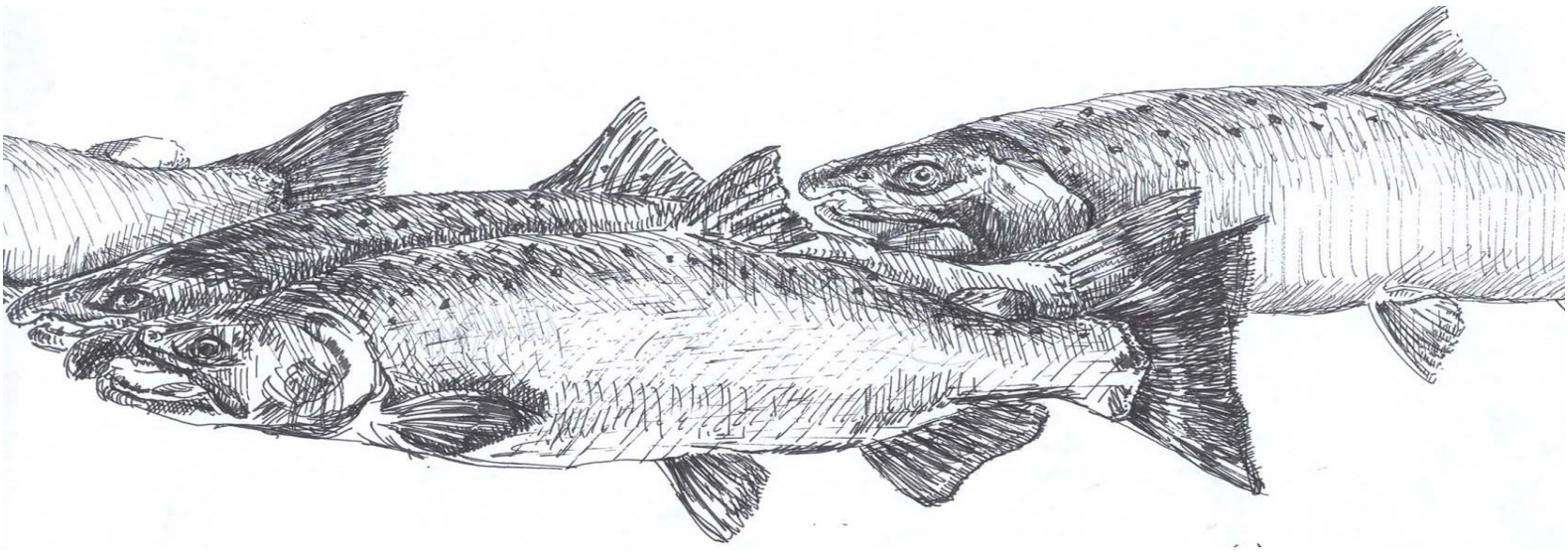
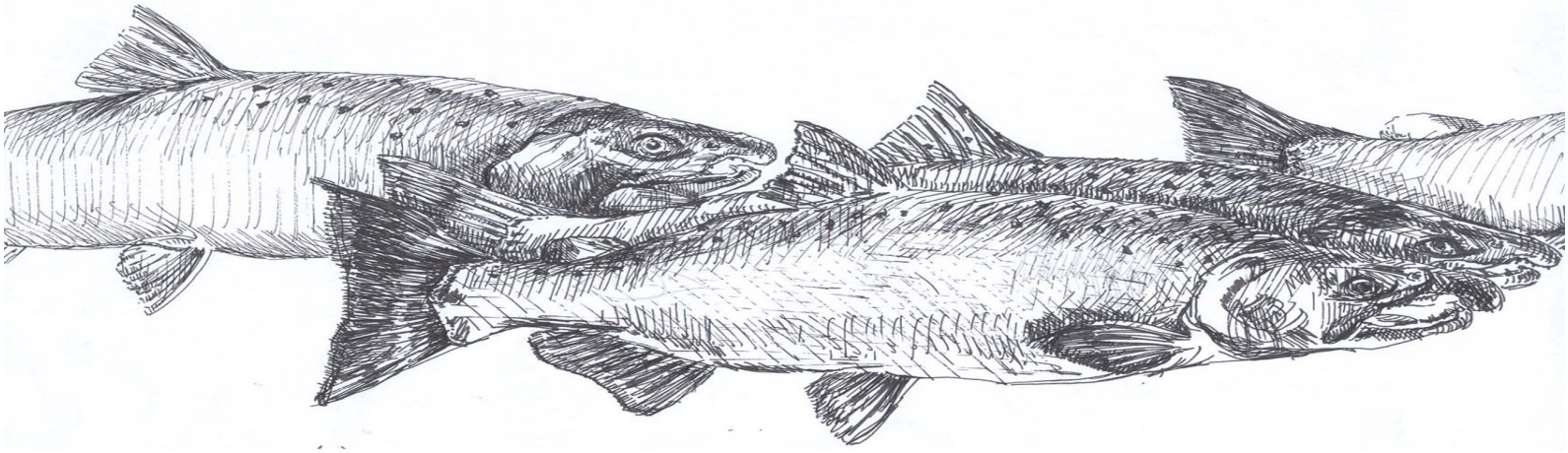


Documenting Biodiversity of Coastal Salmon (*Oncorhynchus* spp.) in Northern California



Final Report

Documenting Biodiversity of Coastal Salmon (*Oncorhynchus* spp.) in Northern California

Sonoma County Water Agency
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Principal Investigator: Dennis Hedgecock

Project personnel:
Michael Banks (former co-P.I., now Assistant Professor, Oregon State University), Kate Bucklin (graduate student research assistant and postdoctoral researcher), Cheryl A. Dean (staff research associate), Will Eichert (programmer), Carolyn Greig (postgraduate researcher), Paul Siri (Marine Life Management Project.), Bruce Nyden (programmer), Jason Watters (graduate student research assistant),

Bodega Marine Laboratory, University of California at Davis
P.O. Box 247, Bodega Bay, CA 94923

Documenting Biodiversity of Coastal Salmon (*Oncorhynchus* spp.) in Northern California

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SUMMARY

This report describes research on the genetic status and relationships among coastal California salmonid populations. The scope of work broadened from the original contract investigating population structure and genetic diversity of coho populations to include research on steelhead and Chinook populations and the development of a Geographical Information System (GIS).

Substantial progress was made in documenting coho population genetic diversity within the California Central Coastal (CCC) ESU. A suite of highly polymorphic microsatellite DNA markers was identified and used to establish genetic diversity within and among 57 collections of coho salmon from 14 watersheds. The samples encompass the southern end of the Southern Oregon / Northern California (SO/NC) ESU, the entire CCC ESU, and the South of San Francisco (SSF) ESU recognized by California's Endangered Species Act. Genetic distances among samples support the present State of California ESU structure, forming statistically significant clusters of samples corresponding to the CCC, the SSF, and the most southerly of samples from the SO/NC ESU (Eel and Mattole Rivers). Samples from the Klamath and Trinity Rivers are significantly separated from the Eel / Mattole River samples and from the CCC and SSF ESUs clusters. Sampling of different year-classes at seven sites reveals that temporal variation is typically significant, though smaller than the geographic component of population genetic structure. The congruence of genetic and geographic distance is surprising in light of the history of coho stock transfers within California and between California and other Pacific Coast states. Stock transfers appear to have left no genetic mark on extant populations. Alternatively, or in addition to stock transfers, the diversifying effects of genetic drift within the relict coho populations of California may be keeping pace with whatever homogenization has been or is being effected by hatchery practices.

We find many significant deviations between observed genotypic composition of coho salmon populations and the composition expected under random mating. These deviations occur both in juvenile samples, in which they might be expected, owing to kinship among individuals, and in adult samples, in which they are not expected based on the population genetic literature for natural populations of Pacific salmon. We discriminate and attempt to correct for the contributions of two different potential causes of deviations from random mating equilibrium – admixture in collections of individuals from genetically differentiated subpopulations and kinship. Partitioning samples based on independent biological information (sex, size, date caught, precise site of collection, whether marked, type of mark) does successfully reduce the deviations within some samples.

In most juvenile samples, many pairs of individuals show statistically significant odds of being full brothers and sisters. Because such samples yield biased and inaccurate estimates of the genetic diversity in the adult spawning population, population geneticists in the past have avoided using juvenile samples. Nevertheless, the depressed state of coho salmon populations often precludes collections of sufficient numbers of adults. Juveniles, on the other hand, are more readily available in large numbers. Of the 57 collections available for this study, 27 comprised juveniles. To salvage these important samples for genetic analysis, we apply methods pioneered in our lab for adjusting samples for family structure to derive unbiased and accurate estimates of adult allele frequencies. Related individuals are either removed and replaced with reconstructed parents or simply removed from a sample, resulting in a sample that is smaller but

usually closer to, if not in random mating equilibrium. Nearly half of the samples used to infer the geographic distribution of genetic diversity in this study are adjusted juvenile samples.

A large fraction of coho samples continues to deviate from random mating expectations after adjusting samples for substructure and kinship. Deviations from random mating proportions in some adult samples could be explained by inbreeding, and a significant excess of individuals homozygous for multiple markers supports this hypothesis. The non-equilibrium state of coho juveniles from Green Valley Creek and their highly aberrant genetic distance to other populations in the CCC ESU is of special concern, as fish from this population are currently being reared for a hatchery-based recovery effort in the Russian River watershed.

In order to estimate the genetic affinities of Chinook salmon in the Russian River with other stocks in California, we examined seven DNA markers in 449 fish from the Russian and Eel Rivers and Lagunitas Creek. Genetic distances show that Chinook salmon in the Russian River are distinct from those in the Eel and Klamath Rivers to which they are more closely related than Chinook from the Central Valley of California.

Jason Watters, a Ph.D. student supported by this contract, examined the development and maintenance of alternative male phenotypes in coho salmon. He showed that the phenotypes of juvenile coho males are affected by rearing habitat and alternative male phenotypes have different reproductive success. Thus, the maintenance of alternative male phenotypes in wild spawning populations could be critical to population viability.

Finally, a web-based GIS that focuses on coastal near-shore processes and allows linkages and integration of marine and coastal stream environmental data was developed. It is the first GIS model to incorporate real-time ocean surface currents measurements derived from coastal high frequency radar stations. This web-based GIS has the potential to deliver up-to-date information to a broad audience in a timely manner. Custom PERL programming scripts were developed in collaboration with the REGIS laboratory at UC Berkeley. A CD Rom containing the database files, software, directory structure and scripts is included with this report.

INTRODUCTION

This report describes research done under contract #TW 99/00-110 a continuation of work initiated under contract #TW 96/97-10 from the Sonoma County Water Agency. The first contract focused on the population genetics of coho salmon (*O. kisutch*) in Northern California, and this continued to be the major emphasis under the second contract. The scope of research on the second contract was expanded, however, to include research on life history variation in coho salmon as well as on the population genetics of steelhead (*O. mykiss*) and Chinook salmon (*O. tshawytscha*). We proposed, moreover, to develop a geographical information system (GIS), to enable synthesis and visualization of environmental and genetic data critical to management of coastal salmonid resources. Progress towards achieving the specific tasks is summarized in the body of this report.

Salmonid conservation requires identification of appropriate management units in a complex, geographically structured hierarchy of populations. Population genetics documents biodiversity at various levels in a population hierarchy and provides a variety of tools for resource management. In the first contract, for example, we developed molecular diagnostic tests that discriminates steelhead, coho and Chinook salmon, which co-occur in juvenile and carcass samples and can be difficult to distinguish morphologically (Greig et al. 2002). Within Pacific salmon species, the challenge is to identify how geographically structured biodiversity is influenced by hatcheries, environmental degradation, and ocean harvests. Finally, at the level of the local spawning run, estimates of effective population size (N_e) from genetic data can help predict the rate of loss of biodiversity and identify foci for recovery efforts. All of these genetic measures are essential components of viable population size (VP) estimates, which are central to management and restoration efforts.

Genetics of geographically structured populations

A brief review of basic population genetic principles will aid in understanding of some exceptional findings to be presented in this report. One of the oldest principles of population genetics, named, after its co-discoverers, the Hardy-Weinberg Principle (Hedrick 2000), describes the expected proportion of genotypes in a randomly mating population. If a hypothetical gene (or **locus**) has two alleles in a population, A_1 and A_2, with relative frequencies of p and q=(1-p), respectively, then the proportions among N adults of the three possible genotypes at this locus are given by the binomial expansion, $N(p+q)^2 = Np^2 + 2Npq + Nq^2$. For example, if alleles A_1 and A_2 have frequencies of 0.7 and 0.3, respectively, then among 100 individuals in a sample of adults, we expect to find 49 A_1A_1 homozygotes, 42 A_1A_2 heterozygotes, and 9 A_2A_2 homozygotes. Populations conforming to this principle are said to be in Hardy-Weinberg (H-W) or random mating equilibrium. The H-W Principle, which is easily extended to the multiple alleles typical of the highly polymorphic microsatellite DNA markers used in this research, simplifies enormously the description of populations, reducing the number of parameters to n alleles per locus, rather than the $n(n + 1)$ genotypes formed by sexual reproduction of diploid organisms.

