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 97145
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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Restoration Project 97145
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Study History: This study was initiated under Restoration Project 96145. This is the second annual report for this multi-year project. Continued support for this project is being requested under Restoration project 98145.

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,* anadromous, resident, allozymes, meristics, mtDNA, microsatellites.

Project Data: (will be addressed in final report)

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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 used a variety of techniques to meet the objectives of this study. These include techniques that are useful for determining genetic, meristic, and life history variation. We used 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 1997, using a variety methods.

Allozyme and DNA analysis of cutthroat trout continued throughout FY97.

Allozyme and DNA analysis of Dolly Varden continued throughout FY97.

Otolith microchemistry and meristics of dolly Varden and coastal cutthroat trout continued through FY97.

Contractual agreements with cooperating agencies were developed.

Results

Field collections of Dolly Varden from 13 sites and coastal cutthroat trout from 10 sites were completed in September of 1997.

MtDNA analysis of Dolly Varden and cutthroat trout were completed.

Initial analysis of allozyme and microsatellite data for subsamples of the entire collection were completed.

Otolith microchemistry of Dolly Varden and coastal cutthroat trout was initiated.

Discussion and Conclusion

We were successful in doubling out sample sizes for Dolly Varden and cutthroat trout during the 1997 field season.

Preliminary results from allozyme and microsatellite data suggests that there are highly significant differences between coastal cutthroat trout from different sampling locations.

However, there is no geographic pattern to these differences. This data is preliminary and may change when sample numbers increase.

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 in coastal cutthroat trout mtDNA, suggesting that microsatellites may be a more appropriate tool for this aspect of the study. We have detected variation in Dolly Varden mtDNA and we will continue to examine the variability of this species with this tool.

Sr/Ca levels in Dolly Varden are variable and suggest variation is consistent with life history types. Sr/Ca patterns in coastal cutthroat trout are highly variable, but do not appear consistent with life history types.

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 FY97, we collected coastal cutthroat trout specimens from 10 sites throughout Prince William Sound in July. Sample sizes consisted of 13-20 cutthroat trout from sites below waterfall barriers and 12-20 individuals from sites above barriers (Table 1). Dolly Varden were collected from 13 sites throughout Prince William Sound in August and September (Table 1). 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.

Fish were collected with baited minnow traps, seines, electrofishing, 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. In addition to these specimens, fin clips were taken from coastal cutthroat trout from five additional sites. Fin clips were immediately stored in 95% ETOH.

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. Analysis of this data was completed with computer software programs BIOSYS-1 and GENEPOP 3.1.

Results of this analysis are presented in Figure 1.

Analysis of mtDNA polymorphism using polymerase chain reaction (PCR) was completed for the cutthroat trout in October of 1997. 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) and for some samples with phenol-chloroform. Three segments of mtDNA- NADH dehydrogenase-1 (ND-1), ND-2 and D-loop- were amplified. Amplified DNA was digested with sixteen restriction enzymes (Table 3) following the methods of Cronin et al. (1993). The same protocol and restriction enzymes were used for a subsample of 94 Dolly Varden from 10 sites.

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 pair 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. A subsample of 95 coastal cutthroat trout was screened with nine microsatellite loci. Peak height and base pair size was determined with genotyper 2.1 software. Analysis of four of these loci is presented in Figure 2.

Microsatellite analysis has been initiated for Dolly Varden using the same primer pairs and methods as those listed above.
Four new primers (Ots4, Oneu8, Sfo12, Sfo23) were screened for Dolly Varden and coastal cutthroat trout.

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 using a scanning electron microprobe was initiated in July of 1997 for 20 Dolly Varden and 20 cutthroat trout.

Otolith microchemistry, allozyme, and DNA work from these collections of Dolly Varden and coastal cutthroat trout will continue through the end of FY98.

Results

Please note that all of the following data is preliminary.

All coastal cutthroat trout possessed a single mtDNA haplotype for sixteen restriction enzymes. Dolly Varden possessed a single mtDNA haplotype for 15 of the restriction enzymes, however they appear to possess multiple haplotypes at a single restriction site.

Allozyme variation in coastal cutthroat trout suggest that there is moderate population variation among sampling areas (Fst=0.10). However, there does not appear to be a geographic pattern to these differences (Figure 1).

In coastal cutthroat trout, we have detected high genetic variation among populations based on analysis of 4 microsatellite loci. Base pair sizes were in the range of those detected by Wenberg (1996). There does not appear to be a geographic pattern to these differences (Figure 2).

