations associated increased risk of immunosuppression, impaired thyroid function, reduced growth, reproductive impairment, DNA damage, and delayed mortality in outmigrant juveniles

Exposure to contaminants might also exert evolutionary pressure on affected stocks in several ways including:

- Selecting for fish that are more resistant to these contaminants
- Selecting for fish that spend less time in the estuary to avoid contaminant exposure, which may contribute to reductions in the life history diversity of salmon stocks
- Selective processes may or may not be advantageous for the long term survival of affected stocks

Persistent organic pollutants in Southern Resident killer whales: Potential sources from Pacific salmon

Sandra O'Neill and James West
Washington Department of Fish and Wildlife
Olympia, Washington

Gina Ylitalo, Donald Brown, Catherine Sloan, Jennie Bolton, and Margaret Krahn
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
gina.ylitalo@noaa.gov

Previous studies indicated that Southern Resident killer whales (*Orcinus orca*) contain higher concentrations of persistent organic pollutants (POPs) than found in northern residents and other north Pacific "fish-eating" killer whales. Elevated contaminant levels in Southern Residents may be due to dietary differences among these whale populations or to regional differences in POP concentrations in their prey. Free ranging populations of anadromous Pacific salmon, known prey of southern and northern residents, generally have low levels of POPs, as most of their growth occurs in open waters of the Pacific Ocean. However, the five Pacific salmon species differ in their oceanic distribution, with some species having a more coastal distribution. Furthermore, populations within species can also differ in their use of estuaries and in oceanic distribution. Overall, concentrations of POPs were higher in coho and Chinook populations that have more coastal distributions than those measured in salmon species with more oceanic distributions. Regional variation in POP exposure was also evident in Chinook salmon and appears to be associated with differences in marine distribution of these species. For example, Fall Chinook returning to Puget Sound had 2 to 6 times more PCBs and 5 to 17 times more PBDEs than other populations of Summer/ Fall Chinook. Similarly, concentrations of PCBs and PBDEs were 3 and 5 times higher in Southern Residents than northern residents. In addition to contamination, regional differences in caloric content of Chinook salmon from Puget Sound further reduce their quality as prey to Southern Resident killer whales.

Does supplementation work? Analysis of long-term effects of hatchery releases on Snake River basin steelhead populations

Melanie Paquin
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington
melanie.paquin@noaa.gov

This study characterizes the genetic structure of Snake River Basin steelhead populations by comparing the distribution of genetic variation in wild steelhead populations (those with little or no hatchery influence) to naturally-produced steelhead populations (those with varying levels of influence from hatchery fish). Fifty-one steelhead populations from throughout three Snake River sub-basins—the Lower Snake, Clearwater, and Salmon—were analyzed. "Wild" and "naturally-produced" populations were used to test the hypothesis that introgression by steelhead hatchery fish has diminished naturally occurring genetic variation among Snake River Basin steelhead populations. Analysis of 14 microsatellite loci was used to estimate levels of gene flow and to identify geographic areas that contain genetically differentiated populations. Results from FST pair wise comparison analysis show overall genetic variation is low within the Lower Snake River sub-basin relative to the other two sub-basins. More importantly, patterns of genetic variation between populations within the sub-basins were similar for wild and naturally-produced populations. This suggests that hatchery introgression, especially from non-indigenous hatchery sources, has not dramatically influenced the genetic structure of the Snake River steelhead populations. The results reported here support recent studies of reproductive success in hatchery-origin steelhead and suggest that non-indigenous or highly domesticated stocks may have relatively little success reproducing in the wild.

Ocean conditions and salmon survival in the northern California Current

William T. Peterson, Edmundo Casillas, and John W. Ferguson
Northwest Fisheries Science Center
Fish Ecology Division
Seattle, Washington
bill.peterson@noaa.gov

Rian C. Hooff
Washington State University
Department of Biological and Environmental Sciences
Vancouver, Washington

Cheryl A. Morgan and Karen L. Hunter
Oregon State University
Newport, Oregon

We developed three sets of ecosystem indicators to aid in understanding the ecological interactions between juvenile salmon and coastal oceanographic conditions for use in predicting adult salmon returns to Pacific Northwest rivers. The first set of indicators is based on large-scale oceanic and atmospheric conditions in the North Pacific Ocean, and consists of the Pacific Decadal Oscillation and the Multivariate El Niño Southern Oscillation Index. These metrics help gauge the influence of basin-scale winds and ocean currents on local ocean dynamics off the coast of Washington and Oregon. The second set of indicators is based on local observations of physical and biological ocean conditions off Newport, Oregon. They include measures of upwelling, water temperature and salinity characteristics, and plankton species compositions. The third set of indicators consists of estimates of juvenile salmonids, forage fish, and Pacific hake abundance. From this combination of physical and biological indicators we forecast adult salmon returns. Almost all of the 14 individual ecosystem indices measured in 2005 point to low adult returns of coho salmon in 2006 and spring Chinook salmon in 2007. This information will be used by fishery managers to set harvest levels, and by river managers to interpret salmon recovery actions taken in freshwater in the context of ocean productivity.

Movement and behavior of steelhead (*Onchorhynchus mykiss*) smolts through Hood Canal

Megan Petrie, Skip Tezak, and Barry Berejikian
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Manchester, Washington

Unexplained declines in Hood Canal and Puget Sound steelhead populations have been detected in the last 10 to 20 years, and have been shown to contrast markedly with the relatively stable condition of populations along the Washington and Oregon coasts. This discrepancy between the health of Coastal as opposed to Puget Sound and Hood Canal steelhead populations indicates that nearshore smolt migration may constitute a major cause of mortality. Many studies have been published on the early marine survival of Pacific Salmon and steelhead species (Healey 1982, Ward 1989, Henderson and Cass 1991), but no known studies have looked at early marine migration of Puget Sound or Hood Canal steelhead. In this study, wild steelhead smolts from the Skokomish River, the Dewatto River, and Big Beef Creek, and hatchery-reared smolts from the Hamma Hamma River population were tagged with acoustic transmitters in order to determine patterns of survival and migration.

Materials and Methods

Wild and hatchery steelhead smolts from four rivers feeding into the Hood Canal were tagged with Vemco V9 acoustic transmitters (9 mm diameter x 20 mm diameter, 3.5 g). Two Vemco VR2 acoustic receivers were situated at each river mouth and at various other locations within Hood Canal. A line of four receivers was hung from the Hood Canal Bridge in an attempt to detect all smolts passing that location. Binary logistic regression was used to determine the factors that best predicted estimated minimum odds of marine survival of the tagged fish. The independent variables in the initial analysis (the 'full model') were population (BBC, Dewatto, Hamma Hamma, or Skokomish), length, release date, smoltification index, and condition factor. A 'reduced model' included those independent variables that explained a large portion ($P < 0.25$) of the variability in the odds of survival. General linear modeling (GLM) was used to investigate the factors that explained the variation in travel rate and relative Hood Canal residence time. The independent variables were population, length, smoltification index, release date, and all interactions. The proportion of fish entering Hood Canal that were detected moving at least once in a southerly direction (i.e, away from the Hood Canal Bridge) was also calculated.

Results

Marine survival

Overall marine survival rate from river mouth to the Hood Canal Bridge was 77% (84 out of 109). The Big Beef Creek population also had the highest marine survival rate (85.4%; Table 1). None of the independent variables explained a significant portion of variability in marine survival ($P > 0.15$), including no differences among populations (Dewatto, $P = 0.152$; Hamma Hamma, $P = 0.632$; Skokomish, $P = 0.179$).