The significance of differences between the observed and expected proportions of genotypes in populations can be tested in a number of ways, classically by a goodness-of-fit χ^2-test but, more recently, by Fisher exact tests, Markov-Chain approximations of the exact test, or permutation tests, which are more appropriate to the small expected numbers generated by many low

frequency alleles. The vast literature on the genetics of Pacific salmon populations shows that natural populations generally conform to the Hardy-Weinberg Principle (*e.g.* Bartley et al 1992a, b), implying that mating is more or less at random among spawning adults. Here, we report many exceptions to random mating equilibrium.

The principle of random mating equilibrium can be extended to multiple genes considered simultaneously. For example, with two genes, A and B, each with two alleles, A_1, A_2 and B_1, B_2, the expected proportion of each gamete at random mating equilibrium can be calculated as the product of the relevant allelic frequencies, *e.g.* the expected frequency of an egg carrying the A_1B_2 combination is the product pr, if the relative frequencies of A_1 and B_2 are p and r, respectively. As for the single-locus equilibrium described by the Hardy-Weinberg Principle, statistical tests of departures in samples from random multi-loci associations of alleles into gametes can be made, usually for pairwise combinations of markers. These tests are commonly called tests of **linkage disequilibrium** or **LD** (though, since physical linkage is not required, they are more properly called tests of gametic-phase disequilibrium; Hedrick 2000). Again, Pacific salmon populations are generally in gametic-phase equilibrium, but we report many exceptions here.

A number of factors can cause deviations from random mating expectations. In order to understand these and the results to be presented in this report, we must first consider how the genetic diversity of a species can be partitioned into components within and among population units, ranging from local, randomly mating populations (or **demes**) to subpopulations to the total species. Wright (1931, 1943) partitioned genetic variation within a species, using F-statistics, which measure the average genetic correlation between pairs of gametes derived from different levels in a population hierarchy. At the basal level of this hierarchy, the correlation between gametes drawn from different individuals within a deme is symbolized as F_{IS}. F_{IS} is zero in a randomly mating subpopulation but is positive when there are excesses of homozygotes relative to H-W expectations. Inbreeding, mating among related individuals, causes excesses of homozygotes and deficiencies of heterozygotes, in which case F_{IS} is positive.

If a species is subdivided into partially isolated, finite subpopulations, mating among individuals in the total population cannot take place at random and there will be genetic drift within each subpopulation. The effect on the proportion of genotypes in the species is analogous to the effect of inbreeding: local populations will tend towards fixation, with a decline in heterozygosity, but genetic diversity will be preserved among rather than within subpopulations. The genetic correlation between gametes drawn from different demes or subpopulations, with respect to allelic frequencies in the total population, is given by F_{ST}, the ratio of the variance of allelic frequencies among subpopulations to the variance in allelic frequencies among all subpoulations. When local populations diverge from one another, there will be an excess of homozygotes and a deficiency of heterozygotes, with respect to random mating expectations, summing across subpopulations. The principle is readily understood at the extreme, in which each subpopulation is fixed for one allele or another (F_{ST}=1.0); in this case, there are no heterozygotes in the total population. Heterozygote deficiency can result artificially from the unwitting admixture, in collections from natural populations, of individuals from genetically differentiated demes. This artificially induced deficiency of heterozygotes, which is known as the **Wahlund effect**, after its discoverer (Hedrick 2000), will be illustrated in the study of coho salmon reported here.

Genetics of juvenile salmon populations

Finally, we consider the consequences of sampling juveniles rather than adults for studies of genetic diversity. The old and very sound advice for students of salmon population genetics is to avoid sampling juveniles:

> "The correct way of approaching the question of possible genetic differences between subpopulations is to sample the spawners. ...it is dangerous to draw conclusions about reproductive isolation between adults by estimating allelic frequencies in their progeny. Differences caused by a small number of reproducing adults without any reproductive isolation can become highly statistically significant when a large number of progeny are sampled." (Allendorf and Phelps 1981).

Nevertheless, the presently depressed state of salmon populations, particularly coho salmon populations in Central and Northern California, often precludes the collection of a sufficient number of adult samples for genetic analysis. Juveniles, either fry or smolts, which are more easily collected in large numbers, are often the only sample available. In such samples, however, the H-W Principle does not apply. Either excesses or deficiencies of heterozygotes with respect to random mating expectations can occur, depending on the number and sizes of families present in a juvenile collection and on the genotypes of their parents. Likewise, linkage disequilibrium can often be generated, owing to the limited number of gametic combinations passed to progeny from a small number of parents; indeed, linkage disequilibrium provides a sensitive indicator of family structure in juvenile samples. Departures from random mating equilibrium will be illustrated for juvenile samples of coho salmon. We have endeavored to correct the allele-frequency estimates for the family structure in these samples, following the approach pioneered by us previously (Banks et al. 2000), thus to salvage these samples for use in our study of coho salmon diversity.

POPULATION GENETICS OF COASTAL CALIFORNIA COHO SALMON POPULATIONS

Introduction

The specific tasks in our scope for work were: 1) to determine relatedness in samples comprised of juveniles, 2) to determine temporal genetic variation among year classes, 3) to estimate genetic divergence among and effective population sizes of spawning runs, 4) to determine genetic change between historical and extant coho populations, to assess influence of hatchery plantings and reductions in abundance, 5) to relate the genetic diversity of California coho populations to environmental and biological factors being measured in the sampling process.

The contract also supported Kate Bucklin's doctoral thesis research on nucleotide sequence diversity and phylogeny across the North Pacific range of coho salmon. However, as described in the annual report for 2001, so little variation was detected at the nucleotide level that this research was not pursued and no results are presented here.

The major objective of this contract and its predecessor was to describe the genetic diversity of coho salmon populations along the central and northern coast of California, using highly polymorphic microsatellite DNA markers. Genetic diversity of coho salmon in this region was previously examined using protein markers, which have low levels of polymorphism and reveal little geographic structure (Bartley et al. 1992a). For our analysis, we selected seven

microsatellite DNA markers for their variability and apparent diversity among populations. The geographic coverage of our samples extends from the Klamath River, Del Norte Co., to Scott Creek, Santa Cruz Co., and includes populations from three Evolutionary Significant Units (ESUs), the Southern Oregon / Northern California ESU, the Central California ESU, and the South of San Francisco ESU, which the State California distinguishes from their Central California ESU. We present results for seven DNA markers, in over 1600 fish from 57 populations of coho salmon. These genetic data provide a context for understanding Sonoma County coho populations.

Materials and Methods

Microsatellite DNA markers

An extensive survey of known salmonid microsatellite DNA markers established a suite for assessing genetic diversity of California coho salmon. Investigation into published primers for the six Pacific species produced 69 microsatellites for testing. The screening processes used samples from Scott Creek (Santa Cruz County), Noyo River (Mendocino County), Eel River (Humboldt County) and Smith River (Del Norte County) to examine variability and assess potential diagnostic power. Sixty-two microsatellites were eliminated leaving seven polymorphic, potentially diagnostic loci (Table 1). Multiplexing the seven microsatellites into three PCR reactions increased efficiency. The microsatellite *iso-Ots-2* is known to have species-specific differences and was included to ensure species identity (Greig et al 2002).

Table 1. Summary of microsatellites examined from six Pacific salmon and other species. Microsatellite screening results are coded as follows: (N) total number examined, (In Use) selected for use assessing populations in California, (ND) not diagnostic in California, (NV) not variable, fewer than 4 alleles, (NW) primers did not work.

A. Markers screened.

Species	N	In Use	ND	NV	NW
Oncorhynchus gorbuscha	7	0	0	5	2
O. keta	3	0	0	1	2
O. kisutch	13	1	2	4	6
O. mykiss	3	0	0	1	2
O. nerka	18	1	0	9	8
O. tshawytscha	22	4	3	10	5
Other	3	1	0	1	1
Total	69	7	5	31	26

B. Markers selected for use.

Microsatellite	Repeat #	# Alleles	Reference
Ots-2	Di	8	Banks et al. 1999
iso-Ots-2	Di	16	Greig et al. 2001
Ots-3	Di	12	Banks et al. 1999
Ots-103	Tetra	35	Nelson and Beacham 1999
Oki-1	Tetra	13	Smith, C. T et al. (1998).
One-13	Di	17	Scribner et al. 1996
P-53	Di	10	Park et al. 1996

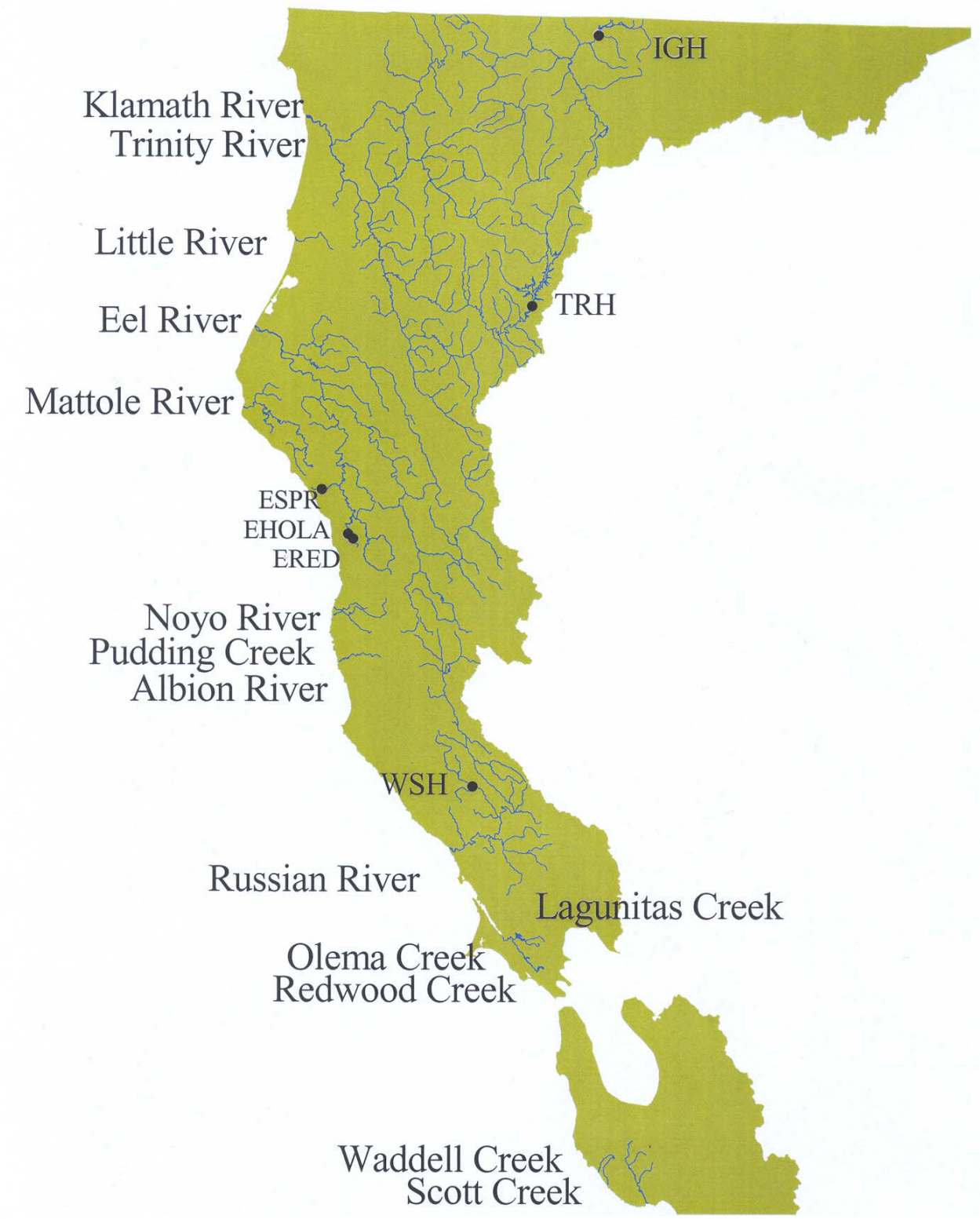

Figure 1. Map of Northern California, showing watersheds and in-stream sites from which coho salmon were collected for population genetic analyses. Site abbreviations are IGH=Iron Gate Hatchery, TRH=Trinity River Hatchery, ESPR=Sproul Creek, EHOLA= Hollowtree Creek, ERED=Redwood Creek, and WSH=Warm Spring Hatchery. See Table 2 for sample sizes.

Table 2. Samples of coho salmon used for genetic analysis. Stages are A= adults, S= smolts, Y= young of the year. Populations are designated by their Name codes in subsequent tables and figures. The criteria for subdividing collections from certain sites or drainages are listed.

