Exxon Valdez Oil Spill
Restoration Project Annual Report

Cutthroat Trout and Dolly Varden in Prince William Sound, Alaska: the Relation Among and Within Populations of Anadromous and Resident Forms.

Restoration Project 96145
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Cutthroat Trout and Dolly Varden in Prince William Sound, Alaska: the Relation Among and Within Populations of Anadromous and Resident Forms.

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Study History: This study was initiated under Restoration Project 96145. This is the first annual report for this multi-year project. Continued support for this project is supported by Restoration project 97145.

Abstract: Dolly Varden and coastal cutthroat trout samples were collected in Prince William Sound. Initial genetic screening of cutthroat trout and Dolly Varden was initiated. Life History analysis of otoliths was initiated. Contract agreements with cooperating agencies were reached.

Key Words: *Exxon Valdez*, coastal cutthroat trout, *Oncorhynchus clarki clarki*, Dolly Varden, *Salvelinus malma*, andromous, resident, allozymes, meristics, mtDNA, microsatellites.

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Executive Summary

Introduction

Dolly Varden (*Salvelinus malma*) and coastal cutthroat trout (*Oncorhynchus clarki clarki*) are distributed throughout Prince William Sound, Alaska. Both are an important part of the recreational fishing opportunities in the state of Alaska (McCarron and Hoffman 1993). The *Exxon Valdez* Oil Spill (EVOS) Trustee Council lists Dolly Varden and coastal cutthroat trout as an injured resource whose recovery is unknown.

There are resident and anadromous, or sea-going forms, of Dolly Varden and cutthroat trout which may occur sympatrically within basins. The level of interactions among these forms is unknown. For instance, it is unknown if resident populations which reside above barriers contribute to below barrier populations. Further, the level of interaction among anadromous populations from different basins is also unknown.

Our proposal (97145) is based on the assumption that the level of interaction between life history forms of fishes within basins as well as among basins is important for the recovery of these species. Knowledge about the relation of resident and anadromous forms within the same watershed will provide insight into the potential response of populations exposed to oil over the long-term. For example, if above barrier populations do contribute to anadromous populations then there may be a buffer against potential long-term population declines due to oil. In such a scenario, it would be important to protect these fishes residing above barriers and their habitat. Insight to the amount of interaction among anadromous forms residing below barriers may also provide information for recovery of these populations. If these populations are being recolonized by adjoining populations careful management of the population segments as a whole would be warranted.

In October 1995, the Exxon Valdez Trustee Council awarded multi-year funding to investigate this problem. In fiscal year 1996 we collected specimens of Dolly Varden and coastal cutthroat trout in Prince William Sound and initiated genetic and life history analysis on these specimens.

Objectives

The objectives of this study are to:

1. Determine for Dolly Varden and cutthroat trout whether anadromous and resident forms in the same watershed are part of one population or different populations.
2. Determine for Dolly Varden and cutthroat trout whether spawning aggregations in different streams in Prince William Sound are part of one population or different populations of a metapopulation.
3. Develop a restoration strategy for Dolly Varden and cutthroat trout based on the results of this study.

Methods

We proposed to meet the objectives of this study with a variety techniques that are useful for determining genetic, meristic, and life history variation. We proposed to use four different techniques: 1) protein electrophoresis, 2) mitochondrial DNA (mtDNA) or microsatellite DNA markers, 3) meristic variation, and 4) otolith microchemistry. As stated in our original proposal, objectives 1 and 2 will be met in the latter part of FY98, and objective 3 will be met by the end of FY98.

Samples were collected in Prince William, Sound in the summer and fall of 1996, using a variety methods.

Allozyme and DNA analysis of cutthroat trout was initiated in July 1996.

Otolith microchemistry and merisics were begun in January 1997.

Contractual agreements with cooperating agencies were developed.

Results

Field collections of Dolly Varden from 12 sites and coastal cutthroat trout from 8 sites were completed in September of 1996.

Preliminary allozyme analysis of 48 loci coding for 20 enzymes was completed for cutthroat trout. Ten loci were polymorphic at the 0.95 level. Samples from the 1997 sampling season will be included in further analysis.

We have detected no polymorphisms in cutthroat trout using 9 restriction fragment enzymes on the mitochondrial region of the genome. We are close to completing our protocol development for microsatellites.