Table 1. Summarized biological, survival, and behavioral data from steelhead implanted with acoustic transmitters and released into Hood Canal Streams.

Population	Mean Length (mm)	Mean Weight (g)	% Survival to RM	Distance from RM to bridge (km)	% Survival from RM to bridge	Travel Rate (km/d)	Residence Time (d)
Skokomish	195	69.8	78%	75.4	71.4	8.36 ± 1.44	17.7 ± 4.7
Dewatto	189	58.6	90%	62.0	66.7	7.12 ± 1.72	11.2 ± 2.6
Hamma	168	43.8	88%	50.8	75.9	8.68 ± 1.83	21.8 ± 4.3
BBC	194	65.3	98%	28.1	85.4	7.60 ± 1.27	15.2 ± 2.5

Travel rate

Travel rate within Hood Canal averaged 8.0 km/d (SE ± 0.84) for all populations combined (Figure 1). None of the variables included in the GLM (population, length, condition factor, release date) explained a significant portion of variability in travel rate (Table 1).

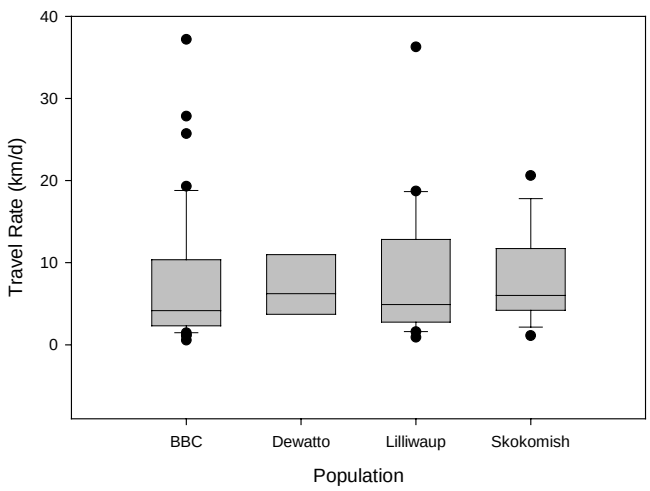


Figure 1: Travel rate of tagged fish from respective river mouths to Hood Canal Bridge.

Residence time

Residence time in Hood Canal averaged 17.2 days, and the amount of time fish spent in Hood Canal was found to be highly variable (Figure 2). The full GLM revealed no significant effects of population, length, release date, or interactions on marine residence (Figure 2).

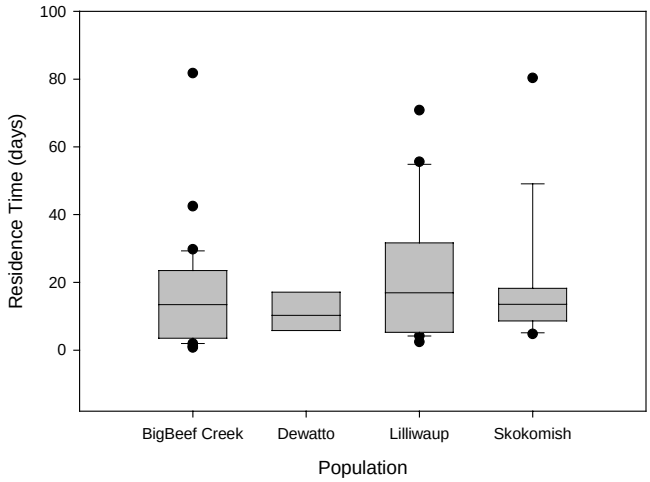


Figure 2. Tagged fish residence time - from the last river mouth detection to the last Hood Canal bridge detection, corrected for distance from the river to the bridge.

The reduced model shows a significant interaction between population and release date (P = 0.027), indicating that relative residence time for BBC fish was significantly shorter for the earliest release group (Figure 3).

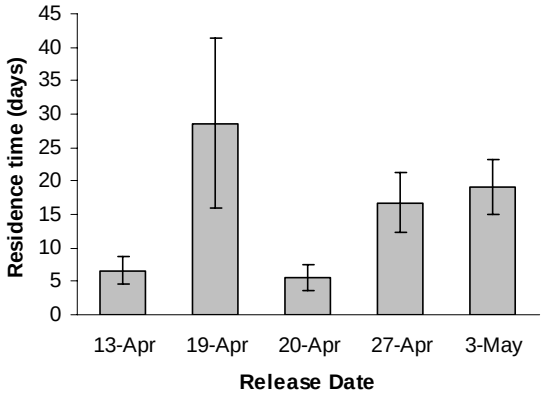


Figure 3: Residence time for Big Beef Creek fish released on different dates

Migration patterns

Thirty-nine of the 82 fish (47%) that were detected both in the estuary and at the bridge were detected at receivers south of where they had at least once been previously detected. Twenty-three out of 40 (57%) of the fish from Big Beef Creek, 3 of 5 (60%) of the fish from the Dewatto River, 10 of 22 (45%) of the Hamma Hamma River fish, and 3 of 15 (20%) Skokomish River fish travelled in a southerly direction south at least once.

Discussion

This is the first study to quantify early marine survival of steelhead in Hood Canal, and builds on recently obtained information on early marine survival of steelhead in the Salish Sea region (Puget Sound and Georgia Basin). Welch et al. (2004) studied steelhead smolt survival in Queen Charlotte Strait, and found a minimum marine survival rate of 55% for wild smolts, though their array did not detect fish migrating by all possible routes. Hood Canal early marine survival rates quantified here (75% for wild and hatchery combined) are high in comparison and represent minimum survival estimates. The two northern-most populations had higher survival to the bridge than the two populations to the south, but the analyses failed to detect significant effects of travel distance, body size, smoltification index or release date on estimated survival. The one hatchery population exhibited survival rates and migratory characteristics within the range of the three wild populations, with the exception of a slightly longer residence time in Hood Canal (non-significant). Since there were many fish that travelled through Hood Canal at rates much slower than the maximum observed rate, it is likely that the majority of the steelhead smolts in this study used the Hood Canal to some degree as a rearing area, and not simply as a migratory corridor. Moreover, nearly half of all fish detected beyond the river mouths were not migrating linearly through Hood Canal.

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Toxic nurseries: Settlement and recruitment of English sole in urban and rural nursery habitats of Puget Sound

Rachel J. Petrik-Finley, Paul M. Chittaro, and Phillip S. Levin
Northwest Fisheries Science Center
Fishery Resource Analysis and Monitoring Division
Science for Ecosystem-based Management Initiative
Seattle, Washington
rachel.petrik-finley@noaa.gov

Urban growth and industrial development in coastal regions are in danger of encroaching on habitats critical to recreationally and commercially important species. Essential nursery habitats for young-of-the-year English sole in Puget Sound may be threatened by high levels of urban and industrial development and heavy inputs of chemical contaminants. We used monthly field surveys to evaluate settlement at sites at sites throughout Puget Sound, and examined a suite of habitat and community variables that may impact growth and survival in juvenile sole. We observed strong latitudinal gradients in settlement of young-of-the-year English sole with northern sites and sites along the western side of Puget Sound receiving the highest abundance of settlers. Settlement was also lower at sites near to and in urban areas. Preliminary analysis of contaminant exposure indicates juvenile English sole are exposed to some toxins (i.e., polyaromatic hydrocarbons) even at nursery sites far from urban development.