Watershed	Tributary or Site	No.	Stage	Yr. Coll.	Name code	Criteria; Collectors
Klamath River	Iron Gate Hatchery	11	A	97/98	KIGHA97a	Ad clip, FL>56cm; CDFG
Klamath River	Iron Gate Hatchery	15	A	97/98	KIGHA97j	FL<56cm; CDFG
Klamath River	Iron Gate Hatchery	36	A	97/98	KIGHA97ll	Left clip, FL>56cm; CDFG
Klamath River	Iron Gate Hatchery	19	A	97/98	KIGHA97nl	No clip, FL>56cm; CDFG
Trinity River	Trinity River Hatchery	17	A	97/98	TRHA97s	FL<45cm; CDFG
Trinity River	Trinity River Hatchery	77	A	97/98	TRHA97l	FL>53cm; CDFG
Little River (Humboldt Co,)	Little River Delta	85	S	2000	LRS00-1	4/3/00-5/6/00; Simpson Timber Co.
Little River (Humboldt Co.)	Little River Delta	11	S	2000	LRS00-2	5/19/00-5/29/00; Simpson Timber Co.
SF Eel River	Hollowtree Creek	16	A	97/98	EHOLA97	Salmon Trawlers Assoc.
SF Eel River	Redwood Creek	92	S	97	EREDS97	Eel River Salmon Restoration Project
SF Eel River	Redwood Creek	22	A	98/99	EREDA98	Eel River Salmon Restoration Project
SF Eel River	South Fork Sproul Creek	34	S	1999	ESPRS99	Eel River Salmon Restoration Project
Mattole River	Mattole River Delta	75	S	98	MATS98-1	5/7/98 and 5/12/98; Mattole Salmon Group
Mattole River	Mattole River Delta	21	S	98	MATS98-2	5/19/1998; Mattole Salmon Group
Pudding Creek	Pudding Creek	33	Y	98	PUDY98h	9/23/1998; Georgia Pacific
Pudding Creek	Pudding Creek	43	Y	98	PUDY98k	10/27/1998; CDFG
Pudding Creek	Upper Pudding Creek	4	Y	98	PUDY98u	9/23/1998; Georgia Pacific
SF Noyo River	Egg Taking Station	47	A	97/98	NOYA97	Bill Cox, CDFG
SF Noyo River	Egg Taking Station	47	A	99/00	NOYA99	CDFG
Albion River	Albion Mainstem	23	A	98/99	ALBA98	Mendocino Redwood Co.
Albion River	Marsh Creek	18	Y	98	ALBY98	CDFG
Russian River	Warm Springs Hatchery	33	A	95/96	RRHA95	CDFG
Russian River	Warm Springs Hatchery	25	A	96/97	RRHA96	CDFG
Russian River	Warm Springs Hatchery	7	Y	97	RRHY97	CDFG
Russian River	Green Valley 10/27	9	Y	97	RRGV97	Michael Fawcett
Russian River	Green Valley	67	Y	98	RRGV98a	Michael Fawcett / SCWA
Russian River	Green Valley10/13	61	Y	98	RRGV98b	Michael Fawcett / SCWA
Russian River	Green Valley	8	Y	99/00	RRGV00	Michael Fawcett / SCWA
Russian River	Estuary	8	S	97	RRDS97	Michael Fawcett

(Table continues next page)

Table 2. Samples of coho salmon used for genetic analysis. Stages are A= adults, S= smolts, Y= young of the year. Populations are designated by their Name codes in subsequent tables and figures. The criteria for subdividing collections from certain sites or drainages are listed.

Watershed	Tributary or Site	No.	Stage	Yr. Coll.	Name code	Criteria; Collectors
Russian River	Estuary	3	S	98	RRDS98	Michael Fawcett
Russian River	Mirabel	2	Y	98	RRM98	SCWA
Lagunitas Creek	Lagunitas Mainstem	9	A	96/97	LAGA96	Trihey Associates
Lagunitas Creek	Lagunitas Mainstem	7	A	97/98	LAGA97	CDFG
Lagunitas Creek	Lagunitas Mainstem	2	A	99/00	LAGA99	MMWD
Lagunitas Creek	Devils Gulch	9	A	96/97	LDGA96	Volunteers
Lagunitas Creek	Devils Gulch	10	A	97/98	LDGA97	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo	32	A	95/96	LSGA95	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo	19	A	96/97	LSGA96	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo	52	A	96/97	LSGA96	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo	61	A	97/98	LSGA97	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo	10	Y	96	LSGY96	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo	12	Y	98	LSGY98	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo Arroyo	36	A	96/97	LSGAA96	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo Arroyo	3	A	97/98	LSGAA97	Bob Chamberlain, Volunteers
Lagunitas Creek	San Geronimo Arroyo	21	Y	98	LSGAY98	Bob Chamberlain, Spring Class
Olema Creek	Mainstem	71(2X)	A	96/97	OLEA96	Natl. Park Service
Olema Creek	Mainstem	34 (2X)	A	97/98	OLEA97	Tomales Bay Assoc.
Olema Creek	Mainstem, Blueline	88	Y	98	OLEY98	Natl. Park Service
Redwood Ck. (Marin)	Mainstem	15	A	97/98	RWMA97	Natl. Park Service
Redwood Ck. (Marin)	Mainstem	24	Y	98	RWMY98	Jerry Smith
Waddell Creek	Mainstem	42	Y	99	WADY99low	RM 3.1 and 3.9; Jerry Smith
Waddell Creek	Mainstem	17	Y	99	WADY99up	RM 4.7; Jerry Smith
Scott Creek	Hatchery	43	A	95/96	SCA95	Monterey Bay Trout and Salmon Project
Scott Creek	Hatchery	57	A	97/98	SCA97	Monterey Bay Trout and Salmon Project
Scott Creek	Hatchery	42	A	98/99	SCA98	Monterey Bay Trout and Salmon Project
Scott Creek	Mainstem, Big & Mill Creeks	40	Y	99	SCY99low	RM 2.55, 3.55, B&M Cks.; Jerry Smith
Scott Creek	Mainstem, Upper Fork	20	Y	99	SCY99up	RM 4.9, Upper Fork; Jerry Smith

Population samples

A study of genetic diversity in coastal coho salmon was conducted on samples taken in 57 collections from 14 watersheds (Table 2; Fig. 1). Sites were chosen as representative of a wide geographic range beginning at the southern end of the Northern California/ Southern Oregon ESU and ending at the southern boundary of Central California Coast ESU (Weitcamp et al. 1995) . California Department of Fish and Game recognizes a split in the Central California Coast ESU at San Francisco Bay and has protected, under the California Endangered Species Act, samples south of San Francisco. All Russian River samples in our possession were included. Redwood Creek on the South Fork of the Eel River, the Noyo River, the Russian River, Olema Creek, Lagunitas Creek, and Scott Creek were sampled in different years, permitting study of temporal genetic variation. Across the 57 samples, 1745 individuals were available for genetic analysis (Table 2); LSGA95 (n=32), one LSGA96 (n=52), and the LSGY96 (n=10) were omitted from further analysis, owing to poor PCR results on the final tray.

Molecular methods

DNA from samples was extracted using the Puregene™ DNA isolation kit (Gentra System), a superior extraction procedure to Chelex 100 (BioRad) particularly when extracting tissue from degraded carcasses. DNA extractions were performed using 96-well trays. We made multiple attempts to extract and amplify samples that were initially unsuccessfully genotyped.

Individuals were genotyped for the seven microsatellites described in Table 1B, by first amplifying each microsatellite marker from genomic DNA via the polymerase chain reaction (PCR) and then separating the PCR products according to molecular size by polyacrylamide gel electrophoresis (PAGE). The forward PCR primer was labeled with a fluorescent phosphoamidite (HEX or fluorescein). PCR products were electrophoresed, 96 at a time, with allelic controls, on a 45.0 cm wide by 22.5 cm high 8% denaturing PAGE gel at 50 W for 150 min. DNA fragments were visualized on the FMBIO® fluorescent imaging system (Hitachi Software Engineering America Ltd). The relative sizes of individual bands were scored, using BIOIMAGE software. To control genotype scoring among trays, we co-electrophoresed eight individuals from each of the 20 trays in one set of gels. The data were double-checked for accuracy and independently verified by at least one other researcher. Individuals that did not produce repeatable genotypes and were difficult to score were not included in the analyses.

Statistical methods

Population genetic parameters. The raw genetic data comprise more than 1600 individual genotypes, such as these:

ID	Ots103	Ots-2	i-Ots2	Ots-3	One-13	P-53	Oki-1
KIGHA97051	236272	180182	217217	147153	000000	169183	088104
KIGHA97053	220268	180180	205251	153153	201203	177181	088104
KIGHA97046	248264	178184	205231	153157	215219	181181	096116
KIGHA97048	220264	180182	205209	147153	000000	173181	096116
KIGHA97019	276280	180182	221221	151153	211219	163169	096096

At the left is an individual identifier. Under each column headed by a marker name is a six-digit figure representing the two alleles scored for that marker in that individual. Each allele is represented by three-digits that correspond, roughly, to the size of the PCR product, in nucleotide base pairs. This number becomes a qualitative category, analogous, say, to alleles at a gene

controlling eye color in the fruit fly. For example, individual KIGHA97051 is heterozygous for the *236* and *272* alleles at the *Ots-103* locus. This same individual is homozygous for the *217* allele at the *iso-Ots-2* locus and is missing information at the *One-13* locus (represented as `000000`, because a six-digit format is required for input into the GENETIX program described below). The fundamental quantitative data of interest are the frequencies of these allelic categories in populations. In this small example, the frequencies of the four alleles observed at the *Ots-2* locus in the five individuals shown above are: $f_{[178]}$=1/10=0.1; $f_{[180]}$=5/10=0.5; $f_{[182]}$=3/10=0.3; and $f_{[184]}$=1/1=0.1. The number of alleles is twice the number of individuals, and their frequencies sum to 1.0. From the H-W Principle, we would expect the frequency of *180180* homozygotes, for example, to be $(0.5)^2$ = 0.25 or 1 out 4; we observe 1 out of 5 such homozygotes in this small data set.

We tested the fit of genotypic proportions within populations to the Hardy-Weinberg equilibrium proportions, using GENEPOP, version 3.3 (available at ftp://ftp.cefe.cnrs-mop.fr/genepop/). Allele frequencies, observed and expected proportions of heterozygotes, and *F*-statistics (F_{IS} and F_{ST}) were calculated, using the program GENETIX version 3.3 (available at http://www.univ-montp2.fr/~genetix/genetix.htm). The significance of pairwise linkage disequilibrium (LD), F_{IS} and F_{ST} tests was determined by performing 500 permutations of the data in GENETIX. For F_{IS} and LD, these permutations were of alleles among individuals within a population; for F_{ST}, the permutations were of multi-loci genotypes among individuals from all populations. Significance was determined by the percentage of permutations yielding a value as large or larger than that observed, with the nominal 5% level being the threshold for rejecting the null hypothesis of F=0. Homogeneous sets of populations within sites were determined by testing the significance F_{ST} among all of the samples from that site; if F_{ST} is significant (*i.e.* 5% or fewer of the permutations yielded an estimate as large or larger as that observed), then the most divergent member of that group was removed and the F_{ST} was re-tested. If significant, the next most divergent member was removed; this process was repeated until a set of homogenous populations with a non-significant amount of inter-population variance was obtained. The matrix of F_{ST} among all pairs of populations is used to determine divergence of members from the rest of the group

Cavalli-Sforza and Edwards (1967) chord measures (CSE) were calculated using GENDIST in the program PHYLIP (Felsenstein 1993). Unweighted pair-group method with arithmetic mean (UPGMA) or average distance trees (Sneath and Sokal 1973) were calculated using NEIGHBOR in PHYLIP. Bootstrap results for assessing the frequency of occurrence, and thus significance, of each tree cluster were obtained using SEQBOOT and CONSENSE in PHYLIP with 1000 replicates. Trees were visualized using TREEVIEW (Page 1996). A Neighbor-Joining tree was constructed in PHYLIP for the individuals in the RRGVY98a sample, based on the allele-sharing distance metric in Msat2 (http:/hpgl.Stanford.edu/info@hpgl.Stanford.edu).

Analysis of kinship and adjustments for family structure in juvenile samples. We examined relatedness in juvenile samples and adjusted these samples for family structure, following the methods previously developed and published by BML (Banks et al 2000). High levels of LD (more than two of 21 loci-pairs significant at 5% or lower) and significant departures from single-locus H-W equilibrium proportions indicated those samples in need of adjustment.

The odds of two individuals being full-siblings rather than unrelated were calculated, using the program KINSHIP 1.2 (Goodnight and Queller 1999). An appropriate baseline of allelic frequencies was derived from a pool of adults collected within the same ESU as the juvenile sample of interest. The log of the odds ratio (LOD-score) classification of a full-sib relationship between two individuals is conservative when applied to winter Chinook test families, *i.e.* the test has low power, detecting a little more than half of true full-sibs, but suitable protection against Type-I error, classifying very few truly unrelated pairs as full-sibs. The number of loci, however, is critical to this test. We first deleted individuals missing more than three loci (though retaining the exceptionally polymorphic combination of *Ots-103*, *iso-Ots-2*, *Oki-1*), to avoid potentially spurious results in evaluating kinship.