Based on otolith microchemistry measures of variance of Sr/Ca are within the detection limits of this technique (Griswold 1996). Initial results suggest that Dolly Varden from three sampling locations from within a drainage basin show distinct patterns that may be related to life history (Figure 3). Coastal cutthroat trout show greater variation in levels of Sr/Ca and do not show distinctive patterns based on life history.

Discussion and Conclusions

Because mtDNA revealed no detectable variation in coastal cutthroat trout we believe that other techniques (microsatellites and allozymes) will provide better information for population structure of this species.

Multiple haplotypes in mtDNA of Dolly Varden based on restriction enzymes suggest there may be mixed lineages of this species in Prince William Sound. We will continue to explore this technique to describe genetic variation of Dolly Varden.
Clustering of sites do not agree between allozyme and microsatellite data. Perhaps when sample sizes increase (both from numbers of samples and number of loci examined) there will be more concordance between the two data sets. Significant amounts of variation among sites of coastal cutthroat trout with no geographic structuring suggest that the populations are isolated and the genetic structure is a result of stochastic events.

We will continue otolith preparation and aging of fish.

We will continue with analysis of meristic, microsatellite, and allozyme data

Acknowledgments

We would like to acknowledge the assistance of Dave Schmid, Ken Hodges, Merlyn Schelske, and Sam Greenwood and numerous volunteers of the USFS Cordova Ranger Station for assistance in selecting study sites and collecting samples. We would also like to acknowledge George Covell for his insight into coastal cutthroat distributions in Prince William Sound and his helpful advice in selecting study sites. We would also like to acknowledge Andy Hoffman, Alaska Department of Fish and Game, Anchorage, AK, for his assistance in obtaining collection permits and selecting study sites. We would also like to acknowledge the expertise of Janet Hanus of the Oregon Cooperative Fishery Unit, Corvallis Oregon in developing the protocol for microsatellites. We would like to thank Marcos Tovar for his help in the Laboratory.

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
Hawkins Island			
	Hawkins Creek, below	22 anadromous	*S. malma*
	Above barrier	28 resident	*S. malma*
Hinchinbrook Island			
	Shelter Bay, below	40 anadromous	*S. malma*
	Above barrier	29 resident	*S. malma*
Knight Island			
	West Arm, Bay of Isles	25 anadromous	*S. malma*
Montague Island			
	Hanning Creek	32 anadromous	*S. malma*
	Stump Lake	37 anadromous	*S. malma*
	Pt. Chalmers	19 anadromous	*S. malma*
Mainland			
	Copper River, Clear Creek	25 anadromous	*S. malma*
	Eshamy Bay	38 anadromous	*S. malma*
	Unakwik Inlet, Cowpen Lake	40 anadromous	*S. malma*
	Shrode Lake	37 anadromous	*S. malma*
	Milton Lake	37 anadromous	*S. malma*
Green Island			
	Green Island Creek	13 anadromous	*O. clarki*
Hawkins Island			
	Hawkins Creek	20 anadromous	*O. clarki*
	above barrier	12 resident	*O. clarki*

Table 1. Continued

Location	Primary Site	N	Species
Hinchinbrook Island			
	Shelter Bay	20 anadromous	*O. clarki*
		27 resident	*O. clarki*
Montague Island			
	Stump Lake	20 anadromous	*O. clarki*
Mainland			
	Milton Lake	23 anadromous	*O. clarki*
Gunboat Lakes		20 anadromous	*O. clarki*
	Unawik Inlet	20 anadromous	*O. clarki*
	Columbia Bay	20 anadromous	*O. clarki*
Knight Island			
	Bay of Isles	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

Table 3. Mitochondrial DNA fragments and restriction enzymes screened for Dolly Varden and coastal cutthroat trout in Prince William Sound.