Inhibition of sortase enzyme activity of *Renibacterium salmoninarum* dramatically reduces adherence and prevents invasion of Chinook salmon cells *in vitro*

Sudheesh Ponnerassery, Samuel Crane*, and Mark Strom
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington
sudheesh.ponnerassery@noaa.gov
mark.strom@noaa.gov

*Present address: Department of Biology
City University of New York and the Institute for Comparative Genomics
American Museum of Natural History
New York, New York

Renibacterium salmoninarum is a Gram-positive bacterium and the causal agent of bacterial kidney disease (BKD), a chronic systemic infection in salmonid fishes worldwide. Genome sequencing of the bacterium identified a sortase homologue and nine open reading frames specifying proteins presumably translocated to the bacterial cell wall mediated by the sortase enzyme. The sortase and sortase substrates identified by the bioinformatic analyses were found to be constitutively transcribed in *R. salmoninarum*. Inhibition of sortase activity by phenyl vinyl sulfone (PVS) significantly reduced the adherence of the bacterium to chinook salmon fibronectin when assayed by an ELISA procedure. In addition, the ability of the PVS treated bacteria to adhere to chinook salmon embryo cells (CHSE-214) *in vitro* was dramatically reduced compared to that of untreated bacteria. More importantly, PVS treated bacteria with reduced adherence properties were also unable to invade and grow inside CHSE-214 cells as demonstrated by an intracellular growth assay and by light microscopy. In addition the PVS-treated bacteria could not produce any cytotoxicity to CHSE-214 cells, whereas, untreated bacteria produced cytotoxicity in few days. These findings clearly show that inhibition of sortase activity by small molecule drugs like PVS can interfere with the ability of *R. salmoninarum* to adhere and colonize fish cells, thereby possibly reducing its virulence. Further research will probe the effect of the drug in reducing the colonization of *R. salmoninarum* in salmonids *in vivo* and as an antivirulence chemotherapeutic drug for the treatment of BKD.

Induced macrolide antibiotic resistance in the salmonid pathogen *Renibacterium salmoninarum*

Sudheesh Ponnerassery and Mark Strom
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington
sudheesh.ponnerassery@noaa.gov
mark.strom@noaa.gov

Bacterial kidney disease caused by *Renibacterium salmoninarum* is a chronic disease affecting salmonids worldwide. The macrolide antibiotics erythromycin and azithromycin have been used to treat the disease in fish with limited success, and the possibility of drug resistance development is a major concern. This study was carried out to investigate the potential of the bacterium to develop induced resistance to these drugs and to identify the genes involved in the context of the recently sequenced genome of the bacteria. *R. salmoninarum* was induced to resist up to 2 mg/ml erythromycin and 2µg/ml azithromycin after growing the bacteria in selective kidney disease medium (SKDM-2) in the presence of macrolide drugs starting from 0.00195 µg/ml and then inoculating the cultures to increasingly higher concentrations of drugs. The transcription of six macrolide resistance genes was analyzed by reverse transcription–PCR. Transcription of a methyl transferase gene and a gene specifying a macrolide efflux protein was induced following drug treatment even at sub clinical doses. The induced macrolide resistance phenotype was found to be stable for two subcultures when the induced bacteria were grown in SKDM-2 medium in the absence of any antibiotic. Induced macrolide resistance was also observed when the bacteria previously grown in the presence of macrolide drugs were used to infect a CHSE-214 cells. The finding that the bacteria were able to resist subclinical doses of these drugs points to possible resistance development in salmon hatcheries after prophylactic macrolide treatment of BKD.

Seed quality: Engineering larval diets to increase growth and reduce disease transmission

Adelaide C.E. Rhodes
University of Idaho
Aquaculture Research Institute
Hagerman Fish Culture Experiment Station
Hagerman, Idaho
adelaide.rhodes@noaa.gov

Michael B. Rust
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington

Diet, disease and economics are the three biggest challenges encountered by hatcheries that desire to optimize post-release fingerling survival and quality. New developments in fingerling production related to innovative diets and associated disease management techniques such as probiotics, immunostimulants, disease prevention and vaccination delivery systems can improve the quality of fingerlings used in stock enhancement. This poster reviews the latest larval feeding and treatment techniques used to reduce the cost of fingerling production, improve larval growth and development, and reduce the risk of disease transmission at the hatchery level.

Disease Prevention

Hatchery production of healthy, hardy fingerlings is crucial to the development of stock enhancement protocols. Not only does mitigation of the risk of disease and genetic change start in the hatchery, fingerlings that are not physically robust will not survive as well when released to the wild. Once environmental parameters are fine-tuned, the hatchery can use the combined strategies of vaccination and nutrition.

Probiotics and Immunostimulants

Probiotics, nonpathogenic opportunistic bacteria of the genera *Bacillus*, *Lactobacillus* and *Streptococcus* have been proposed as a defense against harmful bacteria in the digestive system (Sorgeloos et al., 2001; Gomez-Gil et al., 2000; Skjermo and Vadstein, 1999). For example, *Vibrio sp.*, which are known fish pathogens, constitute a substantial component of the bacterial flora that is naturally associated with *Artemia* (Olafsen, 2001; Verschuere et al., 2000). Olsen et al. (2000) demonstrated a correlation between microbial levels in live feeds and *Hippoglossus hippoglossus* larvae mortality rates. Probiotics are an alternative to antibiotics for controlling pathogenic strains of bacteria found in the culture water (Skjermo and Vadstein, 1999).

Probiotics can easily be incorporated into microparticulate feeds or bio-encapsulated during the enrichment phase of *Artemia* production (Eddy and Jones, 2000; Skjermo and Vadstein, 1999). The introduction of probiotic varieties such as lactic acid bacteria strains already found in fish gut microflora (Ringo and Gatesoupe 1998) could have similar pathogenic control properties to

the strains successfully used for terrestrial animals (Sorgeloos et al. 2001; Gomez-Gil et al. 2000).

Natural immunostimulants (yeast, glucans) have also shown promise for increasing disease resistance in fish, but still lag behind vaccines in their effectiveness (Devresse et al. 1997). For example, Atlantic cod *Gadus morhua* L. do not have completely functioning immune systems until 2 or 3 months post-hatch, making vaccination at earlier life stages impractical. Magnadottir et al. (2006), in a survey of 12 potential immunostimulants, found a reduction in 10 day post-hatch cod mortality under stressful conditions when the larvae were treated with lipopolysaccharide (LPS) isolated from the bacterium *Aeromonas salmonicida* or crustacean polysaccharides. The results were not consistent at all ages, but the application of immunostimulants to early larvae may provide enough benefit to overcome the limitations of the approach.

Environmental Control

Fish reared outside their optimal temperature and salinity and density can develop a range of abnormalities. Incidences of scoliosis, lordosis, exopthalmia, blindness and opercular deformities increase outside of the optimal ranges (Fig. 1). Documenting the incidence of deformity at a range of temperatures and salinities (Fig. 2) helps to separate the incidence of deformity due to lack of appropriate nutrients or disease, and can relate to potential consequences of climate change.

Figure 1. From the top left: Vertebral deformity in California halibut, exophthalmia in sea bass, blindness, opercular deformity, lordosis in white sea bass, and lordosis in sablefish. (Source: Photographs, except for the sablefish, were provided by Mark Drawbridge, Hubbs SeaWorld Research Institute.)