The output of KINSHIP is a triangular half-matrix of relatedness coefficients, LOD scores, or test results from all possible pairwise kinship tests. This latter matrix becomes one input into SIBLINGS, a program written by Will Eichert to analyze the family structure of sampled individuals, following the methods of Banks et al (2000). The other inputs are allele frequencies for the appropriate baseline population and the genotypes of individuals in the juvenile sample. The significance of the relatedness test serves as an initial indicator of possible sibling groups. The SIBLINGS program examines potential sibling groups for violations of Mendelian rules of inheritance (*e.g.* more than 4 alleles at any locus or impossible combinations of genotypes). Any individuals not conforming to Mendelian rules are discarded from the group, though they become candidates for inclusion in other groups. The clustering and discard algorithm has difficulty parsing a sample that comprises many families with complex mixtures of full- and half-sibs, as happened in the RRGVY98a sample. Following Bentzen et al (2001), we partitioned this sample into smaller sets, using a Neighbor-Joining tree of allele sharing among individuals. Once smaller kinship groups were identified, the genotypes of the group's parents are reconstructed. The genotypes of possible parents must be able to produce the genotypes of all offspring (see Table 2, Banks et al 2000). Possible mating pairs are then scored and ranked. The score is a product of the sibling group's probability, under all relevant bi, tri, or tetranomial distributions, and the joint likelihood of the parental genotypes. After forming full-sib groups, SIBLINGS looks for families that have a common parent (half siblings). All individuals in each sibling group are then removed from the dataset, and replaced by their parents.

Adjustments of samples

Adult populations that departed significantly from random mating expectations were further examined for evidence of admixture, *i.e.* that deficiencies of heterozygotes in these samples might have resulted from Wahlund effect. Subdivision of a sample was only possible if independent information, such as size (fork length), collection date, or collection site, was available. In these cases, samples were subdivided, according to criteria specified in Table 2, and each subsample was re-tested for single and multi-loci random mating equilibria. F_{ST} among subsamples was also calculated and tested for significance. Wahlund effect in the original sample would be evidenced by non-significant departures from H-W within subsamples but significant F_{ST} among subsamples. Details on specific populations are given below.

Twenty-seven of the 57 collections comprised young of the year or smolts. Each of these juvenile samples required intensive effort to discriminate the contributions of population admixture (Wahlund effect) and family structure to its departure from random mating equilibrium. We first checked for admixture, if independent criteria permitted subdivision, as

described above for adult samples. We next applied the family adjustment procedure multiple times, altering both stringency of inclusion in kinship groups and minimum sib-group size, in a series of tests designed to find an optimum adjustment that minimized LD and the number of reconstructed parents, while maximizing the number of unrelated individuals. The large amount of family structure revealed in the RRGVY98a sample is detailed in the Results section; detailed accounts of adjustment procedures in each of the other juvenile samples follow. We also applied family adjustment to the Scott Creek adult samples from the Monterey Bay Trout and Salmon Project hatchery, which also showed substantial LD.

KIGHA

Eighty-one Klamath River, Del Norte County samples were collected from returning adults at the Iron Gate Hatchery (IGH) on 11/18, 11/24, or 12/18/1997. Biological data also included sex, fork length and marking type applied at time of release. We separated the 81 individuals into subgroups determined by the relevant and available biological information to determine whether heterogeneity existed among samples. There was no difference among samples based on collection date (F_{ST} = 0.0038, P< 0.159). We separated individuals by mark type and fork length (FL). Returning adults had an adipose clip, a left maxillary clip, or were non-clipped. Adipose clipped fish are likely released from the Cole M. Rivers Hatchery on the Rogue River, OR, which in some cases is verified by the presence of recovered pit tags (personal communication, IGH staff). Non-clipped adults may be wild spawned or hatchery escapees, while left maxillary fish are returning IGH adults. We tested the frequency distribution of size by mark type to determine cut-off points for developing discrete sub-populations (Fig. 2). Thirteen left-clipped, and two non-clipped individuals, constitute a sub-population of precocious males or jacks (FL<56cm) (population KIGHAj, Table 2, where N=15), and likely represent an alternate year class. Large individuals (FL> 56cm) of all mark types generally follow a normal distribution (Fig. 2) and are initially considered as three separate sub-populations within the Klamath system. Sample sizes for large adipose-clipped, left-clipped, and non-clipped adults are 11, 36, and 19 respectively (populations KIGHAal, KIGHAll, and KIGHAnl, respectively). In tests for homogeneity among all four putative populations, only adipose-clipped and non-clipped could be combined F_{ST} =0.0044, P< 0.306 (Table 4). The number of loci-pairs showing significant linkage disequilibrium (P< 0.05) was high when considering the 81 samples represented a single population (8/21 loci-pairs).

TRHA

We analyzed a total of 94 adults collected at the Trinity River Hatchery (TRH) on November 12 or December 1, 1997. All adults were marked with a right-maxillary clip applied by TRH at the time of release. Fork lengths, date of collection, and sex were also provided for each individual. We partitioned the 94 individuals into smaller putative populations based on the available information to test for heterogeneity among samples. Samples collected on the two dates (11/12 and 12/1) were homogenous (F_{ST} = 0.0024, P<0.253). We tested for heterogeneity among different size classes. Fork-length ranged from 36-74 cm, and there was a discrete separation between small males (36-44cm) and large (53-74cm) adults of both sexes. The jacks or small male category (sample TRHAs where N=17) and large category (TRHAl where N=77) were significantly heterogeneous (F_{ST} = 0.0131, P<0.022).

LRS00

Little River, Humboldt County (LRS00) samples, were provided by Simpson Timber Co. from the Little River lower South Fork trap, spanning the dates April 3, to May 29, 2000. All samples were collected from out-migrating smolts. Data included sample collection date for individual samples. Nine loci-pairs out of 21 showed significant LD. We tested whether samples collected from different dates constituted a single homogeneous population. In cases where the number of out-migrating smolts collected on individual dates was insufficient, samples were binned to achieve adequate sample sizes. The 5 putative populations were grouped as follows: 4/3 (N=19), 4/4(N=38), 4/6(N=17), (4/20-5/6) (N=11), and (5/19-5/29) (N=11). The global F_{ST} for these 5 populations was 0.0095 (P<0.014). The most divergent population (5/19-5/29) was removed and the F_{ST} for the remaining 4 populations was 0.0036 (P<0.204). This indicates that the 85 individuals collected between 4/3 and 5/6 constitute a single homogenous (population LRS00-1, Table 2) that is not homogeneous with the 11 individuals collected between 5/19 and 5/29 (LRS00-2). After separating samples into two populations, eight out of 21 loci-pairs showed significant LD in population LRS00-1. We adjusted both populations for potential family structure with the program SIBLINGS. Two individuals were removed from LRS00-1 because they did not meet the minimum requirement of genotype values at four loci (or the acceptable combination of *Ots-103*, *iso-Ots-2*, and *Oki-1*). This reduced N= 85 to N=83 individuals. The SIBLINGS output pedigree for this population included 28 unrelated individuals, and 44 parents representing 23 Sibling groups (23 smolts were replaced by their hypothetical parents), totaling 72 individuals in the adjusted sample. The 11 LRS00-2 individuals were also corrected for family structure. Of the initial 11 individuals, five were unrelated and four parents, representing two sibling groups, replaced six. After adjustment, both sub-populations were subsequently homogenous (F_{ST} = 0.0031 P<0.292), and the LD was reduced from 9/21 to 3/21 significant loci-pairs.

EREDS97

In 1997, out-migrating smolts were collected from Redwood Creek on the South Fork of the Eel River (population EREDS97). Of the 95 samples analyzed, 81 were collected on 4/26/97, and the remaining14, were collected on 4/30/97 (Eel River Restoration Salmon Project, Table 2). There was no available information, to separate the 95 samples into sub-populations. To correct for possible family structure, we analyzed 89 individuals that met the four (or three) locus criteria. The SIBLINGS pedigree included 52 unrelated individuals and 24 hypothetical parents comprising 13 different sibling groups. From an initial 2/21 significant loci-pairs, the adjustments for family structure reduced LD to 1/21 significant associations.

ESPRS99

In 1999, 34 out-migrating smolts were collected from the South Fork of Sproul Creek located on the South Fork of the Eel River (Eel River Salmon Restoration Project, Table 2). Accompanying data included date trapped and fork length. Fork length ranged from 68 to 110mm but showed a gap between 92mm and 96mm; thus, we formed two putative sub-populations of 68 to 92mm and 96 to 110mm. These populations were homogenous (F_{ST} = 0.0066, P<0.20). Samples split into groups based on trap date (4/5-4/22 and 5/10-6/4) were also homogenous (F_{ST} = 0.015, P<0.072). Thirty-four individuals were tested for family associations using SIBLINGS. The program pedigree included 12 unrelated individuals, and 18 hypothetical parents comprising nine

sibling groups (Table 4). LD dropped from an initial 4/21 significant locus-pair associations, to 0/21 after adjustment for family structure.

MATS

Ninety-six Mattole River smolts were collected between 5/7/98 and 6/1/98 from the Mattole mainstem at river mile three by screw trap (Mattole salmon Group). Fork length and collection date were available. Three putative populations were constructed based on collection time 5/7-5/11, N=47, 5/12-5/16, N=28, and 5/19-6/1, N=21. The global F_{ST} for three putative populations was 0.0077, P<0.032. Removal of 21, late-migrating individuals (5/19-6/1) resulted in a homogenous population (MATS-1) of early out-migrants (F_{ST} = 0.0047, P<0.148). LD was significant (5/21 loci-pairs), but lower than the initial 8/21 significant loci-pairs. The N=21 MATS-2 sub-population, exhibited an LD value of 1/21 significant loci-pairs. Before adjusting family structure in MATS-1, two individuals were dropped due to insufficient data. The SIBLINGS output pedigree included 27 unrelated individuals, 26 sibling groups, and 1 shared parent. However, the LD value remained high at 6/21 significant loci-pairs. To reduce LD, we selected only the 27 unrelated individuals and tested homogeneity with the MATS-2 sub-population (F_{ST} = 0.0048, P<0.21). This yielded a homogeneous population of 48 unrelated individuals (MATS).

PUDY98

Eighty Pudding Creek 1998, young of the year samples were acquired by two collectors, from different portions of the watershed on 9/23/98 and 10/27/98 (PUDYh N = 37, PUDYk N =43, Table 2). PUDYh samples were further divided into two groups based on collection location. Upper Pudding Creek samples (PUDYu N = 4) and one individual with insufficient data, were dropped from further analysis, making N = 32 for PUDYh. The global F_{ST} for PUDYh and PUDYk was not significant at –0.0055 (P<0.844). However, after adjustment for family structure in SIBLINGS, LD was reduced only slightly to 9/21 from an initial 10/21 significant loci-pairs. Taking the two sub-populations separately, LD for PUDYh and PUDYk respectively, was 4/21 and 6/21 significant loci-pairs. To further reduce the LD, we removed all hypothetical parents from the separate SIBLING pedigrees and jointly analyzed only unrelated individuals. We specifically tested whether the 44 unrelated individuals from the two sub-populations were homogenous (F_{ST} = -0.0062, P< 0.860). The calculated LD for the adjusted population PUDY was 5/21 significant loci-pairs.

ALBY98

Eighteen young of the year samples were collected on 10/30/98 from Marsh Creek, a tributary of the Albion River (CDFG). Linkage disequilibrium was moderate (3/21 loci-pairs) for these 18 individuals. We corrected for family structure given that they were collected in-stream, from few pools, over a short distance. SIBLINGS detected two sibling groups, consisting of three individuals each. Six individuals were replaced with their hypothetical parents, which reduced the number of significantly associated loci-pairs from 3/21 to 1/21.

RRGV98a

Sixty-seven young of the year samples were collected from Green Valley Creek, a tributary to the Russian River on 7/20/98. These samples were collected from a relatively small area and were not likely to be heterogeneous (see RRGV98b below). A substantial number (15/21) of

loci-pairs had significant LD. We were unsuccessful in reducing LD to less than 3/21 significant loci-pairs using the SIBLINGS program. We removed individuals with missing information (scored for all 7 loci) and subdivided the remaining 59 individuals into four putative sibling groups using a dendrogram based on allele sharing (see Fig. 4). After identifying closely related individuals based on the number of shared alleles, we corrected for family structure using SIBLINGS. Of the four SIBLING pedigree outputs, the largest identified sibling group contained 25 individuals, which were replaced by their two hypothetical parents. SIBLINGS also identified three groups of two siblings, four groups of three siblings, and two groups of four siblings. In all cases, two hypothetical parents replaced each sibling group. The adjusted N of combined tests was 25 individuals. The LD for adjusted RRGV98a samples was 1/21 significant loci-pairs.

RRGV98b

Sixty-one young of the year samples were collected from Green Valley Creek, a tributary to the Russian River on 10/13/98. These samples were collected from the same location as population RRGV98a, which was collected three months earlier (Fawcett, Table 2). Individuals collected at the later date could have been the same individuals sampled on the earlier date, but we were unable to confirm this, because all individuals collected at the later date possessed intact caudal fins (a caudal fin genetic sample was taken on 7/20). We initially tested whether samples collected from different pools constituted a homogenous population. No heterogeneity was detected among RRGV98b samples, collected from different pool sites (F_{ST} = -0.0083, P<0.816). In the unadjusted sample, 15/21 loci-pairs showed significant associations. To correct for family structure, we ran all individuals simultaneously through the program SIBLINGS (for comparison, see RRGV98a, MATERIALS). SIBLINGS created a total of 18 sibling groups, the two largest groups of which consisted of 15 and 8 full-siblings. There were also 11 sibling groups consisting of three individuals each, four sibling groups with four individuals each and one group with five (see Table 4). The adjusted N for this sample dropped from 61 to 39 including hypothetical parents. After adjustment for family structure, LD dropped from 15/21 significant loci-pairs to 7/21. We were unable to reduce LD further.