Discussion and Conclusion

Preliminary results from allozyme data suggests that there may be population level variation in coastal cutthroat trout. Sample sizes are small, however, therefore our goal for this season's field sampling is to increase our sample sizes in our 1996 sample locations.

In our original proposal we stated that we would explore two DNA techniques and use the one most promising for detecting population level variation. To date, we have seen no variation with mtDNA, suggesting that microsatellites may be a more appropriate tool for this study. We will continue, however, to screen additional mtDNA fragments with the intention of finding polymorphic haplotypes.

Annual Report

Introduction

Dolly Varden (*Salvelinus malma*) and coastal cutthroat trout (*Oncorhynchus clarki clarki*) are important ecological and recreational resources in Prince William Sound. Populations of each species are found throughout Prince William Sound. Anadromous, or sea-going forms, and resident forms of each fish may be found within a single watershed. Anadromous individuals spend varying amounts of time in freshwater (up to 4 years) before going to the marine environment (Armstrong 1971, Scott and Crossman 1979). There, both species feed in nearshore and estuary areas (Scott and Crossman 1979, Morrow 1980). Cutthroat feed on fish and Dolly Varden feed on crustaceans, small invertebrates, and fish, such as sandlance (*Ammodytes hexapterus*) and capelin (*Mallotus villosus*) (Narver and Dahlberg 1965). Resident forms of these fishes live out their entire life history in freshwater. They may co-occur with the anadromous forms or they may be isolated from the anadromous form by geographic barriers, such as waterfalls. Both are a popular sport fish and are an important part of the recreational fishing opportunities in the state of Alaska (McCarron and Hoffman 1993).

Areas used by the anadromous forms of these fish were impacted by petrogenic hydrocarbons from the *T/V Exxon Valdez* oil spill. Benthic organisms in nearshore areas are particularly susceptible to petrogenic hydrocarbons (Teal and Howarth 1984). In Prince William Sound, the size of epifauna and numbers of amphipods, which are food sources for Dolly Varden, decreased in areas exposed to the spill (Jewett and Dean 1993, Jewett et al.1993). Hepler et al. (1993) found that Dolly Varden and cutthroat trout populations in oiled areas had slower growth rates compared to populations in unoiled streams from 1989 to 1990, the year of the spill. A similar pattern was observed for cutthroat trout in 1990 to 1991. However, growth rates of Dolly Varden in oiled areas did not differ from those in unoiled areas during that period (Hepler et al. 1993). Survival rates for each species from 1989 to 1990 were less in oil impacted areas than in unimpacted areas (Hepler et al. 1993). Hepler et al. (1993) hypothesized that chronic starvation and/or direct exposure to petrogenic hydrocarbons were responsible for the differences in growth and survival of the species in oiled and unoiled areas. The *Exxon Valdez* Oil Spill (EVOS) Trustee Council officially lists these species as injured resources whose recovery is unknown. Coastal cutthroat trout may be of particular concern as Prince William Sound is the northern extent of their range (Johnston 1981).

Reduced growth and survival rates could have long-term impacts on populations of Dolly Varden and cutthroat trout in areas exposed to oil. These species may live up to 8 years (Morrow 1980) and the expected persistence of oil in the nearshore environment (Lee et al. 1979) suggests the potential exists for long-term impacts to these species. Decreased survival would have obvious population implications. The extent would depend on population size; smaller populations would be most susceptible to eventual extinction (Rieman et al. 1993). There may be less obvious impacts also. The potential for loss of genetic variability, which is needed for long term adaptation, increases as population size decreases (Nelson and Soule 1987). Reduced growth rates of individuals can lead to increased susceptibility to mortality and

decreased reproductive potential (Adams 1990). If any of these impacts were to occur for extended periods, even at low levels, affected populations would face increased probability of extinction.

Collections of interacting populations of the same species can be termed a metapopulation (Hanski and Gilpin 1991). Features of such populations include local populations that are more likely to interbreed and interact among themselves than with other groups, but exchange of individuals occurs through various dispersal mechanisms. There may be local extirpation of populations as a consequence of catastrophic events. Surrounding populations then serve as sources of individuals for recolonization and recovery of impacted populations (Brown and Kodric-Brown 1977, Sjogren 1991). The dynamics of metapopulations are particularly important to the persistence and recovery of populations following catastrophic events (Yount and Niemi 1990).