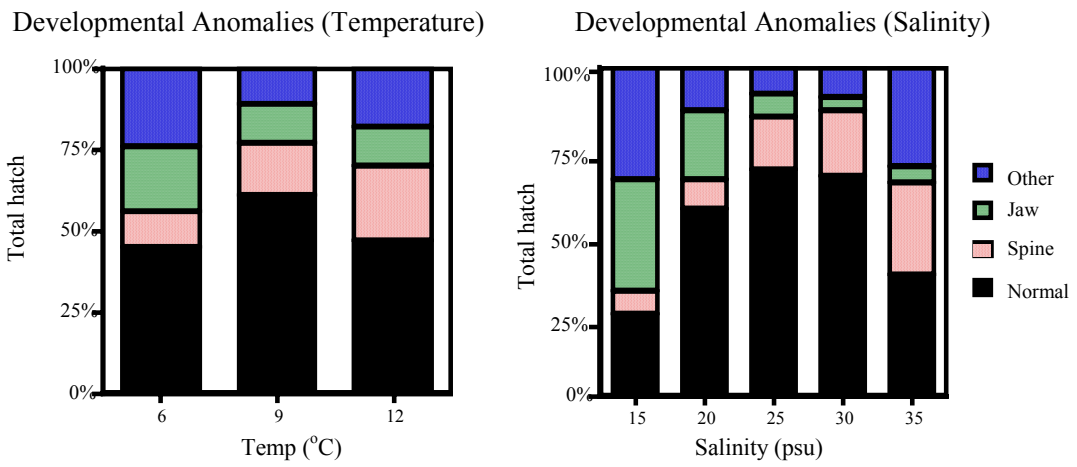

Figure 2. Rates of deformity in lingcod *Ophiodon elongatus* when reared at suboptimal salinity and temperature (M. Rust, unpublished data).

Nutrition Deficiencies

Rates of deformity in hatchery-reared gilthead sea bream (*Sparus aurata)* exceed the 4% observed in wild caught larvae (Boglione et al. 2001). Very few of the fish can survive extreme deformity. Table one lists some of the more likely nutrient deficiencies which lead to deformities. Some nutrients, as noted, were harmful in excess.

Table 1: Nutritional deficiencies which lead to deformities in hatchery-reared fish. Adapted from Cahu, C., Zambonino-Infante, J. and Takeuchi, T. 2003.

Deformity	**Species**	**Nutrient (citation)**
Scoliosis	Chum salmon	Tryptophan (Akiyama et al., 1986)
Skeletal malformation	Seabream, *Sparus aurata*	Peptides (Kolkovski and Tandler, 2000)
Bone deformities	Japanese flounder, *Paralichthys olivaceus*	Excess Vitamin A (Dedi et al., 1995)
Gill arch pathology and caudal fin deformation	Common carp	Vitamin C (Dabrowski et al., 1988)
Twist jaw and scoliosis	Ayu, *Plecoglossus altivelis*	Phospholipid (Kanazawa et al., 1981)
Malpigmentation	Halibut, *Hippoglossus hippoglossus*	HUFA's (Shields et al., 1999)
Opercular malformation	Milkfish, *Chanos chanos*	DHA (Gapasin and Duray, 2001) and ascorbic acid (Gapasin et al., 1998)

Treating Live Feeds

Artemia (brine shrimp) and rotifers can have nutritional limitations, as well as introduce unwanted microbial organisms and pathogens into the larval rearing system. These live feeds can be improved nutritionally with focused enrichments that provide the essential fatty acids and vitamins which ensure good growth and normal development, or they can be replaced by

alternative live feeds such as copepods. The development of alternative live feeds such as copepods or polychaete worms, which can be sterilized before introduction may reduce disease transmission (Figure 3).

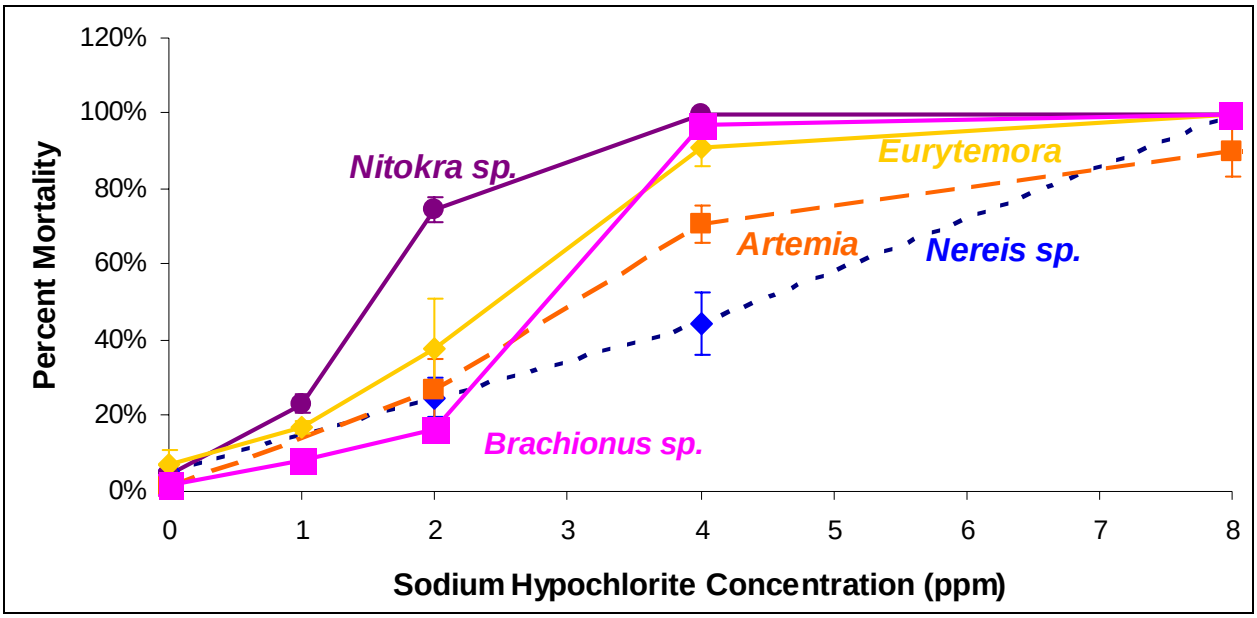

Figure 3. After 24 hours exposure to sodium hypochlorite, the prey items *Nitokra lacustris* (harpacticoid copepod), *Eurytemora americana* (calanoid copepod), *Brachionus plicatilis* (rotifer), *Artemia*, and *Nereis virens* (polychaete worm) were all still viable. At dosages are sufficient to kill most pathogenic bacteria (2 ppm for 10 minutes is typical for drinking water), the live feeds all survived to some extent. Rinsing and disinfecting the live feeds before introduction to larval tanks could reduce pathogen introduction and proliferation (A. Rhodes, unpublished data).

Microparticulate Feeds

The early introduction of microparticulate diets needs to be further developed to reduce potential disease vectors, to increase growth and reduce economic costs to the hatcheries. At the NMFS Montlake facility, researchers have developed a method for the direct comparison of the uptake of live and microparticulate feeds (Cook et al. in press). Food items are marked with inert metal oxides that do not affect food quality while allowing for the detection of feed preference and digestibility (Fig. 4).