LSGAY98

In 1998, 21 young of the year samples were collected from San Geronimo Arroyo (BML spring class, Table 2). These samples displayed an LD value of 6/21 significant locus-pair associations. After adjustment for family structure using SIBLINGS, only 2/21 loci-pairs were significant. The adjusted population comprised 16 unrelated individuals and two hypothetical parents replacing a sibling group of three individuals.

OLEY98

Eighty-eight Olema 1998 young of the year samples were collected from four reaches spanning the area just downstream of Vendata to, and including, Blueline Creek. We initially tested whether samples collected from the five different reaches constituted a single homogenous population. The samples collected from Reach 5 were least like the downstream samples but were not significantly heterogenous (F_{ST} = 0.0030, P< 0.20). Five out of 21 loci-pairs showed significant LD. We corrected family structure with SIBLINGS, which constructed a population of 53 unrelated individuals and 10 sibling groups. The largest sibling group included eight

individuals, and there were five groups with four individuals and four groups consisting of three individuals each. After adjustment, 4/21 loci-pairs still showed LD.

WADY99

In 1999, fifty-nine young of the year samples were collected from three distinct areas of Waddell Creek. Twenty-three samples were collected at or around river mile (RM) 3.1, 19 samples were collected from RM 3.9, and 17 samples were collected from RM 4.7 (Smith, Table 2). The among-site global F_{ST} was highly significant at 0.0370, $P<0.00$. Samples originating from RM 4.7 were heterogeneous to both RM 3.1 and 3.9 and were removed (WADY99up, Table 2). The F_{ST} for the remaining 36 samples (RM3.1 and 3.9) was not significant at 0.005, $P<0.27$ (WADY99low, Table 2). The WADY99up population had LD of 2/21 loci-pairs, while the WADY99low population had 7/21 significant locus-pair associations. We corrected WADY99low for family structure. The adjusted WADY99low population consisted of 15 unrelated individuals, and eight sibling groups, the largest of which represented 7 full siblings. The adjustment reduced LD to 3/21 significant loci-pairs. After adjustment, the WADY99low population was still heterogeneous with WADY99up ($F_{ST} = 0.059$, $P<0.00$) and could not be combined.

SCA95A

Forty-one returning adult coho were collected at the hatchery on Scott Creek in 1995 (MBTSP, Table 2). Five out of 21 loci-pairs had significant LD, potentially caused by family structure. Adjustments for family adjustment proceeded, using SIBLINGS. Seventeen unrelated individuals and 11 sibgroups were formed, 10 were derived from sibling groups consisting of two individuals each, and one group had four siblings (Table 4). After adjustment, LD dropped to 1/21 significant loci-pairs.

SCA97A

Fifty-six adults returning to Scott Creek were trapped at the hatchery in 1997. Fifteen out of 21 loci-pairs had significant LD. Adjustments for family structure proceeded, using SIBLINGS which produced a pedigree comprising 16 unrelated individuals, four groups of sibling pairs, nine groups of three siblings, and 1 group of four siblings. The LD after adjustment was reduced to 4/21 significant loci-pairs.

SCA98A

Forty-two adults returning to Scott Creek were trapped at the hatchery in 1998. To reduce possible family structure in these samples (LD = 11/21 loci-pairs), we used SIBLINGS. Four samples were deleted from further analysis due to insufficient data. The SIBLINGS pedigree consisted of five unrelated individuals, and 18 hypothetical parents. The largest sibling group consisted of six individuals while the majority had three siblings (Table 4). Adjustments for family structure reduced the LD to 4/21 significant loci-pairs.

SCY99

Sixty young of the year coho were collected from various regions within the Santa Cruz Scott Creek watershed, in 1999 (Smith, Table 2). Ten individuals were collected from each of the following mainstem areas; RM 2.55, RM 3.55, RM 4.9, and tributaries, Big Creek, Mill Creek and Upper Fork totaling N=60. The global F_{ST} for 60 samples separated by collection site was

highly significant (F_{ST} = 0.036, P< 0.00). Upper Fork samples were the most heterogeneous and were removed. The F_{ST} for the remaining sample sites was 0.0191 and still significant (P<0.030). The further removal of RM 4.9 samples (SCY99up) yielded a homogenous population consisting of RM 2.55, RM 3.55, Big and Mill Creek samples (SCY99low, Table 2). Six out of twenty-one loci-pairs showed significant LD in the SCY99low population. The adjusted SCY99low population consisted of 12 unrelated individuals, and seven sibling groups, in most cases consisting of six to eight siblings per group (Table 4). After family adjustment, SCY99low was not homogenous with SCY99up, but the LD value was reduced to 3/21 significant pair associations.

Results

Genetic diversity within California Coastal Coho

Preliminary analyses of the genetic data suggested widespread departures from random mating expectations, as measured by tests of single-locus and multi-loci equilibria (2001 annual report). Although many of these deviations were observed in juvenile population samples, which are expected to deviate from random mating expectations, many samples of adults also appeared to depart from random mating equilibrium. First, we investigate the possibility that departures from random mating equilibrium within adult samples might have resulted from artificial admixture of fish from genetically different subpopulations.

The 1997 sample of 81 adults from the Klamath River Iron Gate Hatchery illustrates the Wahlund effect. F_{IS} for this sample is 0.076, a value that is attained in none of the 500 permutations of the alleles among individuals (*i.e.* P = 0.0), and seven of 21 pairwise LD tests are significant at the 5% level. The distribution of fork lengths in the KIGHA97 sample shows a clear separation into jacks (males less than 56cm FL) and older adults (Fig. 2). The sample can also be subdivided by the presence and kind of mark (no mark, which could be either wild or

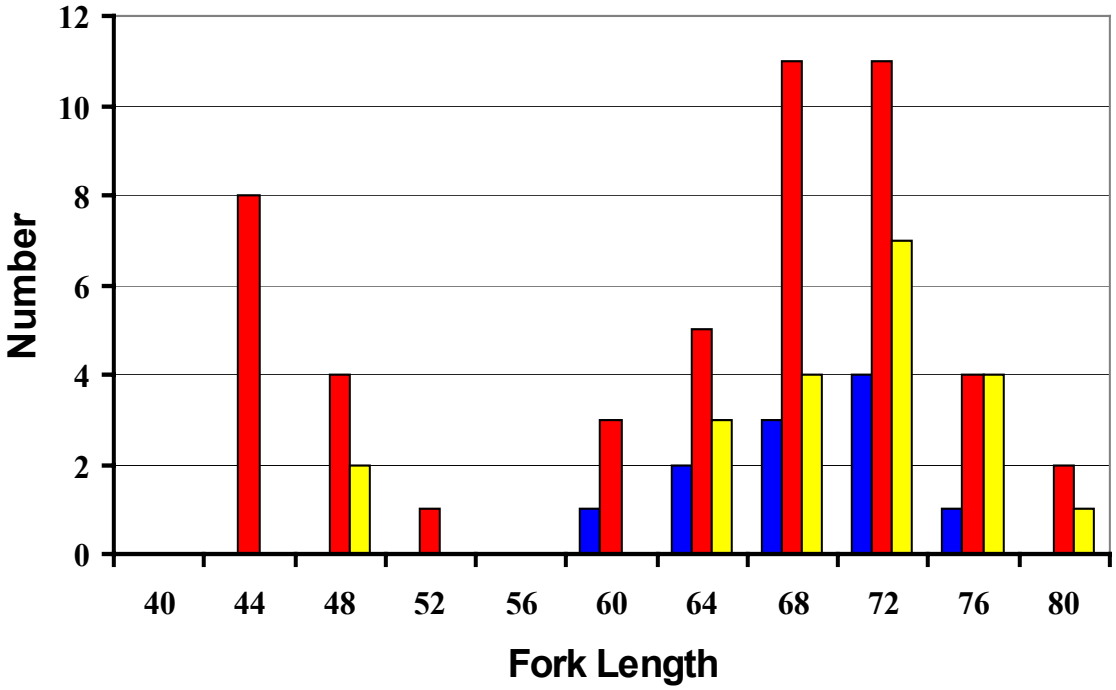

Fig. 2. Distribution, by fork length (cm) and mark, of adult coho salmon sampled from returns to the Iron Gate Hatchery, Klamath River, in 1997; blue bars are adipose fin clipped (Rogue River hatchery mark), red bars are left maxillary notched (the IGH mark), yellow bars are unmarked fish (wild or hatchery).

Table 3. Deviations from random mating genotypic proportions, by locus (*, P<0.05; **, P<0.01; ***, P<0.001) and over all loci (F_{IS}, P), and proportion of loci pairs showing linkage disequilibrium (LD) for 49 samples of coho salmon. NA indicates sample not analyzed because too few individuals were amplified successfully, or the locus was insufficiently polymorphic.

Population	N	*Ots-103*	*Ots-2*	*Iso-Ots2*	*Ots-3*	*One-13*	*P-53*	*Oki-1*	F_{IS}	*P*	LD
KIGHA97a	11								0.009	0.470	2/21
KIGHA97j	15			*					0.019	0.326	4/21
KIGHA97ll	36		*	**		*			0.073	0.012	3/21
KIGHA97nl	19								0.089	0.010	1/21
TRHA97s	17								0.024	0.276	2/21
TRHA97l	77	***		***				*	0.062	0.000	4/21
LRS00-1	85	***		***	***		**		0.080	0.000	9/21
LRS00-2	11			**					-0.014	0.668	6/21
EHOLA97	16	*							0.064	0.132	3/21
EREDS97	92		*	**					0.058	0.000	2/21
EREDA98	22	***							0.056	0.066	2/21
ESPRS99	34								-0.020	0.720	4/21
MATS98-1	73						*	*	0.030	0.112	7/21
MATS98-2	21					*			0.054	0.850	3/21
PUDY98h	32	*					*		0.068	0.022	5/21
PUDY98k	43	**	*						0.070	0.012	9/21
NOYA97	44				**			*	0.064	0.012	1/21
NOYA99	43	*		*					0.076	0.010	1/21
ALBA98	22					***		*	-0.012	0.642	6/21
ALBY98	18						*	*	-0.023	0.706	3/21
RRHA95	33	**							0.057	0.018	3/21
RRHA96	25						*		-0.046	0.914	4/21
RRHY97	7						*		0.120	0.060	5/21
RRGVY97	8		NA					**	-0.032	0.588	0/19
RRGVY98a	70	***	*	***		**	*	***	-0.047	0.978	15/21
RRGVY98b	58	***	*	***		*	*	***	0.019	0.202	15/21
RRGVY00	8		NA					*	-0.257	1.000	0/15
LAGA96	8							NA	-0.062	0.734	0/15
LAGA97	7								0.052	0.194	2/21
LDGA96	9								0.165	0.012	0/21
LDGA97	10							*	0.086	0.106	2/21
LSGA96	5								0.138	0.096	0/21
LSGA97	61								-0.014	0.718	4/21
LSGY98	12								-0.062	0.870	1/21
LSGAA96	25								0.000	0.538	0/21
LSGAA97	3								-0.042	0.672	0/21
LSGAY98	21						*	*	0.000	0.442	7/21
OLEA96	70				***			*	0.105	0.006	6/21
OLEA97	34				*		*	*	-0.010	0.610	3/21
OLEY98	88		*	**					-0.010	0.560	5/21
RWMA97	15	**		**	NA				0.113	0.090	0/18
RWMY98	24	*							-0.002	0.480	0/21
WADY99low	42	**		**	***				0.011	0.356	7/21
WADY99up	17						*		-0.085	0.900	2/21
SCA95	41								-0.051	0.958	5/21
SCA97	57	*		*	**				-0.047	0.966	15/21
SCA98	38	***		*	*	*	**	*	0.099	0.010	11/21
SCY99low	40								-0.028	0.780	7/21
SCY99up	20								-0.030	0.690	2/21

unmarked hatchery fish; left maxillary, which are from the IGH; adipose fin, which are likely from a Rogue River hatchery). For further analysis, the KIGHA97 sample is subdivided into three subsamples of adults greater than 56cm fork length differentiated by marks (adipose, left maxillary marks, no marks) and a jack subsample comprised of 13 left maxillary marked and 2 unmarked fish. F_{IS} is non-significant in two of the four subsamples but remains significant in the KIGHA97ll (left mark, large) and KIGHA97nl (no mark, large) subsamples; LD is reduced to low levels in two of the subsamples but remains moderately large, four and three of 21 pairwise comparisons, for the KIGHA97j (jacks) and KIGHA97ll subsamples, respectively (Table 3). There is significant variance (F_{ST}) in all but one of the six pairwise comparisons among the four subsamples (see Table 6), suggesting that the original sample was an admixture of samples from genetically differentiated subpopulations. Similar adjustments for Wahlund effect were made in the Trinity River Hatchery 1997 adult sample and in the course of adjusting several of the juvenile samples (Table 2, "Criteria" column).

