

## Pristane Monitoring in Mussels

Project Number: 02195

Restoration Category: Research and Monitoring

Proposers: Jeffrey W. Short and Patricia M. Harris  
NMFS, Auke Bay Laboratory  
ABL Program Manager: Dr. Stan Rice

Lead Trustee Agency: NOAA

Cooperating Agencies: None

Alaska Sea Life Center: No

Duration: Indefinite

Cost FY02: \$ 55,000

Cost FY03: \$ 55,000

Cost FY04: \$ 55,000

Geographic Area: Prince William Sound

Injured Resource/Service: Pink Salmon

### ABSTRACT

This project has focussed on elucidating the transport mechanism of pristane from *Neocalanus ssp* copepods into mussels in PWS for the previous 6 years. In FY00 and FY01 the utility of monitoring the response of pristane in mussels to mass-release of juvenile pink salmon from PWS hatcheries was successfully initiated, using pristane concentration levels. This project will continue with this direction to assess feeding conditions for juvenile pink salmon during the critical period of initial marine residence, and will forecast survivals through this period. Forecasts will be compared to actual returns to assess reliability.

## INTRODUCTION

Predicting recruitment is a fundamental goal of fisheries management, but an adequate understanding of the factors modulating recruitment are rarely achieved. This project has been funded in the hope that it would elucidate recruitment factors during the early marine phase of salmon and herring in Prince William Sound (PWS). Project results, augmented by agency-sponsored research, indicate that monitoring pristane in mussels may provide a basis for predicting marine survival of pink salmon, which might also be applicable to other salmon species (especially chum salmon). In 2000 this project advanced to the validation stage, and compared pink salmon survival forecasts with actual returns, to assess reliability. Preliminary analysis of 2000 data indicates that pristane monitoring provides fisheries managers with a new tool to improve salmon and ecosystem management in PWS.

Monitoring the response of pristane in mussels to mass-releases of juvenile pink salmon from PWS hatcheries indicated the key causal link required to predict marine survival. Prince William Sound Aquaculture Corporation (PWSAC) hatcheries in PWS have adopted a strategy of releasing juvenile salmon *en masse* in recent years to minimize predation. Numbers of released juveniles usually range from 20 to more than 100 million per release. Released juveniles immediately begin searching for adequate prey, and they become increasingly vulnerable to predation until prey adequate to support rapid growth are located.

By far the most available prey during spring in PWS are *Neocalanus* copepods, which contain pristane concentrations of about 1% (dry weight basis). High-density patches of these copepods accumulate near shorelines in response to wind-driven surface currents, and juvenile pink salmon remain close to shorelines during their first few weeks of marine residence searching for prey. Abundant fecal material rich in pristane is produced when large numbers of released pink salmon encounter concentrated near-shore patches of copepods. This pristane-laden fecal material is readily incorporated by mussels, so monitoring the increase of pristane concentrations in mussels near PWSAC hatcheries 2 to 3 weeks following releases of juveniles provides an indication that the released fish have located adequate prey. Conversely, failure to detect pristane increases in mussels anywhere within 25 km of hatcheries following a mass release strongly suggests low prey availability, leading to high vulnerability of fry to predators.

Most aspects of the transport pathway linking pristane generation in copepods to consumption by mussels have been validated by field and laboratory experiments. Field studies have demonstrated that high *Neocalanus* copepod abundance alone does not result in much pristane accumulation by mussels, hence direct incorporation of pristane dissolved into seawater from copepods, or of pristane in feces produced by these copepods, are negligible pathways to mussels. Other zooplanktivorous fishes may also produce pristane-laden feces during Spring, but are unlikely to pose significant confounding because compared with pink salmon they are not as abundant near hatcheries just after releases of pink salmon, and other these zooplanktivorous fishes are less closely associated with the shoreline. Shoreline association is important because both field and laboratory studies showed that effective incorporation of pristane by mussels requires production of feces just above mussel beds at higher tidal stages. Laboratory studies also showed that mussels accumulate pristane within hours when exposed to pristane-laden feces, attaining thousand-fold concentration increases within a few days, and that depuration occurs

much more slowly over a period of a few weeks.