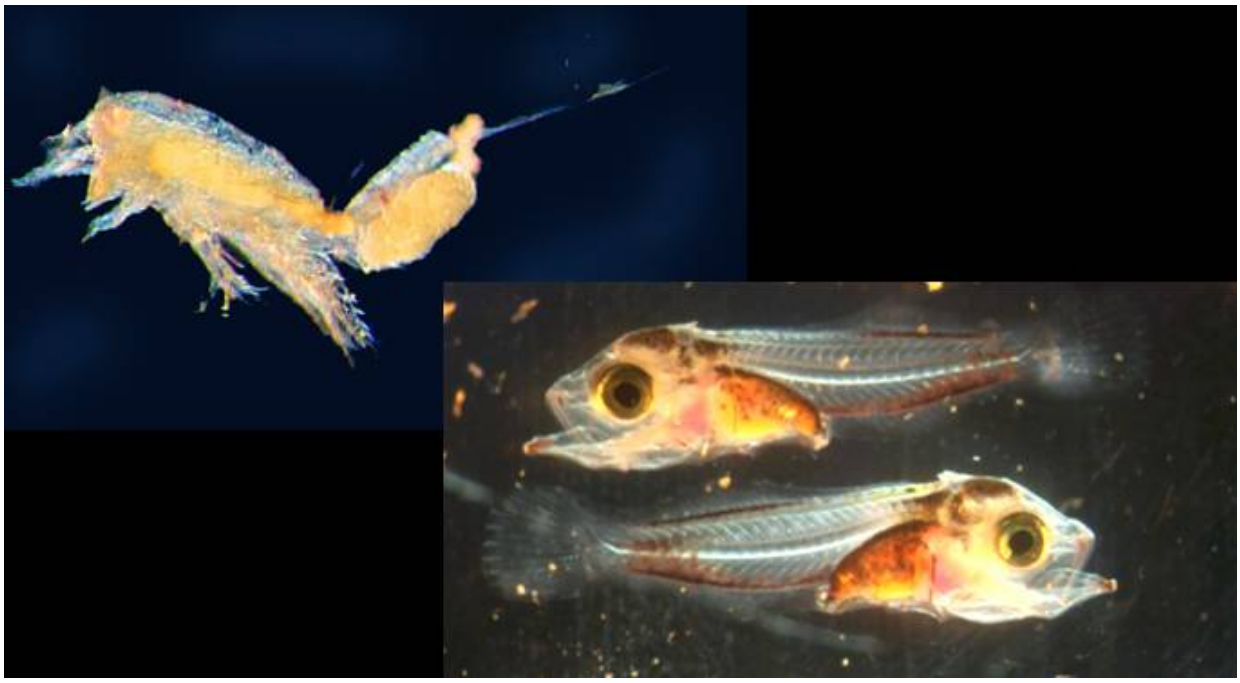

Figure 4. Top left: Copepod that has taken up yttrium, automatic feeder. Bottom right: Yelloweye rockfish (*Sebastes ruberrimus)* that have consumed *Artemia* marked with yttrium. (Photo by Mark Tagal, Hubbs SeaWorld Research Institute.)

Discussion

Future investigations will focus on the development of healthier fish through a variety of these methods. Utilizing the inert metal oxide uptake protocol, we should be able to track the ingestion and digestion of a variety of inert and living feeds (Cook et al., in press). The nutritional value of the feeds and optimum growth conditions will be analyzed by recording the incidence and type of deformities found in rockfish, lingcod and other species raised at Montlake. Live feeds are potential vectors of disease, as well as handy delivery mechanisms for immunostimulants or probiotics. Therefore, we will be using a combined strategy of live feed treatment to remove unwanted organisms and enrichments to add advantageous microorganisms to the live feeds.

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Abundance, length, stock origin, and pathogen infection in marked and unmarked juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the nearshore surface waters of Puget Sound

Casey Rice and Correigh Greene
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
correigh.greene@noaa.gov

Paul Moran, David Teel, and David Kuligowski
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, Washington

Linda Rhodes, Shelly Nance, Surafel Gezahegne, and Colleen Durkin
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington

Reg Reisenbichler
U.S. Geological Survey
Western Fisheries Research Center
Ecology Section
Seattle, Washington

Eric Beamer
Skagit River System Cooperative
La Conner, Washington

Northwest Fisheries Science Center
Fish Ecology Division
Seattle, Washington

To better understand major anthropogenic influences on wild Puget Sound Chinook salmon (*Oncorhynchus tshawytscha*), we studied seasonal and geographic patterns in the abundance, size, stock origin, and pathogen infection prevalence in marked (known hatchery) and unmarked (majority natural origin) juvenile Chinook. Monthly surface trawl sampling was conducted from April to October in 2003 at 52 sites in Eastern Puget Sound ranging from Bellingham Bay to the Nisqually Reach. Unmarked Chinook in the northern regions of the study area showed broader seasonal distributions of abundance and length than both marked Chinook from all areas and unmarked Chinook in central and southern Puget Sound. Unmarked fish tended to be smaller than marked fish. For genetic analysis, data from 13 standardized microsatellite DNA loci surveyed in over 60 spawning populations in Washington and British Columbia were used as a

baseline to estimate the stock composition of a subset of 424 unmarked individuals. The genetic results, combined with coded wire tag (CWT) data from 283 fish, showed that juveniles in all sampling areas included individuals from a wide range of populations, and that fish from different source populations vary in terms of movement patterns and apparent residence time. Prevalence of infection by *Renibacterium salmoninarum* was related to capture location rather than stock origin. These results demonstrate that juvenile Chinook use neritic waters in Puget Sound during much of the year, but suggest more extensive use of estuarine environments by wild rather than hatchery Chinook, and differential use of various geographic regions of greater Puget Sound by all Chinook. In addition, length differences and infection associations have implications for interactions between hatchery and wild Chinook throughout Puget Sound.

Describing the Southern Resident killer whale watching industry of Greater Puget Sound

Suzanne M. Russell and Morgan Schneidler
Northwest Fisheries Science Center
Conservation Biology Division
Seattle, WA 98112

Orca whales (*Orcinus orca*), also known as killer whales, are cultural icons for the human residents of the Pacific Northwest's Puget Sound. They recently received an endangered listing under the Endangered Species Act, and are protected under the Marine Mammal Protection Act. In a research atmosphere where ecosystem science is emphasized, it is instinctive to discuss the biological elements of an ecosystem. However, it is important to recognize that humans are part of the ecosystem as well. While biological research on the Southern Resident Killer Whale (SRKW) population is beginning to answer ecosystem questions, there is little information on the connection of these animals to human populations.

As social scientists, we study the human dimension of ecosystems, working to better understand people and their relationships to marine resources. In this instance, we are interested in better understanding the relationship of people to the SRKW. Specifically, we have identified a unique relationship between the whale watching industry of the Greater Puget Sound and the SRKW. We aim to more clearly understand this connection. As with fishermen who depend on healthy fish stocks, this industry depends on a healthy and resilient population of SRKW. This poster summarizes the ongoing research efforts aimed at describing the people involved in the whale-watching industry. Insights into the reasons people participate in the industry as well as the backgrounds of those participants will clarify the connection to the significant marine resource that is the SRKW.

Objectives

The purpose of this research is to create a sociocultural description of the U.S. whale watching industry in the greater Puget Sound area. It is important to note that in addition to the U.S. industry there is a Canadian whale watching industry that targets the same whales. This study currently focuses singularly on the U.S. industry. This research will also support and inform ecosystem based management and science, provide baseline data for future studies, help inform the discussion of industry impacts in the event of regulatory action, and highlight future research topics and data gaps.