Metapopulation dynamics are an important consideration in the development of conservation and restoration programs (Murphy and Noon 1992, Noon and McKelvy 1992). Restoration strategies for a metapopulation would differ from those for single populations in regards to such features as recolonization potentials, time to recovery, etc. Importantly, a recovery strategy that considers metapopulations may require less investment of resources than that required for single populations.

Many salmonid populations exist as part of metapopulations. Homing and fidelity to spawning and nursery areas results in some isolation of populations (Ricker 1972). Local adaptations provide further isolation. Dispersal among groups may be maintained through straying of migrating adults (Simon 1972, Labell 1992), density displacement of individuals (McMahon and Tash 1988, Northcote 1992), or maintenance of pioneering or colonizing phenotypes (Northcote 1992). Geologic barriers provide unique circumstances for isolation among species within a watershed (Northcote and Hartman 1988) and the level of contribution from above to below barrier populations is unknown (Johnston 1981).

The amount of interaction among anadromous Dolly Varden populations and coastal cutthroat trout populations is unknown. Further, it is unknown if resident populations of these fishes contribute to anadromous populations. These relationships have important implications for the management and potential recovery of these fish. For example, if resident forms of a species contribute to the anadromous forms then there may be a buffer against potential long-term declines of anadromous forms. In such a case, the most prudent restoration activity may be to protect these resident populations and their habitat in streams with populations exposed to the oil spill. Knowledge about the relation among populations of each species will provide additional insight into the potential long-term impacts of exposure to oil. If the populations are a metapopulation, any long-term impacts on a population segment could possibly be mitigated by recruitment from other population segments. Conversely, if the populations are unique this indicates that there is little exchange with nearby populations. Consequently, the ability of surrounding populations to aid a declining population would be reduced. Mitigation measures focused on individual populations would be required in such a case.

In October 1995, the Exxon Valdez Trustee Council awarded multi-year funding to investigate this problem. We have initiated the first steps of this research. This has consisted of collection of Dolly Varden and cutthroat trout samples (July-October 1996), preparing samples for otolith, allozyme, and DNA analysis (October- November 1996), screening mtDNA and developing protocols for microsatellite analysis (July-April 1997) and preparation for field sampling in 1997.

Objectives

The objectives of this study are to:

1. Determine for Dolly Varden and cutthroat trout whether anadromous and resident forms in the same watershed are part of one population or different populations.
2. Determine for Dolly Varden and cutthroat trout whether spawning aggregations in different streams in Prince William Sound are part of one population or different populations of a metapopulation.
3. Develop a restoration strategy for Dolly Varden and cutthroat trout based on the results of this study.

Methods

We proposed to meet the objectives of this study with a variety techniques that are useful for determining genetic, meristic, and life history variation. We proposed to use four different techniques: 1) protein electrophoresis, 2) mitochondrial DNA or microsatellite DNA markers, 3) meristic variation, and 4) otolith microchemistry. As stated in our original proposal objectives 1 and 2, will be met in the latter part of FY98, and objective 3 will be met by the end of FY98.

In FY96, we collected coastal cutthroat trout specimens from 8 sites throughout Prince William Sound in July. Sample sizes consisted of 20 cutthroat trout from sites below waterfall barriers and 25-30 individuals from sites above barriers (Table 1). Dolly Varden were collected from 12 sites throughout Prince William Sound in August and September (Table 1). Several sites did not contain the target species (either Dolly Varden or cutthroat trout). A complete account of the locations and presence and absence of target species was reported to the Alaska Department of Fish and Game in Juneau, Alaska.