Marine survival of juvenile pink salmon released *en masse* from PWS hatcheries was found to be significantly associated with pristane concentration increases in mussels near hatcheries 2 - 3 weeks following releases. Pristane concentrations have been monitored during Spring at a network of 30 stations for each of the last 6 years in PWS. Comparison of marine survival determined from adults returning to these hatcheries, with pristane concentration increases in mussels collected from sampling stations within 25 km of hatcheries before and 2 - 3 weeks following release of juveniles, showed that 33% of the interannual survival variability is explained by pristane increases ( $P < 0.05$ ,  $df = 13$ ). These results strongly suggest that continued monitoring of pristane in mussels may have predictive value to forecast marine survival of hatchery-released pink salmon.

This proposal emphasizes a shift from a research project to a validation project. This transition exploits the results of the research phase to optimize the monitoring design. Six samplings are proposed, biweekly beginning early April through end of June, to address the temporal variability of the spring zooplankton production and hatchery release strategies. The network of sampling stations was increased in 2000 by 11 to optimize geographic coverage near the hatcheries. Two stations were dropped because of the difficulty of access. The current network of stations permits assessment of the relation of marine survival estimates for hatchery pink salmon to wild stocks in PWS.

## NEED FOR THE PROJECT

### A. Statement of Problem

Pink salmon are a recovering species in PWS. This project will assess feeding conditions for juvenile pink salmon during the critical period of initial marine residence, and will forecast survivals through this period. If these forecasts are sufficiently reliable, they may help improve management of salmonids in PWS. Improved management will aid the full recovery of this species.

### B. Rationale

Pristane in PWS mussels has been monitored for the last 6 years to assess whether seasonal variability of tissue concentrations may be related to recruitment of salmon. Pristane is an environmentally persistent hydrocarbon naturally produced by *Neocalanus* copepods in PWS. These copepods account for nearly all of the planktonic biomass available as prey for zooplanktivorous fishes during early Spring, especially juvenile pink salmon during initial marine residence. Laboratory and field experiments have confirmed that these fishes excrete some of the pristane ingested with *Neocalanus* copepods in feces, and the feces are subsequently ingested by mussels. The time scale for pristane accumulation by mussels exposed to pristane-laden feces is a few days, and for depuration of accumulated pristane a few weeks. Monitoring pristane concentration increases in mussels during Spring thus indicates the conversion of nearby copepods into fish feces, implying growth of the zooplanktivorous predators. Rapid growth during early life history is essential for high survival. Verification of survival forecasts will

permit more precise assessment of human impacts on this species.

### C. Location

Mussel samples will be collected in Prince William Sound and will be analyzed for pristane concentrations at the Auke Bay Laboratory, Juneau, Alaska. Marine survival forecasts for pink salmon will help improve management of salmonids in PWS.

## COMMUNITY INVOLVEMENT

We will continue to involve Prince William Sound residents in this project to share knowledge and interest in PWS ecosystems and to reduce sampling costs. Since 1994, the Prince William Sound Aquaculture Association has collected mussels near their 4 hatcheries at the appropriate times and stored them until the end of the season for pick-up. If Youth Area Watch program continues in 2002 (EVOS Project 02210), students will again be collecting mussels near their hometowns, Tatitlek, Whittier, Chenega Bay, Valdez, Cordova, and Seward, and may be assisting with collections at other sites. We will provide materials for each participating school that explains the rationale of the project, and compares specific results for each school with the results for the whole effort. The underlying biology of this project gets to the heart of how the Sound turns sunlight into fish, which we believe can provide a very useful local teaching resource. Youth Area Watch students will also continue to participate in a 1 day workshop at Auke Bay Laboratory on laboratory analysis techniques for pristane in mussels.