To investigate further the genetic characteristics of samples, we also dropped small samples that could not be combined with other samples in preliminary tests of homogeneity (*i.e.* PUDY98u, RRDS97, RRDS98, RRM98, Table 2), as well as those individuals in juvenile samples with insufficient data for testing relatedness. Of the 1745 samples in Table 2, we were left with 1587

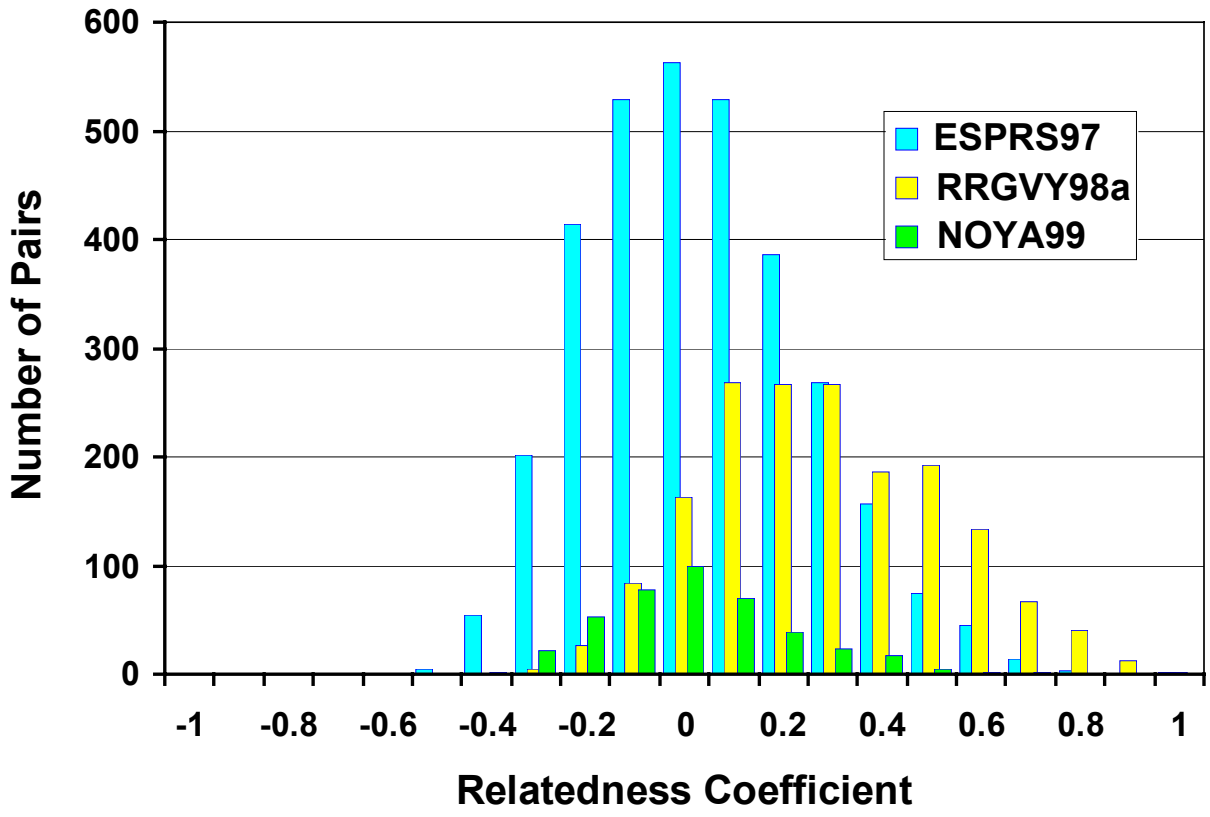

Fig. 3. Relatedness coefficients calculated for all pairwise comparisons among individuals in three samples of coho salmon. The coefficient should have a mean of zero for unrelated individuals. The distribution for the Eel River has a mode at zero (n=3240) but is skewed towards pairs with coefficients above 0.5, the expected relatedness of full-sib pairs. Relatedness of Russian River Green Valley juveniles (n=1711) appears consistent with many full- and half-sib relationships. Even the distribution for Novo River adults (n=406) is skewed towards high relatedness.

individuals in 49 populations for our initial analyses (Table 3). Of the 27 adult samples in Table 3, 9 or 33% have significant F_{IS} ($P < 0.05$) and 9 or 33% have more than three significant pairwise LD tests. All three Scott Creek adult samples have high levels of LD; two have significant excesses and one has a significant deficiency of heterozygotes. By contrast, of the 22 juvenile samples in Table 3, only 4 or 18% have significant F_{IS}, but 13 or 59% have more than three significant pairwise LD tests. High levels of LD and relatedness, such as these are atypical for Pacific salmon populations (*cf* Bartley et al 1992a, b). Juvenile samples with high LD and the Scott Creek adult samples are adjusted for the effects of family structure.

Family structure is evidently strong in the RRGV98 samples, which have very high levels of linkage disequilibrium (15 of 21 loci-combinations) and, in the RRGV98a sample at least, a significant excess of heterozygotes (Table 3). More than 40% of the pairwise tests of the full-sib hypothesis in the RRGVY98a sample are above the α=0.01 level of significance. SIBLINGS is unable, however, to form kinship groups out of the total sample, owing to the apparent complexity of family structure and the large number of discard permutations that has to be checked. We made the problem tractable for SIBLINGS by first subdividing the sample according to the degree of allele sharing among individuals. After determining kinship and sibling groups for the two major branches on the Neighbor-Joining tree (Fig. 4), we find that only 9 individuals are unrelated (the red branches on Fig. 4) and that the rest of the sample can be replaced by 16 sets of full-sib parents and 1 shared parent (Table 4). We similarly adjusted the RRGVY98b sample, which was collected only three months later than the RRGVY98a

Table 4. Samples adjusted for family structure. Min sib size is a SIBLINGS variable; UNR, unrelated; N, intial sample size; Nw, samples with sufficient data; NF, final sample size.

			Min	Number of sibs per group								# Shared	
Population	**N**	**Nw**	**sib size**	**2**	**3**	**4**	**5**	**6**	**N**	**# UNR**	**# Parents**	**Parents**	**NF**
LRS00-1	85	83	3	0	18	4	1	0	0	28	42	2	72
LRS00-2	11	11	3	0	2	0	0	0	0	5	4	0	9
EREDS97	92	89	4	0	0	11	1	1	0	52	24	1	77
ESPRS99	34	34	3	0	6	3	0	0	0	12	18	0	30
MATS98-1	73	73	3	0	0	0	0	0	0	27	0	0	27
MATS98-2	21	21	-	-	-	-	-	-	-	21	0	0	21
PUDY98h	32	32	3	NA	NA	NA	NA	NA	NA	21	0	0	21
PUDY98k	43	43	3	NA	NA	NA	NA	NA	NA	23	0	0	23
ALBY98	18	18	3	0	2	0	0	0	0	12	4	0	16
RRHY97	7	7	2	1	0	0	0	0	0	5	2	0	7
RRGV98a	67	59	2-3	3	4	2	0	0	25	9	16	1	25
RRGV98b	61	61	3	0	11	4	1	0	15, 8	8	23	8	39
LSGAY98	21	21	2	1	0	0	0	0	0	13	4	0	17
OLEY98	88	88	3	0	4	5	0	0	8	53	18	1	72
WADY99low	42	42	3	0	2	5	0	0	7	15	16	0	31
WADY99up	17	17	-	-	-	-	-	-	-	17	0	0	17
SCA95	41	41	2	10	0	1	0	0	0	17	22	0	39
SCA97	57	57	2	4	9	1	0	0	0	16	24	2	42
SCA98	38	38	2	3	5	0	0	1	0	5	18	0	23
SCY99low	40	40	4	0	0	1	0	5	8	12	8	3	23
SCY99up	20	20	-	-	-	-	-	-	-	20	0	0	20
Totals:	908	903											652

sample and likely contains the same families. Owing to the complexity of family structures in these two samples, however, we did not attempt to combine them but left them separate for further analyses of geographic pattern. Substantial adjustments for family structure were made to 16 other juvenile samples besides the RRGVY98 samples and to the three Scott Creek adult samples (Table 4). These adjustments result in a net loss of 257 individuals, owing to the discarding of full sibs and their replacement by reconstructed parents. Juvenile samples from the Mattole River and Pudding Creek could not be satisfactorily adjusted; only unrelated individuals from these samples are used in further analyses.

One of the full-sib families revealed in the RRGVY98a sample comprises 25 individuals (blue branches in Fig. 3). This family provides evidence for the Mendelian inheritance of the microsatellite DNA markers in coho salmon (Table 5). Moreover, knowing the distribution of

Table 5. Fit between observed and expected Mendelian proportions of genotypes at seven microsatellite DNA markers in a full-sib family of 25 juveniles from Green Valley, Russian River.

Locus	Inferred P1 genotype:	Inferred P2 genotype:	F_1 Genotypes	Obs.	Exp.	Total	χ^2	P
Ots-103	224,236	228,232	224,232	5	6.25	25	2.040	0.564
			224,228	4	6.25			
			228,236	8	6.25			
			232,236	8	6.25			
Ots-2	180,184	180,188	180,180	6	6.25	25	3.320	0.345
			180,184	4	6.25			
			180,188	5	6.25			
			184,188	10	6.25			
iso-Ots-2	205,247	213,227	205,213	5	6.25	25	2.040	0.564
			205,227	4	6.25			
			213,247	8	6.25			
			227,247	8	6.25			
Ots-3	145,153	145,157	145,145	8	6	24	1.667	0.644
			145,157	7	6			
			145,153	5	6			
			153,157	4	6			
One-13	197,209	197,219	197,197	10	6.25	25	3.320	0.345
			197,209	5	6.25			
			197,219	6	6.25			
			209,219	4	6.25			
P-53	181,181	173,181	181,181	11	11.5	23	0.861	0.835
			173,181	12	11.5			
Oki-1	092,100	096,112	092,096	9	6	24	3.000	0.392
			092,112	7	6			
			096,100	4	6			
			100,112	4	6			

family sizes within the Green Valley sample, we can estimate the effective number of breeders (N_b) in this tributary, following the methods of Hedrick et al. (2000). The estimated N_b is 10, suggesting that this population is propagated by few adults and may be undergoing rapid genetic drift.

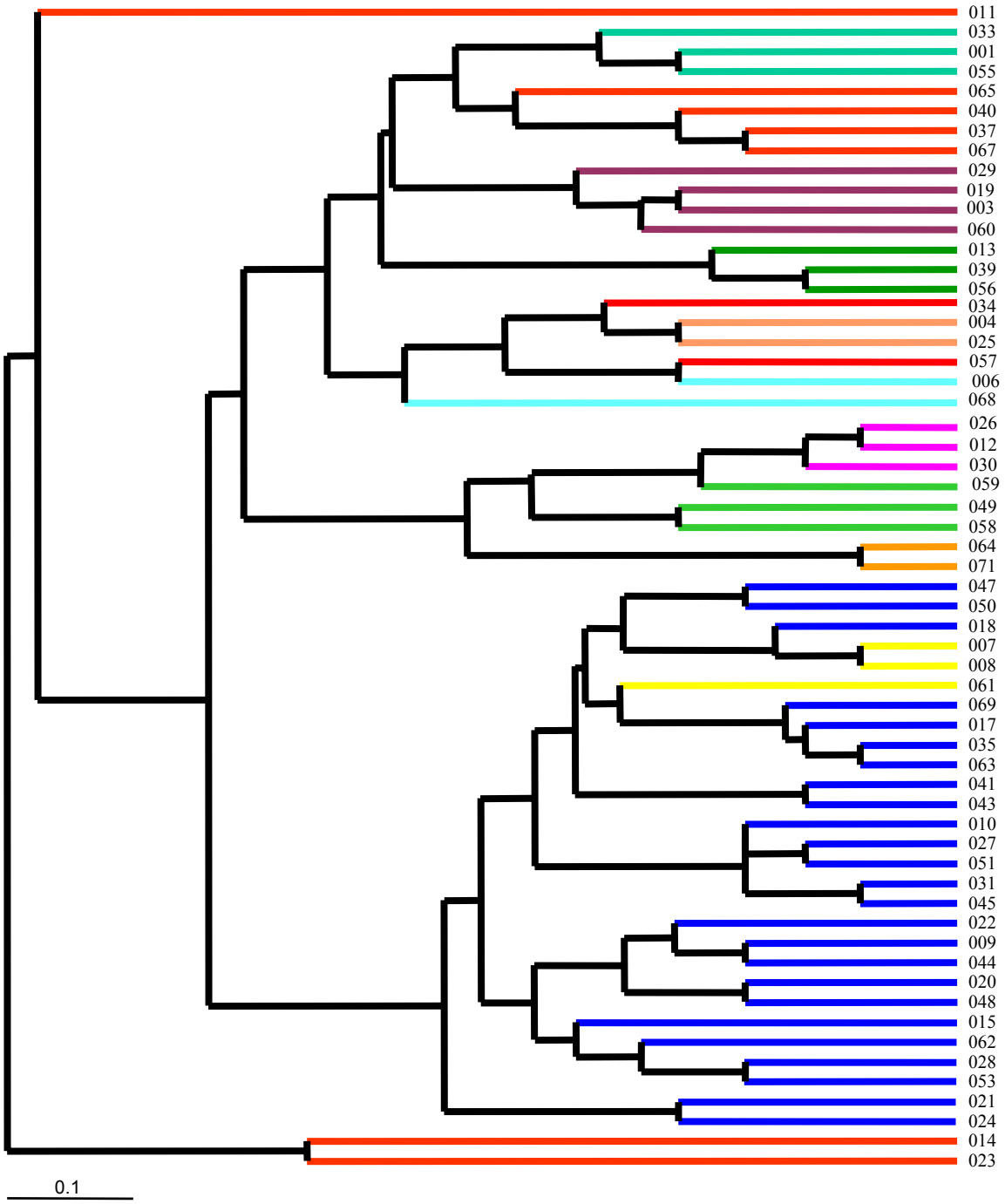

Fig. 4. A Neighbor-Joining tree based on allele-sharing among the 59 individuals in the RRGVY98a juvenile sample. Red branches lead to individuals that are not significantly related to any other individual; other colors depict full-sib groups formed by SIBLINGS (see text).