ND-1	ND-2	D-loop
AluI	*AluI*	*Bg III*
Ava II	*Hind III*	*Dpn II*
Bgl II	*MseI*	*Hha I*
DpnII		*Mse I*
Hae III		
HindIII		
Msp I		
Taq I		

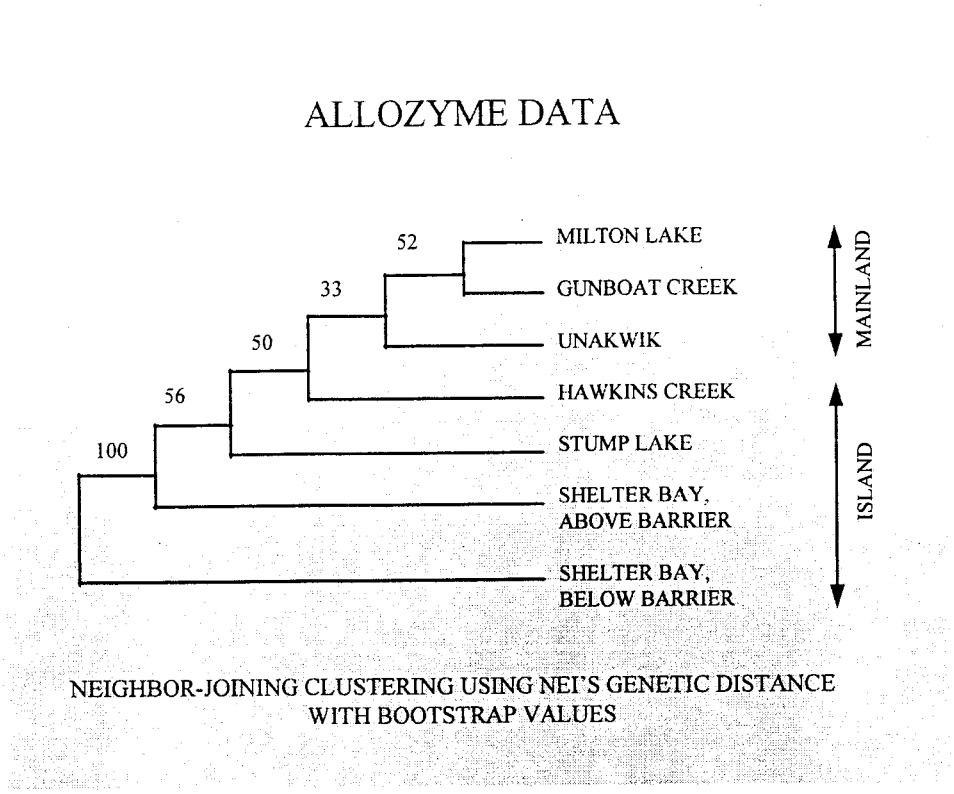

Figure 1. Allozyme data of coastal cutthroat trout from Prince William Sound, Alaska.

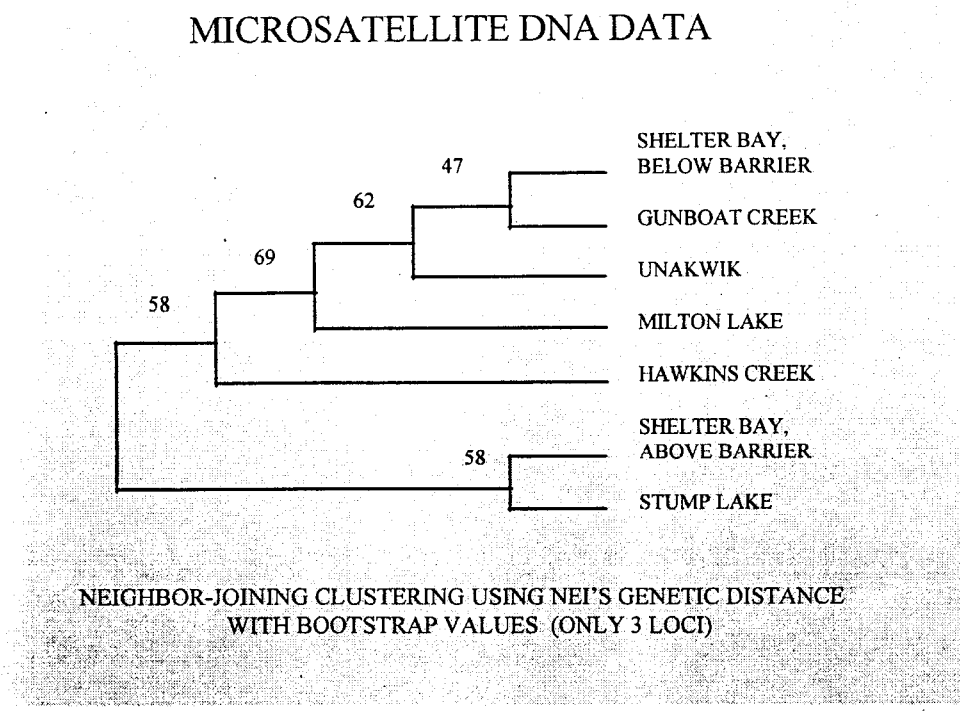

Figure 2. Microsatellite data of coastal cutthroat trout from Prince William Sound, Alaska.