## PROJECT DESIGN

### A. Objectives

In 2002 and onward this project has 1 objective:

1. Forecast marine survival of pink salmon in PWS.

### B. Methods

The project objective will be addressed by determining the variability of pristane concentrations in mussels (*Mytilus trossulus*) from 40 sites in PWS during April through June. Collected mussels will be stored frozen and analyzed for whole-body pristane concentration. Mussels (15) will be collected from selected mussel beds and placed into a plastic bag together with collection documentation (i.e. date, time, location, collector). Selected mussels will ideally be in the length range 20 - 45 mm. Mussels are collected along a transect parallel with the shoreline; 1 mussel is collected every consecutive meter. Previous results archived in the **Exxon Valdez** restoration database for hydrocarbons indicates that pristane concentrations in mussels collected in this way are representative of entire mussel beds.

Maximum increases of pristane concentrations in mussels at stations within 25 km of hatcheries 2 weeks following mass-releases of pink or chum salmon will be normalized to the size of the

release. This ratio, denoted as the pristane index (PI), will be regressed against marine survival of released hatchery salmon (denoted S). Historical regressions of S vs. PI for each hatchery will be used as the basis for survival predictions. Pristane increases at more distant stations will be compared with increases near hatcheries to evaluate wild-stock survivals. The regression relation of S vs. PI for all hatcheries combined will be used as the basis for wild-stock survival estimates. Wild-stock estimates will be made for each salmon management district within PWS.

The chemical analysis of pristane involves pentane extraction of macerated tissues, lipid removal with silica gel, and separation and measurement of pristane by gas chromatography equipped with a flame ionization detector. Pristane measurement will use the internal standard method, with deuterated hexadecane and deuterated eicosane added to the pentane initially as the internal standard. Pristane identification will be based on retention time relative to the internal standard. Quality control samples include method blanks, spiked method blanks, and reference sample analyzed with each batch of 20 samples to verify method accuracy, precision, and absence of laboratory introduced artifacts and interferences. Recovery of the internal standard will be determine by adding a second internal standard prior to instrumental analysis. Method detection limits will be assessed annually for the mussel tissue matrix, and these detection limits will be assumed for the other matrixes analyzed. Based on previous performance, we anticipate accuracy of  $\pm 15\%$  of National Institute of Science and Technology (NIST)-certified values for the spiked blank and reference samples, precision of 95% of reference samples within  $\pm 15\%$  of sample means, and laboratory artifacts below detection limits more than 99% of the time. This level of analytical performance will insure that variability due to sample analysis is negligible compared with variability among replicate mussel samples.

Percent moisture will also be determined in samples so that results may be analyzed on dry weight weight bases. Dry weights will be determined by heating samples at 60 C to constant final weight.

Because there is no other practical way of estimating energy conversion from *Neocalanus* to their near-shore predators over a broad geographic area such as PWS, there are no alternative methodologies to consider here.

### C. Contracts and Other Agency Assistance

It will be necessary to contract a chemical technician to process samples.

## SCHEDULE

### A. Measurable Project Tasks for FY02

FY01:

Apr 1 - June 30: Collect mussel samples.

Jul 1 - Sep 30: Analyze 2002 samples for pristane, summarize results in a report

B. Project Milestones and Endpoints

Write report by Dec. 31, 2002

C. Completion Date

Dec. 31, 2002

PUBLICATIONS AND REPORTS

An annual report will be produced by December 31, 2002.

NORMAL AGENCY MANAGEMENT

NOAA/NMFS has statutory stewardship for most living marine resources; however, if the oil spill had not occurred, NOAA would not be conducting this project. NOAA/NMFS proposes to make a significant contribution (as stated in the proposed budget) to the operation of this project, making it truly cooperative.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

We are cooperating closely with Youth Area Watch (02210), which is providing us with samples and to whom we are providing training and educational materials.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

There are no major changes in the study design from the 2001 proposal.

PROPOSED PRINCIPAL INVESTIGATOR

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National Marine Fisheries Service, NOAA  
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PRINCIPAL INVESTIGATOR

Jeffrey W. Short

Education:

BS, 1972, University of California, Riverside (Biochemistry & Philosophy)

MS, 1982, University of California, Santa Cruz (Physical Chemistry)

Relevant Experience:

1989- Present: Established and managed the hydrocarbon analysis facility at ABL to analyze hydrocarbon samples generated by the *Exxon Valdez* NRDA effort (about 20% of these samples were analyzed at ABL).