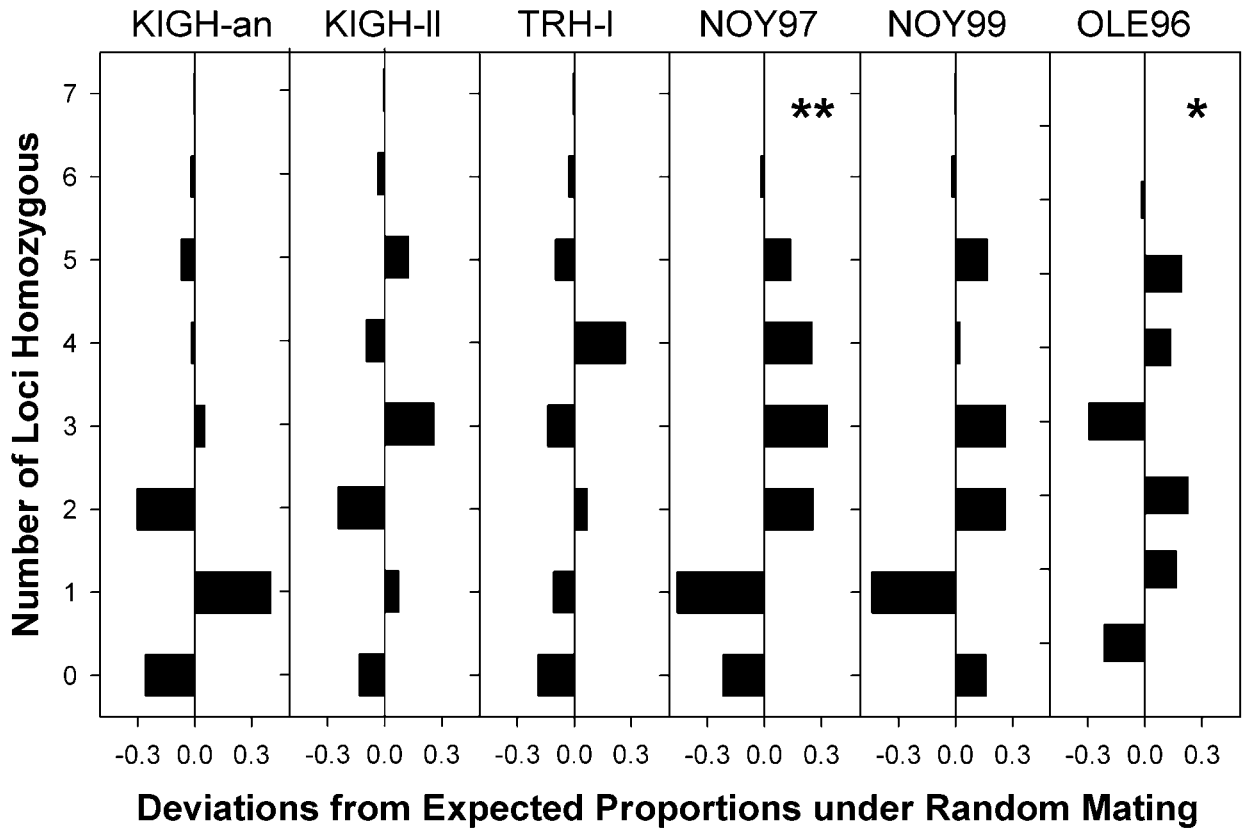

Fig. 5. Deviations between observed and expected proportions of individuals in eight multi-loci genotypic categories, from zero through seven microsatellite DNA markers homozygous per individual, for six adult populations with significant F_{IS} (see Table 7). Expectations are derived from binomial distributions, assuming random mating (see text). Deviations are expressed as the difference between observed and expected numbers divided by the relevant sample size; a square-root transformation (conditional on sign of the deviation) was used to make small deviations visible. Two populations, NOYA97 and OLEA96, show statistically significant deficiencies of individuals with no homozygous loci and excesses of individuals with two to five homozygous loci.

Finally, in addition to the general departures from random mating expectations that we document above, we find significant excesses of multi-loci homozygotes within two of six adult samples examined, NOYA97 and OLEA96 (Fig. 5). This analysis is done on all individuals scored for at least six of the seven markers; individuals are categorized into eight genotypic classes, from individuals homozygous for none of the markers (or heterozygous at all seven markers) to those homozygous for all seven markers (or heterozygous at none of the markers). The expected number of individuals in each category is computed as the product of the probabilities of homozygosity at each locus (from Appendix 1); the probabilities of all possible genotypes are pooled into homozygosity classes and multiplied by the sample size. Significant excesses of individuals homozygous for three to five or six of these highly polymorphic markers in two adult populations suggests that these coho salmon populations are not in random mating equilibrium.

Genetic diversity among coho salmon populations

We next test for heterogeneity among samples within 14 drainages or sites in which multiple samples, either spatial or temporal, were collected (Table 6). Heterogeneity is tested by the significance of F_{ST} among all samples within each drainage or site (indicated by "none" under

the "Sample excluded" column in Table 6. If the initial test is significant ("P-value" < 0.05, Table 6), then samples are removed sequentially, one at a time, with re-testing of the heterogeneity at each step, until a homogeneous pool remains or until all samples are shown to be significantly different from one another (those excluded are tested against each other for homogeneity). Seven pools of homogeneous populations are formed in this manner (underlined in Table 6). Pooling maximizes the sample size within sites and reduces the number of populations for analysis of genetic distance among sites, drainages, and ESUs. The heterogeneity of jacks and older adults in the KIGHA samples suggests significant variance among year classes. The homogeneity of samples from Lagunitas Creek from different year classes and tributaries contrasts with the heterogeneity of samples in other drainages of the Central California ESU.

Table 6. Homogeneity of samples within drainages or sites, as determined by sequential exclusion of samples from the initial pool, with re-testing of the significance of F_{ST} by random permutation of individuals among samples remaining in that pool. Samples are pooled if the significance of F_{ST} is greater than 0.05.

Drainage, site(s)	**pool, if formed**	**Pool Size**	**Sample excluded**	F_{ST}	***P*-Value**
Klamath River, Iron Gate Hatchery		4	None	0.0285	0.000
		3	KIGHA97ll	0.0188	0.000
	Pool: KIGHA97a, KIGHA97nl	2	KIGHA97j	0.0044	0.306
Trinity River, Trinity River Hatchery		2	None	0.0131	0.020
Little River (Humboldt Co.), Little River Delta					
	pool: LRS00-1, LRS00-2	2	None	0.0031	0.326
South Fork Eel River		4	None	0.0285	0.000
		3	EHOLA97	0.0232	0.000
		2	ESPRS99	0.0088	0.022
Mattole River, Mattole River Delta					
	pool: MATS98-1, MATS98-2	2	None	0.0048	0.204
Pudding Creek Pudding Creek					
	pool: PUDY98h, PUDY98k	2	None	-0.0062	0.826
South Fork Noyo, Egg Taking Station		2	None	0.0115	0.000
Albion River, Mainstem and Marsh Creek		2	None	0.0283	0.002
Russian River, Warm Springs Hatchery and Green Valley		7	None	0.0486	0.000
		6	RRGV00	0.0418	0.000
		5	RRGV97	0.0373	0.000
		4	RRGV98b	0.0353	0.000
	pool: RRHA95, RRHA96, RRHY97	3	RRGV98a	0.0089	0.080
Lagunitas Creek, Devils Gulch, San Geronimo, S. G. Arroyo		10	None	0.0124	0.002
	pool: samples from LAG, LDGA, LSG, LSGA	9	LSGAY98	0.0057	0.100
Olema Creek, Mainstem and Blueline		3	None	0.0092	0.000
	pool: OLEA97, OLEY98	2	OLEA96	-0.0001	0.560
Redwood Creek (Marin Co.), Mainstem		2	None	0.0978	0.000
Waddell Creek, Mainstem		2	None	0.0559	0.000
Scott Creek, Hatchery, Mainstem, Upper Fork, Big and Mill Creeks		5	None	0.0170	0.000
		4	SCY99up	0.0134	0.000
		3	SCY99low	0.0094	0.018
	pool: SCA97c, SCA98c	2	SCA95c	0.0021	0.538

After partitioning admixed samples, adjusting the composition of samples having family structure, and then pooling homogeneous samples within sites, we are left with 33 populations for analysis of genetic diversity among populations. The level of departure from random mating expectations remains striking in these samples. Eight samples, including seven adult samples, still show significant F_{IS} and numerous deviations from random mating genotypic proportions at single loci; five of these samples and 10 others with non-significant F_{IS} have high levels of linkage disequilibrium (LD > 2; Table 7). The frequencies of all alleles observed for each of the seven markers, in each of these 33 populations, are given in Appendix 1, together with observed and expected heterozygosities, F_{IS} values, and the significance of F_{IS}. F_{ST} between pairs of populations within the three ESUs are given in Table 8; all are significant except that between the RRGVY98 samples.

Table 7. Deviations from random mating genotypic proportions, by locus (*, P<0.05; **, P<0.01; ***, P<0.001) and over all loci (F_{IS} and associated P), and proportion of di-loci pairs showing linkage disequilibrium (LD) for 33 populations of coho salmon formed after adjustment for family structure and pooling of homogeneous samples within drainages.

Population	**N**	***Ots-103***	***Ots-2***	***iso-Ots2***	***Ots-3***	***One-13***	***P-53***	***Oki-1***	F_{IS}	***P***	**LD**
KIGHA97an	30								0.061	0.032	1/21
KIGHA97j	15			*					0.019	0.362	4/21
KIGHA97ll	36		*	**		*			0.073	0.028	3/21
TRHA97s	17								0.024	0.266	1/21
TRHA97l	77	***		***				*	0.062	0.004	3/21
LRS00	81		*	*					-0.018	0.818	5/21
EHOLA97	16	*							0.064	0.142	2/21
EREDS97	77			*					0.001	0.452	0/21
EREDA98	22	***							0.056	0.086	2/21
ESPRS99	30		*						-0.057	0.942	3/21
MATS98	48							*	0.017	0.278	2/21
PUDY98	44	*	*						0.067	0.016	5/21
NOYA97	44				**			*	0.064	0.014	1/21
NOYA99	43	*						*	0.076	0.002	2/21
ALBA98	22					***			-0.012	0.598	6/21
ALBY98	16						*	**	0.055	0.106	1/21
RRHA	65								0.025	0.118	8/21
RRGVY97	8							**	-0.032	0.628	0/19
RRGVY98a	25					*			-0.006	0.618	1/21
RRGVY98b	39								-0.049	0.952	7/21
RRGVY00	8							*	-0.257	1.000	0/15
LAG	140					*			0.014	0.186	5/21
LSGAY98	17		*					*	-0.023	0.712	1/21
OLEA96	70				***	*	*	*	0.105	0.000	6/21
OLEA9798	106				*		*	*	0.021	0.142	6/21
RWMA97	15	**		***					0.113	0.120	0/18
RWMY98	24	*							-0.002	0.492	0/21
WADY99lo	31			*					-0.021	0.696	3/21
WADY99up	17						*		-0.085	0.908	2/21
SCA95c	39								-0.051	0.934	1/21
SCA9798c	65	*		***	***		***	**	0.210	0.000	4/21
SCY99low	23								-0.081	0.976	3/21
SCY99up	20								-0.030	0.698	2/21

Table 8. Pairwise F_{ST}, a standardized measure of allele frequency variance between populations, for samples of coho salmon within three geographical regions corresponding to federal and state ESUs (A-C). All values are significant by permutation tests, except for the F_{ST} between the two 1998 samples of juveniles from Green Valley Creek, Russian River, in panel B.