Methods

Paperwork Reduction Act (PRA) authorization was received from the Office of Management and Budget (OMB) granting authorization to proceed with information collection in survey form. Literature reviews included pertinent social and biological references to the SRKW and the applicable whale watching industry. Initial information collection included a focus group with marine mammal scientists from both the Northwest Fisheries Science Center (NWFSC) and the Northwest Regional Office marine mammal programs, and informational meetings with staff of

the Whale Museum. Communications were also established with board members of the Whale Watching Operators Association Northwest (WWOANW).

The primary study tool - a detailed survey - was developed and administered to members of the whale watching industry. Surveys were primarily administered in person, but were also available on-line and via mail. Survey participants were identified through the WWOANW membership structure, association with whale watching companies, and collaboration with the Whale Museum Soundwatch Boater Education staff. Owners of companies were contacted with informational letters and an invitation to participate in the study, followed by contact to set up appointments to administer the survey. Access to the staff of the specific companies was facilitated by the owners of the companies. Unstructured ethnographic interviews were conducted during survey administration.

Field sites for survey administration were determined based on the locations of the whale watching businesses. Site visits were conducted in Friday Harbor, Roche Harbor, and Snug Harbor on San Juan Island, Deer Harbor and Orcas Ferry Terminal on Orcas Island, Seattle, Everett, LaConner, Bellingham, Anacortes, and Port Townsend. Multiple visits were conducted to several field sites to allow flexibility of survey participants schedules, to maximize contact with survey participants, and to capture any participants whom may have been early or late arrivals to the whale watching season.

Opportunities were provided by several whale watching companies to accompany several vessels on a whale watching tour. This opportunity allowed for the observance of the communication between whale watching vessels, the interaction between staff on the vessels with tourists, the interaction between different staff occupying different roles on a single vessel, the interaction between different whale watching vessels on the water, an access to staff to administer the survey and conduct interviews.

In addition to the commercial whale watching companies, the Soundwatch Boater Education Program provided the opportunity to observe and participate in boater education efforts aboard their small boat. This allowed for a greater understanding of the data collection which occurs both in the presence and absence of the whales, the interaction between the Soundwatch vessel and the commercial whale watching vessels, and the interaction between the Soundwatch vessel and private and recreational vessels.

Social Survey Instrument

The survey instrument was designed to include five sections. The sections are Demographics, Individual Participation, Business Characteristics, Industry Trends, and Effects on the Community. Each section contained questions designed to gather information elaborating on the theme of the particular section, as indicated by the section header. Survey questions were designed to solicit demographic, behavioral, and attitudinal responses. Question structure varies, including questions soliciting a discrete response, multivariate response, or ranked response utilizing a Lickert Scale.

Below are a few reasons why you may participate in the SRKW watching industry. Indicate the extent you agree or disagree with each statement. (**Please mark <u>one</u> per statement)**

A reason to participate in the SRKW watching industry is:	**Strongly agree**	**Agree**	**Neutral**	**Disagree**	**Strongly disagree**
a) to make money	O	O	O	O	O
b) to transition from another maritime position	O	O	O	O	O
c) to work in the region and on the water	O	O	O	O	O
d) to educate the public about SRKW and Puget Sound natural history	O	O	O	O	O
e) to interact with co-workers who share my values	O	O	O	O	O
f) to work outside and "on the water" instead of in the office	O	O	O	O	O
g) to work a seasonal job in the tourism industry	O	O	O	O	O
h) to spend time with the SRKW and other marine animals on a daily basis	O	O	O	O	O
i) other *(please indicate)*	O	O	O	O	O

Figure 1. Example of a Lickert scale formatted question on Individual Participation from Section B of the survey instrument.

Status of Research

This research is currently ongoing and in the analysis phase. Data is being analyzed utilizing SPSS statistical software and Atlas.ti qualitative coding software. The survey instrument completion deadline was November 30, 2006. The majority of the surveys were administered and completed from June through September of 2006. Response rates are currently under analysis. However, preliminary response information at the time of this event resulted in the distribution of 186 surveys, the receipt of 105 completed surveys, and the completion of 93 unstructured ethnographic interviews. This yielded a preliminary response rate of 56.5%.

Interesting themes are coming to light in the initial analysis and will be further analyzed. Such themes include the influence of popular cultural elements like the film "Free Willy" as major sources for tourist knowledge of the killer whale and in the tourist desire to view whales in the wild. Many respondents also indicated that a major benefit of working in this particular industry is the opportunity to educate the public on various environmental issues. Initial analysis also shows that the U.S. Industry has a high percentage of larger vessels with higher occupancy capacity.

Discussion

The whale watching industry not only is comprised of motorized vessels, but also includes land-based whale watching companies, and kayak companies. Some companies offer more than one type of whale viewing opportunity; while others specialize in only one type of whale viewing whales. For example, some companies only offer motorized vessel tours while others may provide both motorized vessel tours and kayak tours. The opportunity to participate in this study was extended to members of all company types. Some companies target the viewing of SRKW with 100% of their effort. Other companies provide the opportunity to view whales amidst general wildlife viewing activities. These unique differences result in a diversity of views within the industry, where some issues may result in distinct differences, and others may result in commonalities.

This research has resulted in the compilation of both qualitative and quantitative data through both the survey instrument and interviews. Both types of data will be analyzed and reported. Support for this research was received from the NWFSC Socioeconomics Program and the NWFSC Marine Mammal Program.

Non-indigenous species of the Pacific Northwest: An overlooked risk?

Beth Sanderson
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
beth.sanderson@noaa.gov

Michelle Rub and Katie Barnas
Northwest Fisheries Science Center
Conservation Biology Division
Seattle Washington

Non-indigenous species are recognized as one of the major threats to global diversity and have been cited as a cause of decline in 42% of species listed under the U.S. Endangered Species Act. The Pacific Northwest is home to more than a thousand aquatic and terrestrial non-indigenous species, yet the effects of most of these species on native populations, communities and ecosystems remain unknown. During their life cycle, salmonids traverse large geographic areas spanning freshwater, estuary and ocean habitats where they encounter numerous non-native species. To date, the cumulative impact of non-indigenous species on salmonids has not been described or quantified. We examine the extent to which introduced species are a potentially important risk to threatened and endangered salmon, ultimately by contributing to higher levels of life-cycle mortality. We identify and categorize all documented introduced species in the Pacific Northwest, including fish, invertebrates, birds, plants, amphibians and others. Where data exist, we quantify the impact of non-indigenous and range-expansion species on populations of threatened and endangered salmonids. For example, birds and fish predators are reported to consume 0-40% of juvenile salmon in some habitats. These data indicate that the impact of non-indigenous species on salmon is equal to or greater than commonly addressed impacts (habitat, harvest, hatcheries and hydro-system) and suggest that managing non-indigenous species impacts may be imperative for the recovery of these fish.

Environmental chemistry and toxicology studies in the Environmental Conservation Division: Past, present, and future

Carla Stehr, Lyndal Johnson, Gina Ylitalo, Don Brown, John Incardona, and Tracy Collier
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
carla.m.stehr@noaa.gov

The Environmental Conservation Division (ECD) was established in 1972 to study chemical pollution and effects on the health of marine organisms. At that time, it was thought that fish could not metabolize many chemical contaminants. However, in 1975, ECD scientists showed that polycyclic aromatic hydrocarbons (PAHs) were indeed metabolized by fish. In 1976, the National Analytical Facility was established to analyze contaminants in the marine environment. In the 1980's, ECD scientists showed a link between PAH exposure and liver tumors in fish. Indicators of contaminant exposure were developed, validated and used in laboratory and field monitoring studies. Sublethal effects such as reproductive impairment were identified. In 1989 and the 1990's, our expertise was called on to help assess the extent of environmental damage after oil spills such as that of the MV Exxon Valdez. Damage assessment studies were also conducted in industrial areas of Puget Sound. New technologies continue to be added, including utilizing zebrafish as a vertebrate model system to quickly evaluate effects of contaminants on fish, and adapting molecular biology tools to identify mechanisms of cellular damage. Analytical methods are updated or developed to measure emerging contaminants in fish and marine mammals. Currently, the roles of chemicals, including those found in storm water run-off, are being studied with regard to salmon recovery. We continue to develop and apply new technologies as we build on more than 30 years of ecotoxicology studies to determine effects of human activities on the health and fitness of wild fish, especially Pacific Salmon and marine fish.