Sample sizes ranged from 14-40 individuals at each site. For both species, fish were collected with baited minnow traps, baited vexar traps, and hook and line. After collection they were given lethal doses of MS-222. Specimens were weighed, measured and tagged with a unique identifying number. Muscle, liver, heart and eye tissues were extracted in the field from fish larger that 250 mm and immediately place on dry ice. Fish smaller than 250 mm were frozen whole on dry ice in the field. Specimens and tissues were stored in a Alaska Department of Fish and Game -80° freezer in Cordova, Alaska until they were transported on dry ice to a Oregon

Cooperative Fishery Research Unit Laboratory (OCFRU) in Corvallis, Oregon. In addition to these specimens, fin clips were taken from coastal cutthroat from five additional sites. Fin clips were immediately frozen on dry ice. Tissues were removed from the frozen whole specimens and transferred to 1.7 ml microcentrifuge tubes in the OCFRU laboratory.

Allozyme analysis of coastal cutthroat trout was initiated in July of 1996. We screened 48 loci encoding 20 enzymes (Table 2) following the methods of Aebersold et al. (1987). Allele designations of the cutthroat trout were determined relative to the mobility of the common allele in a rainbow trout.

Procedures of analysis of mtDNA polymorphism using polymerase chain reaction (PCR) was initiated for the cutthroat trout in October of 1996. We used muscle samples from eight samples from eight sites in Prince William Sound for this screening. We extracted DNA with the cell lysis method as reported by Olson (1996). Three segments of mtDNA- NADH dehydrogenase-1 (ND-1), ND-2 and D-loop- were amplified. Nine restriction enzymes were used to digest ND-1 following the methods of Cronin et al. (1993).

The development of microsatellite protocol was initiated in July 1996. Selection of primer pairs for microsatellite analysis was based on Wenberg's (1996) work with coastal cutthroat trout and steelhead. Primer pairs were synthesized and labeled with three specific fluorescent tags, which allows for more than one primer pairs to be separated on a gel at a time. We amplified DNA from cutthroat trout using PCR with the primer pairs that follow: Sfo8, Omy77, Ssa85, Oneμ11, Oneμ14, Ots1, Oneμ2, Omy325, Ssa14. PCR products from these primer sets were separated on a denatured polyacrylamide gel using a Perkin Elmer Applied Biosystems, Inc. (ABI) 377 automated sequencer and analyzed using ABI GeneScan 672, anlysis software, version 2.0.2. Protocol development consisted of optimizing the parameters for PCR conditions for microsatellites. These included, but were not restricted to optimizing cycle parameters of temperature and time, MgCl concentrations, and primer concentrations.

Microsatellite analysis has been initiated for Dolly Varden using the same primer pairs and methods as those listed above. Other primer pairs will screened in the future.

Life history analysis has been initiated with examination of otoliths. Saggital otoliths were removed from coastal cutthroat and Dolly Varden in the lab and prepared for aging and microchemistry following the methods of (Secor et al.1992).

Otolith microchemistry, allozyme, and DNA work from these collections of coastal cutthroat trout will continue through the end of FY97. Collection of specimens in Prince William Sound will begin in July 1997 and continue through September 1997. The goal of field sampling this year is to re-sample locations from 1996 to increase sample sizes as well as increase the number of sites.

Results

We detected variation in ten loci using protein electrophoresis at the 0.95 level.

No variation was detected in nine restriction enzymes in ND-1 *AluI, Ava II, BglII, BstUI, Dpn II, Hae III, HindIII, Msp I, TaqI.* Results from ND-2 and D-loop amplifications were unsatisfactory and reamplification of ND-2 and D-loop from DNA extracted using phenol-chloroform is currently underway.

Protocol development for microsatellite analysis is near completion. We have detected variation in microsatellite loci with allele sizes within the range as those detected by Wenburg et al. (1996).

Discussion and Conclusions

We will continue with analysis of allozyme data in FY 1997 and 1998. We will increase our sample size for allozymes during the 1997 field season.

We will continue our protocol development for microsatellites of coastal cutthroat trout and Dolly Varden during fiscal year 1997. In addition, we will complete mtDNA screening of coastal cutthroat trout and Dolly Varden.

We will continue otolith prepartion and aging of fish.

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Table 1. Location and sample size of 1996 collection sites for Dolly Varden (*Salvelinus malma*) and coastal cutthroat trout (*Oncorhynchus clarki clarki*) in Prince William Sound, Alaska.