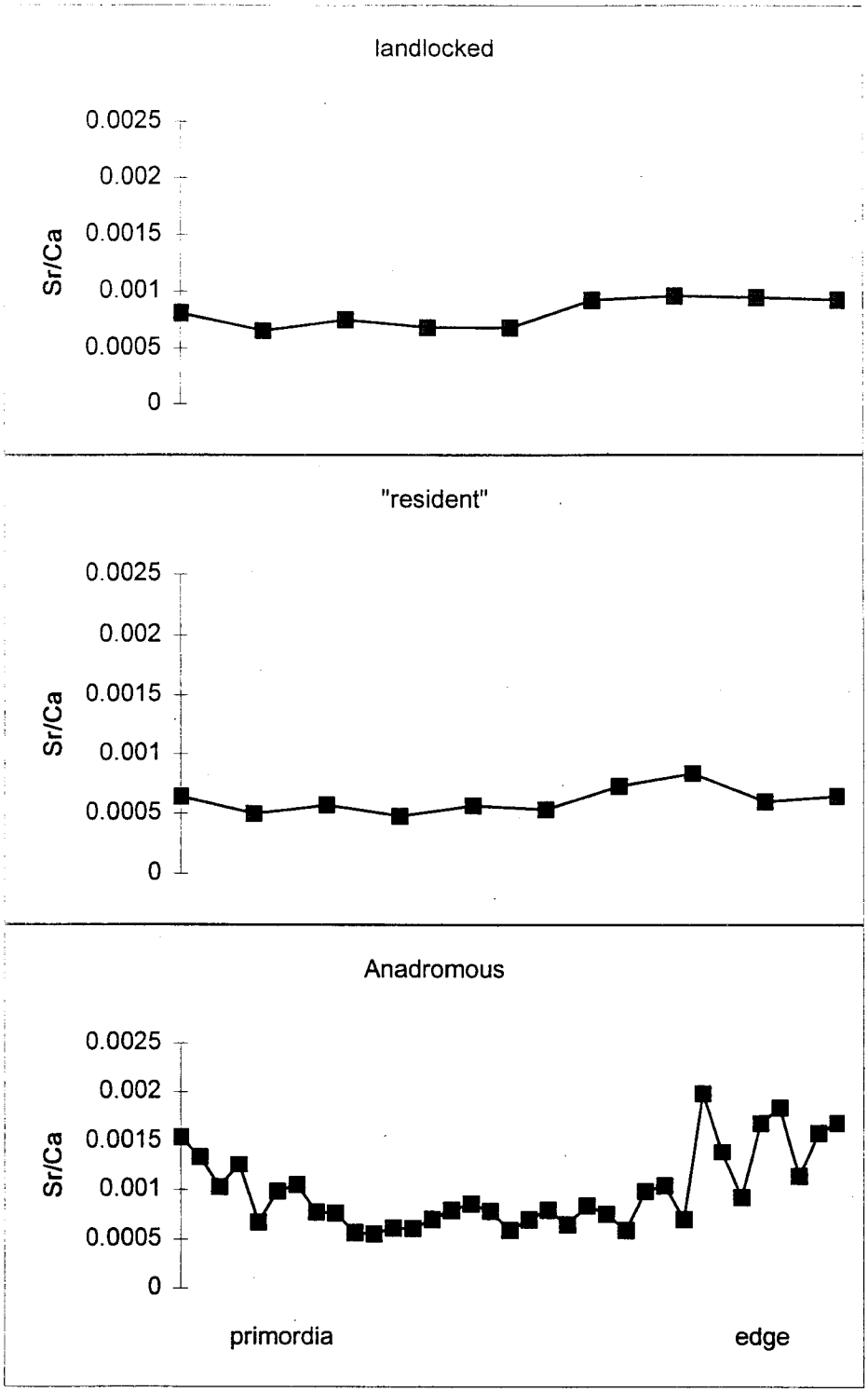

Figure 3. Otolith microchemistry of Sr/Ca in Dolly Varden from Power Creek, near Cordova, Alaska. Landlocked sample a) came from above a waterfall barrier, resident and anadromous samples b) and c) were collected from below a waterfall barrier.

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