The *Renibacterium salmoninarum* genome sequencing project

Mark S. Strom
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington
mark.strom@noaa.gov

Greg Wiens
U.S. Department of Agriculture/ARS
National Center for Cool and Cold Water Aquaculture
Leetown, West Virginia

Dan Rockey
Oregon State University
College of Veterinary Medicine
Corvallis, Oregon

Donald Chen and Samuel Crane
University of Washington
Undergraduate Research Program
Seattle, Washington

Renibacterium salmoninarum, a Gram-positive bacterium and member of the Micrococcaceae family, is the causative agent of bacterial kidney disease (BKD) in salmonids. BKD remains a high priority for hatcheries, aquaculture, and conservation programs designed to maintain salmon stocks listed under the Endangered Species Act. Treatment of BKD remains problematic, and currently available antibiotics and vaccines are not completely efficacious. In order to identify virulence factors, vaccine candidates, and targets for novel therapeutics to control the pathogen in salmon, a project to completely sequence and annotate the genome of *Renibacterium salmoninarum* was carried out. Here we summarize the process and first findings from *in silico* analysis of the complete genome of *R. salmoninarum*.

Reproductive performance of adult salmon exposed to an environmental estrogen during juvenile life history stages

Penny Swanson
Northwest Fisheries Science Center
Resource Enhancement and Utilization Technologies Division
Seattle, Washington
penny.swanson@noaa.gov

Jon T. Dickey, Larissa Felli, Kathy Cooper, and Graham Young
University of Washington
School of Aquatic & Fishery Sciences
Seattle, Washington

Irvin B. Schulz
Battelle Marine Sciences Laboratory
Pacific Northwest Division
Sequim, Washington

A variety of environmental contaminants disrupt reproduction by binding to estrogen receptors. The most biologically potent is ethynylestradiol (EE2), a synthetic estrogen used in oral contraceptives that is present in surface waters as a result of sewage discharges. The objective of this study was to determine if exposure to EE2 during salmonid juvenile life history stages alters survival after seawater entry, growth and reproduction. Individually tagged 1+ age coho salmon were exposed to three concentrations of EE2 (0, 0.25, 2.5, and 25 ng/L; 2 tanks per treatment, 60 fish per tank) via tank water for 2 weeks during the later phases of smoltification. The duration of exposure was intended to mimic a typical downstream migration period. Two weeks after the exposure, tissue and blood samples were collected from a subset of fish to assess acute effects of the exposure on the reproductive endocrine system. The remaining fish were transferred to a common tank of seawater and reared to maturity at age 3 when fish were transferred back to fresh water for spawning. Our results demonstrated that water borne exposure to EE2 for two weeks induces inappropriate hormone production in juveniles, including suppression of genes involved in steroid biosynthesis. Despite the disruption of the endocrine system at juvenile life-history stages, there were no significant effects on survival, growth, fecundity or fertility of adults. These results suggest that the reproductive endocrine system of smolts is disrupted by EE2, even at very low levels. However, the acute disruption did not appear to have long-term effects on adult fertility and survival.

Assessing nutrient limitation in salmonid streams of the Salmon River basin in Idaho

Chau Tran, Beth Sanderson, Kate Macneale, and Holly Coe
Northwest Fisheries Science Center
Environmental Conservation Division
Seattle, Washington
chau.tran@noaa.gov

Spawning adult salmonids deliver marine-derived nutrients vital to the ecology of nutrient-poor streams throughout the Pacific Northwest. In Idaho, declining stocks of endangered Chinook salmon has affected stream productivity of the Salmon River basin. To determine the magnitude and mechanisms of nutrient limitation we placed in-stream nutrient diffusing substrates to measure algal biomass accrual in control, N, P, and N+P treatments. Past results indicate primary N limitation and N+P co-limitation in most study streams, though patterns of significantly lower chlorophyll *a* in P treatments versus control treatments reoccur yearly, and across multiple streams. We continue to explore this inhibitory algal growth response in P treatments in 2006 using a spectrum of P concentrations and the addition of tea tree oil as a possible bacteria inhibitor. In contrast, we also present data from a similar experiment we conducted in Washington State, where endangered salmonids reside in P-limited streams. Understanding and identifying specific patterns of nutrient limitation is a critical first step in assessing whether nutrient additions are appropriate restoration measures for streams with declining adult salmon returns.

Historical bycatch trends and changes in the at-sea hake fishery

Vanessa J. Tuttle
Northwest Fisheries Science Center
Fishery Resource Analysis and Monitoring Division
Seattle, Washington

Background

Some of the significant factors which influence bycatch species composition in fisheries are: fishing depth, geographical area, and time of day. In the at-sea Pacific hake (*Merluccius productus*) fishery, recent changes in average fishing depth seem to have had impacts on the bycatch species composition and amount during the last few years.

New fishery bycatch limits for canary rockfish (4.7 mt) and widow rockfish (200 mt) were implemented in the hake fishery in 2005. Although the new limits were potentially season-limiting, there was no negative impact on the 2005 or 2006 season. Hake fishery bycatch rates from 1991 through 2006 average 1% of the total catch weight.

Methods

Total catch and bycatch monitoring of the at-sea hake fishery started in the 1970s when vessels started carrying fisheries observers on board (Methot and Dorn 1995). Observer coverage levels are 100% of all fishing days, and beginning in 2002 each vessel started carrying two observers for all fishing days, therefore sampling for species composition averages 99% of hauls in recent years. Species composition samples currently average 50% of the total weight of each haul, which means this fishery has an exceptional amount of high quality data.

The data presented here represent only the at-sea sector of the fishery, which is comprised of motherships and catcher-processors. This data set starts with the domestication of the fishery in 1991 and ends with the 2006 season, and does not include any tribal data.

Results

Sixteen years of historical catch data indicate high interannual variability in overall rockfish bycatch with recent sharp declines, while the overall hake catch has increased in recent years (Figure 1). Total hake catch has varied in the at-sea hake fishery since 1991. The lowest points in 2002 and 2003 were due to hake being listed as an over-fished species (Helser et al 2004). Hake have since been re-assessed and the quota has been increased to levels consistent with the 1990s.

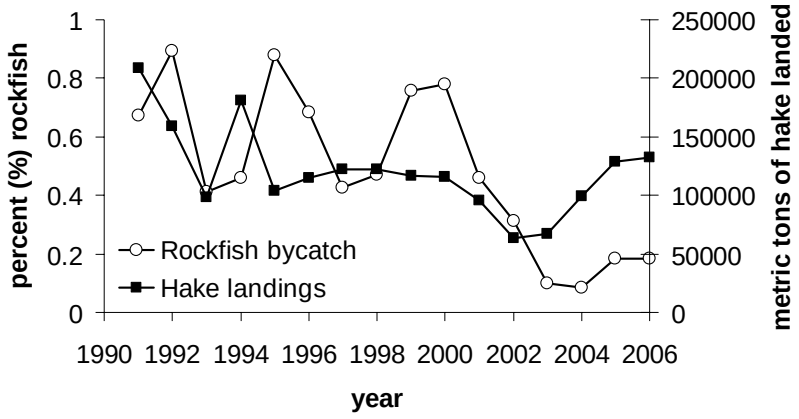

Figure 1. Total hake caught in metric tons and percent of total catch which is rockfish bycatch, between 1991 and 2006.