Location	Primary Site	N	Species
Green Island	Green Island Creek	14 anadromous	*S. malma*
Hawkins Island	Hawkins Creek	32 anadromous	*S. malma*
	Above barrier	31 resident	*S. malma*
Hinchinbrook Island	Shelter Bay	40 anadromous	*S. malma*
Knight Island	West Arm, Bay of Isles	41 anadromous	*S. malma*
Montague Island	Hanning Creek	40 anadromous	*S. malma*
	Stump Lake	40 anadromous	*S. malma*
Mainland	Copper River, Clear Creek	22 anadromous	*S. malma*
	West- Eshamy Bay	18 anadromous	*S. malma*
	Power Creek	41 anadromous	*S. malma*
	Power Creek, above barrier	23 resident	*S. malma*
	Unakwik Inlet, Cowpen Lake	19 anadromous	*S. malma*
Hawkins Island	Hawkins Creek	20 anadromous	*O. clarki*
	Hawkins Creek,	25 resident	*O. clarki*
	above barrier	25 resident	*O. clarki*
Hinchinbrook Island	Shelter Bay	20 anadromous	*O. clarki*
		30 resident	*O. clarki*
Montague Island	Stump Lake	20 anadromous	*O. clarki*
Mainland	East- Milton Lake	20 anadromous	*O. clarki*
	Gunboat Lakes	20 anadromous	*O. Clarki*
	North- Unawik Inlet	20 anadromous	*O. Clarki*

Table 2. Enzymes examined to date for Coastal cutthroat trout (*Oncorhynchus clarki clarki).* Enzyme names from the International Union of Biochemistry (IUB). Tissues include: L-liver, E-Eye, H-Heart. Buffer systems: TBE- a Tris-borate EDTA gel and tray buffer (Markert and Faulhaber 1965), TBCLE-a Tris-citrate gel buffer and lithium hydroxide borate tray buffer (Ridgeway et al. 1970), and ACE- an amine-citrate-EDTA gel and tray buffer (Clayton and Tretiak 1972).

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Aspartate aminotranferase	mAAT-1	E	ACE
	mAAT-2	E	ACE
	sAAt-1,2	M	ACE
	sAAT-3	E	ACE.
Alcohol dehydrogenase	ADH	L	TBCLE
Adenylate kinase	AK-1	E	ACE
	AK-2	E	ACE
Aconitate dehydratase	sAH	L	ACE
Creatine kinase	CK-A1	M	TBCLE
	CK.A2	M	TBCLE
	CK-B	E	ACE
	CK-C1	E	ACE
	CK-C2	E	ACE
Fructose-biphosphate aldolase	F-BALD-1	E	TG
	F-BALD-2	E	TG
Glyceraldehyde-3-phosphate dyhydrogenase	GAPDH-2	H	ACE
	GAPDH-3	H	ACE
	GAPDH-4	H	ACE
	GAPDH-5	H	ACE
Guanine deminase	GDA-1	L	TBCLE
	GDA-2	L	TBCLE
Glycerol-3-phosphate dehydrogenase	G3PDH	M	ACE

Table 2. Continued.

I.U.B. Enzyme Name	Locus	Tissue	Buffer
Glucose-6-phosphate isomerase	GPI-A	M	TBCLE
	GPI-B1	M	TBCLE
	GPI-B2	M	TBCLE
Isocitrate dehydrogenase ($NADP^+$)	sIDH-1,2	H, L	ACE
	mIDHp-1	H	ACE
	m IDHP-2	H	ACE
L-lactate dehydrogenase	LDH A1	M	TBCLE
	LDH A2	M	TBCLE
	LDH-B1	E	TG
	LDH-B2	E, L	TBCLE
	LDH-C	E	TG
Malate dehydrogenase	sMDH-A1,2	H, L	ACE
	sMDH-B1,2	H, L	ACE
Malic enzyme	MEP-1	M	ACE
	sMEP-1	M	ACE
	SMEP-2	L	ACE
Dipeptidase	PEP-A	M	TG
Proline dipeptidase	PEP-D	E	ACE
Phosphogluconate dehydrogenase	PGDH	M	ACE
Phosphoglucomutase	PGM-1	L	ACE
	PGM-2	L	ACE
Superoxide dismutase	SOD-1	L	TBCLE
Triosephosphate isomerase	TPI-1	M	TG
	TPI-2	M	TG
	TPI-3	E	ACE
	TPI-4	E	ACE

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