1989 - 1992: Principal Investigator, Exxon Valdez project Air/Water #3: Determination of petroleum hydrocarbons in seawater by direct chemical analysis and through the use of caged mussels deployed along the path of the oil spill.

1991 - 1996: Principal Investigator, Exxon Valdez project Subtidal #8: Development of computer-based statistical methods for global examination of sediment and mussel hydrocarbon data produced for the Exxon Valdez NRDA effort for systematic bias, and for identification of probable sources of hydrocarbons. In addition, this project produced both hard-copy and computer display maps of all the sediment and mussel hydrocarbon data.

1994 - 1995: Initiated data analysis and pilot projects that established the role of pristane in Prince William Sound.

1996-2001 Principal Investigator 96195, 97195, 98195, 99195, 00195, and 01195

OTHER KEY PERSONNEL

Patricia M. Harris

Education: University of Alaska Fairbanks; B.S. Biological Science 1966  
Graduate work at U of A Fairbanks, U of A Southeast, University of British Columbia

Relevant Experience:

1989-1992: Co-principal investigator of NRDA study Subtidal 3, was responsible for field logistics and sample collection and assisted in data analysis and report preparation; also assisted other NRDA projects in field collections.

1992 -1996: participated in study design, field work, proposal preparation, data analysis, and report preparation for mussel bed monitoring and restoration (R103-96090).

1994-2001 Participated in logistic planning, sampling, and community involvement coordination for the pilot pristane project, 96195-001195

Relevant publications: Co-author of final reports for NRDA study Subtidal 3 and several publications pertaining to distribution of *Exxon Valdez* oil in mussels and underlying sediments. Several public presentations of oil-related scientific research.

Responsibilities: Coordinate sample collection logistics and collect mussel samples; data analysis; report and proposal preparation; and preparation of science educational materials, posters, and reports.





**2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

October 1, 2001 to September 30, 2002

<i>Budget Category:</i>	Authorized FY2001	Proposed FY 2002				
Personnel	\$21.9	\$17.4				
Travel	\$26.2	\$26.2				
Contractual	\$1.0	\$5.0				
Commodities	\$2.5	\$3.4				
Equipment	\$0.0	\$0.0	LONG RANGE FUNDING REQUIREMENTS			
Subtotal	\$51.6	\$52.0			Estimated FY 2003	Estimated FY 2004
General Administration	\$3.4	\$3.0				
Project Total	\$55.0	\$55.0			\$55.0	\$55.0
Full-time Equivalents (FTE)	0.3	0.2				
Dollar amounts are shown in thousands of dollars.						
Other Resources	23.3K	29.2k				
<p>Comments:</p> <p>NOAA contribution: Principle Investigator, Senior Research Chemist Jeff Short 2 months@20 K, Zoologist Pat Harris 1.5 mo @ 9.9K for a total NOAA contribution of 29.9K.</p>						

**FY02**

Prepared: 4/11/01

Project Number: 02195  
 Project Title: Pristane Monitoring in Mussels  
 Agency: National Oceanic and Atmospheric Administration



**2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

October 1, 2001 to September 30, 2002

<b><i>Contractual Costs:</i></b>		
Description		
Temporary labor to analyze pristane samples		
When a non-trustee organization is used, the form 4A is required.		<b><i>Contractual Total</i></b>
<b><i>Commodities Costs:</i></b>		
Description		
Chemicals, glassware and chemistry laboratory supplies to analyze samples, field supplies		
		<b><i>Commodities Total</i></b>

**FY02**

Prepared: 4/11/01

Project Number: 02195  
 Project Title: Pristane Monitoring in Mussels  
 Agency: National Oceanic and Atmospheric Administration

**2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

October 1, 2001 to September 30, 2002

<i>New Equipment Purchases:</i>		Number of Units	Unit Price	
Description				
Those purchases associated with replacement equipment should be indicated by placement of an R.			<i>New Equipment Total</i>	
<i>Existing Equipment Usage:</i>		Number of Units		
Description				
	GC/MS HPLC			

**FY02**

Prepared: 4/11/01

Project Number: 02195  
 Project Title: Pristane Monitoring in Mussels  
 Agency: National Oceanic and Atmospheric Administration