Shifts in the bycatch species composition over time can be seen between 1994 when 77% of all bycatch was rockfish, declining to 50% rockfish in 1999, and declining even further to 20% rockfish in 2006. Other trends include the decrease in jack mackerel and Pacific mackerel bycatch from 8% in 1994 to 1% in 2006, and the consistency of the approximately 1% salmonid bycatch rate over the years. Notably, squid bycatch increased 90% between 2003 and 2004, and comprises the majority of the bycatch from 2003 through 2006.

Serious bycatch avoidance tactics started in 2003 and resulted in deeper fishing depths which contributed to a large decrease in the yellowtail and widow rockfish bycatch (Figure 2). Subsequently the rougheye rockfish bycatch rate has showed increasing variability (Figure 3), likely a result of the deeper depths they inhabit (Love et al. 2002).

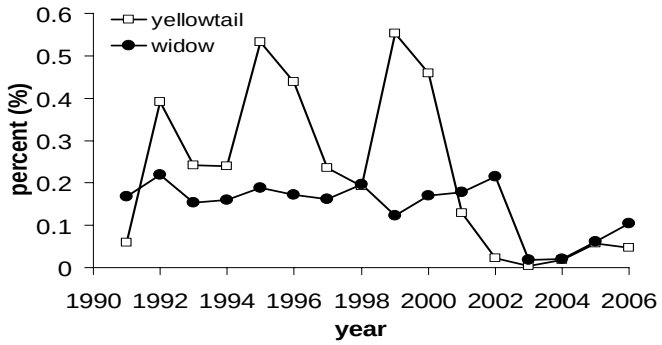

Figure 2. Bycatch rates of yellowtail and widow rockfish as a percent of total catch from 1991 through 2006.

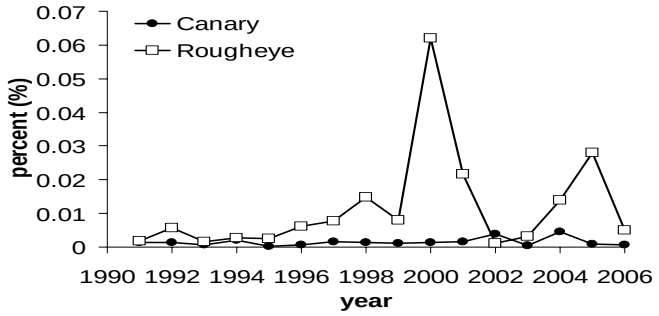

Figure 3. Bycatch rates of canary and rougheye rockfish as a percent of total catch from 1991 through 2006.

Canary bycatch has remained consistent over the years and has never exceeded, even historically, the recent bycatch cap of 4.7 metric tons (Figure 3).

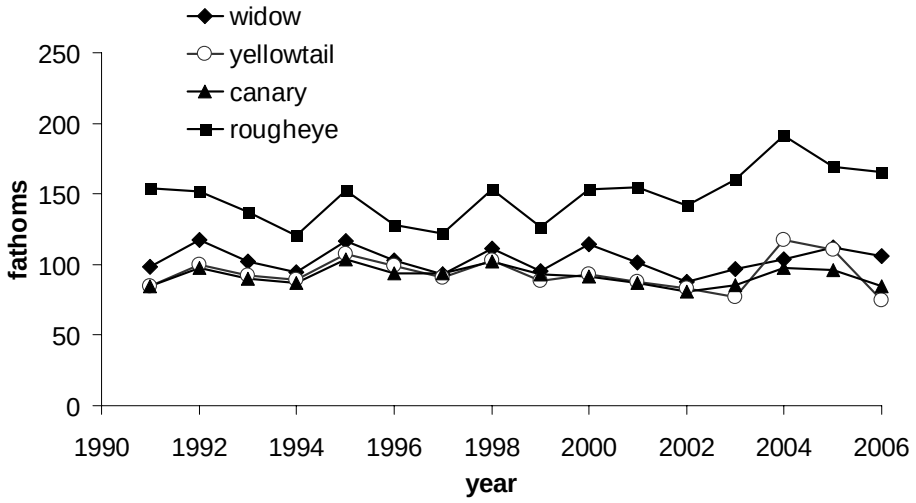

Figure 4. Average capture depth by species in fathoms for widow, yellowtail, canary, and rougheye rockfish.

Average capture depths of rockfish vary by species (Love et al. 2002). Figure 4 illustrates widow, yellowtail, and canary rockfish overlapping in capture depth, ranging between 74 and 117 fathoms, and rougheye rockfish ranging between 120 and 190 fathoms.

Comparison of fishing depth to the distance between the net and the sea floor shows a distinct pattern change starting in 2001 (Figure 5). Between 2002 and 2005 fishing was occurring in deeper waters and the net was closer to the sea floor. In 2006 fishing depths got slightly shallower, but the distance from the sea floor was greater then it has been since 2000, meaning fishing is taking place over deeper water, in general. The shallower fishing depths in 2006 might

account for the increase in widow rockfish bycatch over recent years, and the decline in rougheye rockfish bycatch from 2004 and 2005.

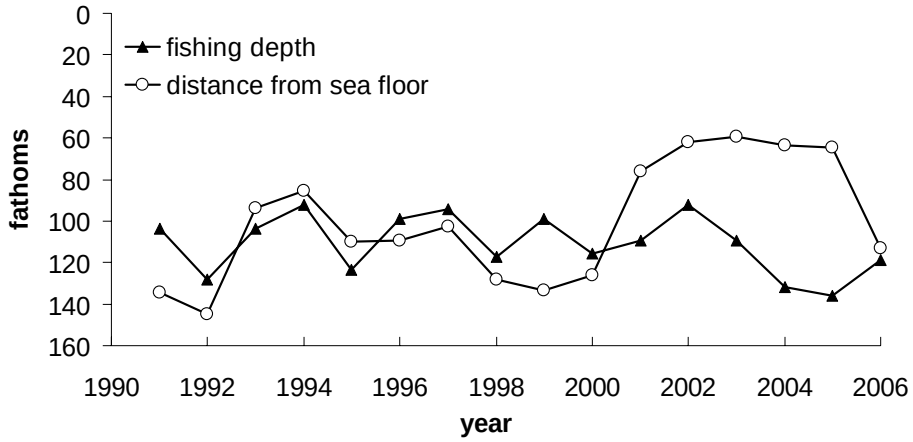

Figure 5. Average fishing depth and distance between the net and the sea floor.

Conclusions

With the implementation of fishery bycatch limits, voluntary measures to prevent the limits from being reached have been instituted with varying degrees of success. Fishing deeper appears to reduce bycatch of some rockfish species, but increases bycatch of other species. In order to effectively reduce rockfish bycatch as a whole, a combination of altering multiple aspects of the fishing effort needs to be addressed. This might include a combination of area, depth, and temporal factors. However, the impact on other bycatch species, particularly salmonids, needs to be carefully considered.

Acknowledgments

Thanks to the at-sea hake observers for collecting the data.

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