A. Samples of coho salmon from the Southern Oregon / Northern California ESU.

Population	KIGH97j	KIGH97l	TRHA97s	TRHA97l	LRS00	EHOLA97	EREDS97	EREDA98	ESPRS99	MATS98
KIGHA97a	0.025	0.024	0.029	0.036	0.041	0.113	0.097	0.118	0.095	0.112
KIGHA97j		0.047	0.046	0.055	0.030	0.115	0.103	0.118	0.090	0.101
KIGHA97l			0.031	0.021	0.061	0.154	0.129	0.155	0.121	0.141
TRHA97s				0.013	0.054	0.113	0.103	0.122	0.104	0.136
TRHA97l					0.069	0.137	0.126	0.147	0.113	0.145
LRS00						0.083	0.077	0.093	0.077	0.071
EHOLA97							0.038	0.051	0.041	0.064
EREDS97								0.009	0.028	0.063
EREDA98									0.043	0.069
ESPRS99										0.070

B. Samples of coho salmon from the Central California ESU.

Population	NOY97	NOY99	ALBA98	ALBY98	RRH	RRGV97	RRGV98a	RRGV98b	RRGV00	LAG	LSGA98	OLE96	OLE9798	RWM97	RWM98
PUDY98	0.028	0.032	0.022	0.011	0.020	0.066	0.068	0.064	0.103	0.017	0.040	0.038	0.033	0.034	0.085
NOYA97		0.012	0.026	0.019	0.009	0.066	0.064	0.065	0.079	0.026	0.041	0.050	0.044	0.042	0.098
NOYA99			0.025	0.027	0.006	0.043	0.079	0.075	0.100	0.019	0.045	0.047	0.035	0.051	0.090
ALBA98				0.028	0.026	0.058	0.092	0.085	0.127	0.026	0.055	0.036	0.026	0.020	0.083
ALBY98					0.020	0.075	0.052	0.051	0.108	0.014	0.033	0.040	0.037	0.063	0.110
RRHA						0.048	0.050	0.048	0.078	0.012	0.030	0.035	0.030	0.042	0.076
RRGVY97							0.079	0.093	0.170	0.053	0.083	0.056	0.048	0.143	0.118
RRGVY98a								0.002	0.113	0.061	0.069	0.057	0.073	0.095	0.146
RRGVY98b									0.096	0.060	0.063	0.060	0.073	0.091	0.142
RRGVY00										0.109	0.101	0.134	0.132	0.160	0.211
LAG											0.027	0.015	0.009	0.053	0.068
LSGAY98												0.040	0.037	0.085	0.093
OLEA96													0.010	0.053	0.101
OLEA9798														0.073	0.064
RWMA97															0.098

C. Samples of coho salmon from South of San Francisco, Central California

Population	WADY99u	SCA95c	SCA9798c	SCY99low	SCY99up
WADY99low	0.056	0.014	0.019	0.017	0.046
WADY99up		0.074	0.076	0.041	0.120
SCA95c			0.013	0.017	0.026
SCA9798c				0.020	0.024
SCY99low					0.024

We conclude our analysis of diversity among coho salmon populations with a series of phylograms or trees depicting genetic distances among samples. Three trees are presented, one for 49 unadjusted samples with 15 or more individuals (Fig. 6), one for the 33 samples formed after adjustment and homogeneity testing (Fig. 7), and the last for a subset of 27 samples (Fig. 8). In all of these trees, genetic distance is measured by Cavalli-Sforza and Edwards (1967) chord distance. The significance of nodes in these trees is tested by bootstrap analysis, in which genetic distance is estimated 1000 times among samples, using a random collection of markers, producing 1000 trees. A node is considered significant if it is recovered in more than half (500) of the bootstrap trees; bootstrap values greater than 500 are placed on the tree.

The tree, showing the relationships among 49 unadjusted samples (Fig. 6), though complex and noisy, shows considerable congruence of genetic diversity and geography. The samples from South of San Francisco (SSF) form a tight cluster. A significant node separates the Central California (CC) ESU from the Southern Oregon / Northern California (SO/NC) ESU. Samples from the SO/NA ESU are found in two significant clusters, with the exception of the Little River (Humboldt Co,) smolts, which cluster with the CC ESU. Scattered over and even outside of these clusters are the samples from Green Valley Creek of the Russian River watershed and from Redwood Creek in Marin County. Although several external nodes separating samples from the CC ESU are supported, few of the deeper nodes separating CC samples are supported.

The tree, showing the relationships of the 33 samples formed after adjustments for admixture and family structure and pooling of homogeneous samples within drainages and sites, suggests an even greater congruence of genetics and geography (Fig. 7). The SSF ESU and a large proportion of the CC ESU form significant clusters, though the node separating these two clusters is not supported. Two groups of SO/NC samples are evident, those from the Klamath / Trinity drainages, now including the Little River smolts, (though the clustering of only three of these is significant) and those from the Eel and Mattole Rivers at the southern end of the SO/NC ESU, a cluster which is recovered in 78% of the bootstrap trees. Green Valley and Redwood Creek samples remain obvious outliers on this tree. Removal of these outliers yields the final tree (Fig. 8), which strongly supports the recognized ESUs for coastal coho salmon. Significant clusters are found within each of the SO/NC, CC and SSF ESUs. Still, the node separating the CC and SSF clusters is not supported by the bootstrap analysis. Likewise, the separation of Klamath / Trinity samples from Eel / Mattole samples is not supported on this unrooted tree.

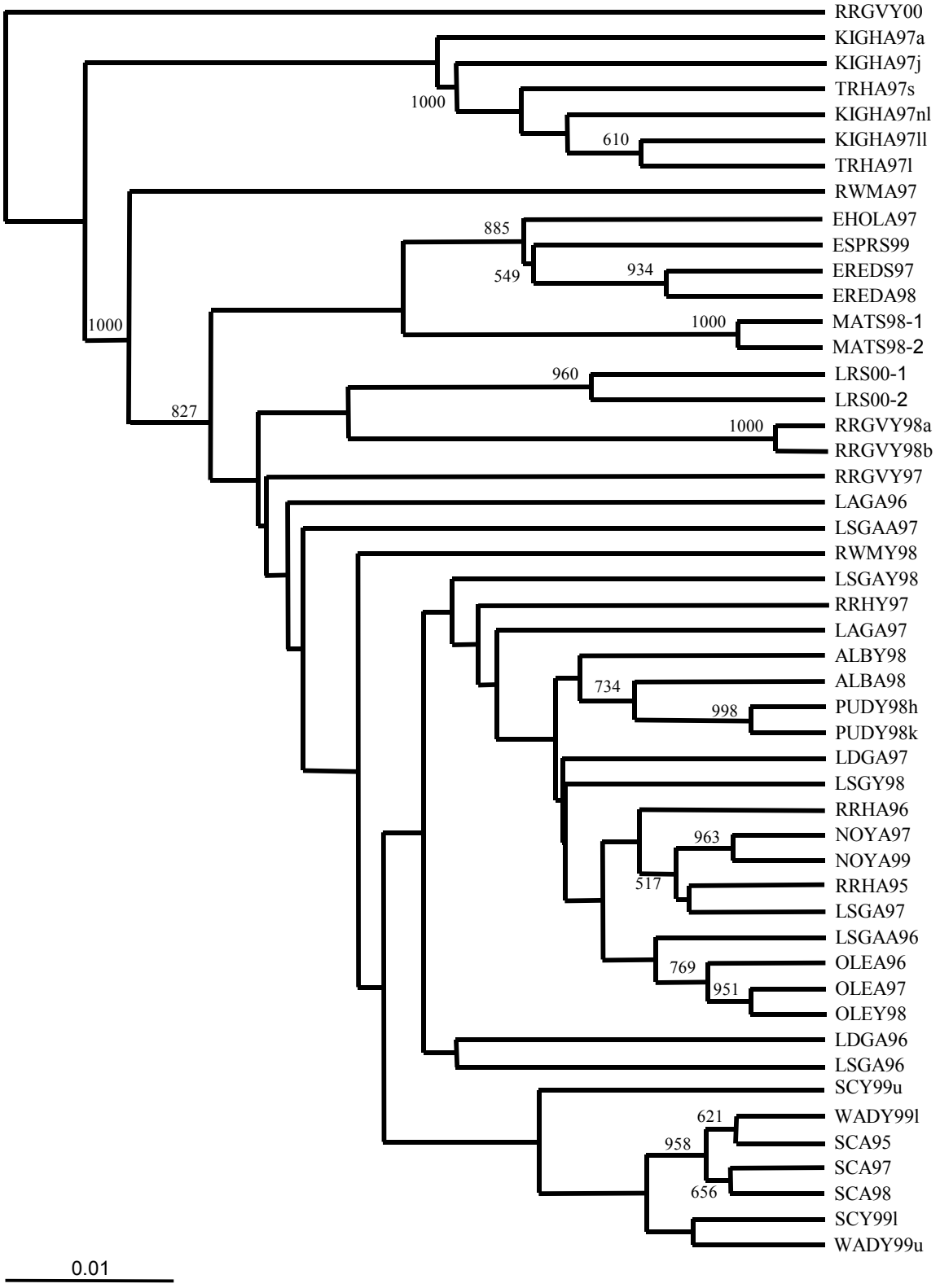

Fig. 6. Unrooted UPGMA phylogram, showing chord distances (Cavalli-Sforza and Edwards 1967) among 49 California coho salmon populations of sample size greater than 15 individuals. Nodes supported by bootstrap values greater than 500 out of 1000 are shown.

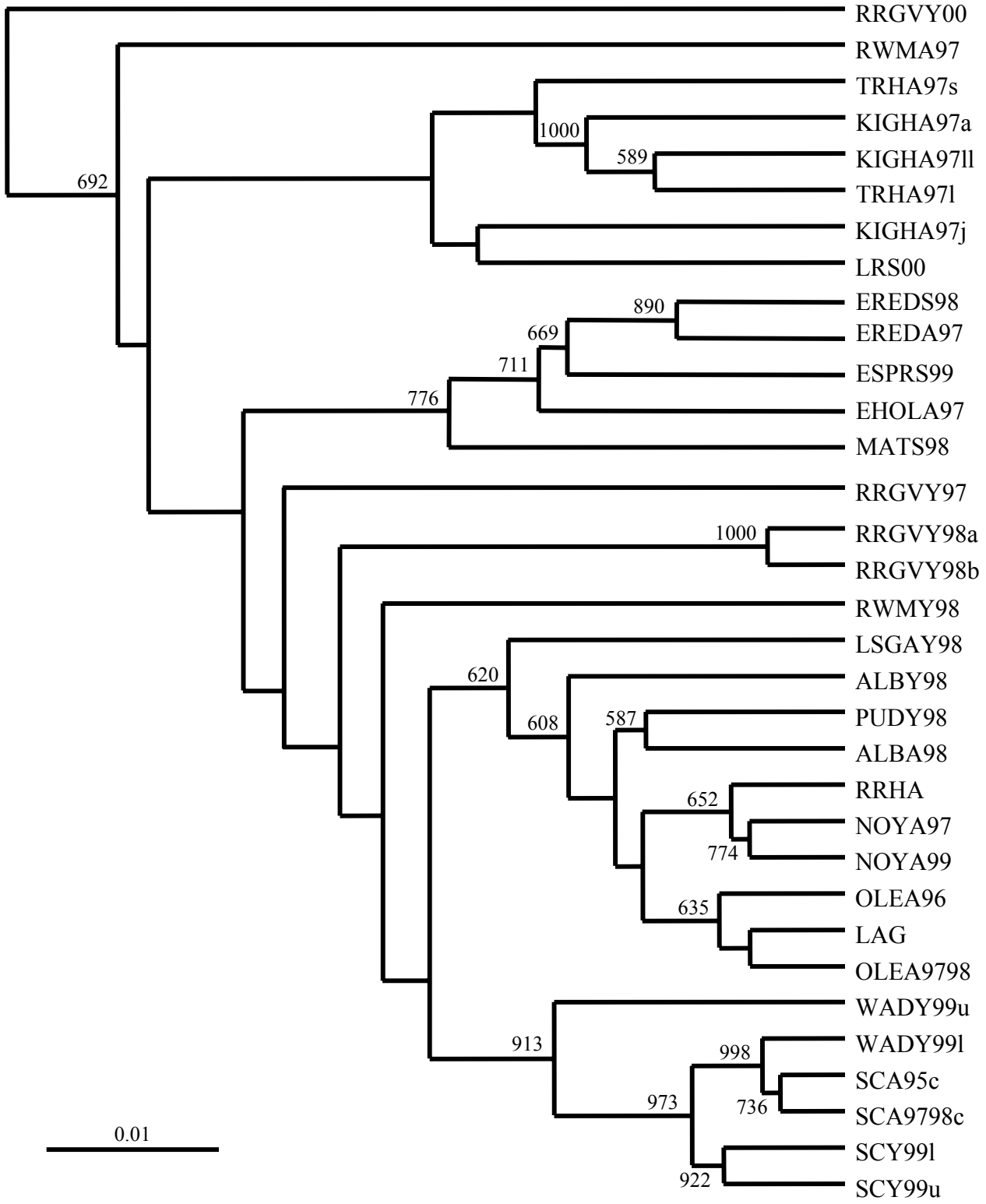

Fig. 7. An unrooted UPGMA phylogram, showing chord distances (Cavalli-Sforza and Edwards 1967) among 33 California coho salmon populations formed after adjustments for admixture and family structure and pooling of homogeneous samples within drainages and sites. Bootstrap values greater than 500 out of 1000 are shown.

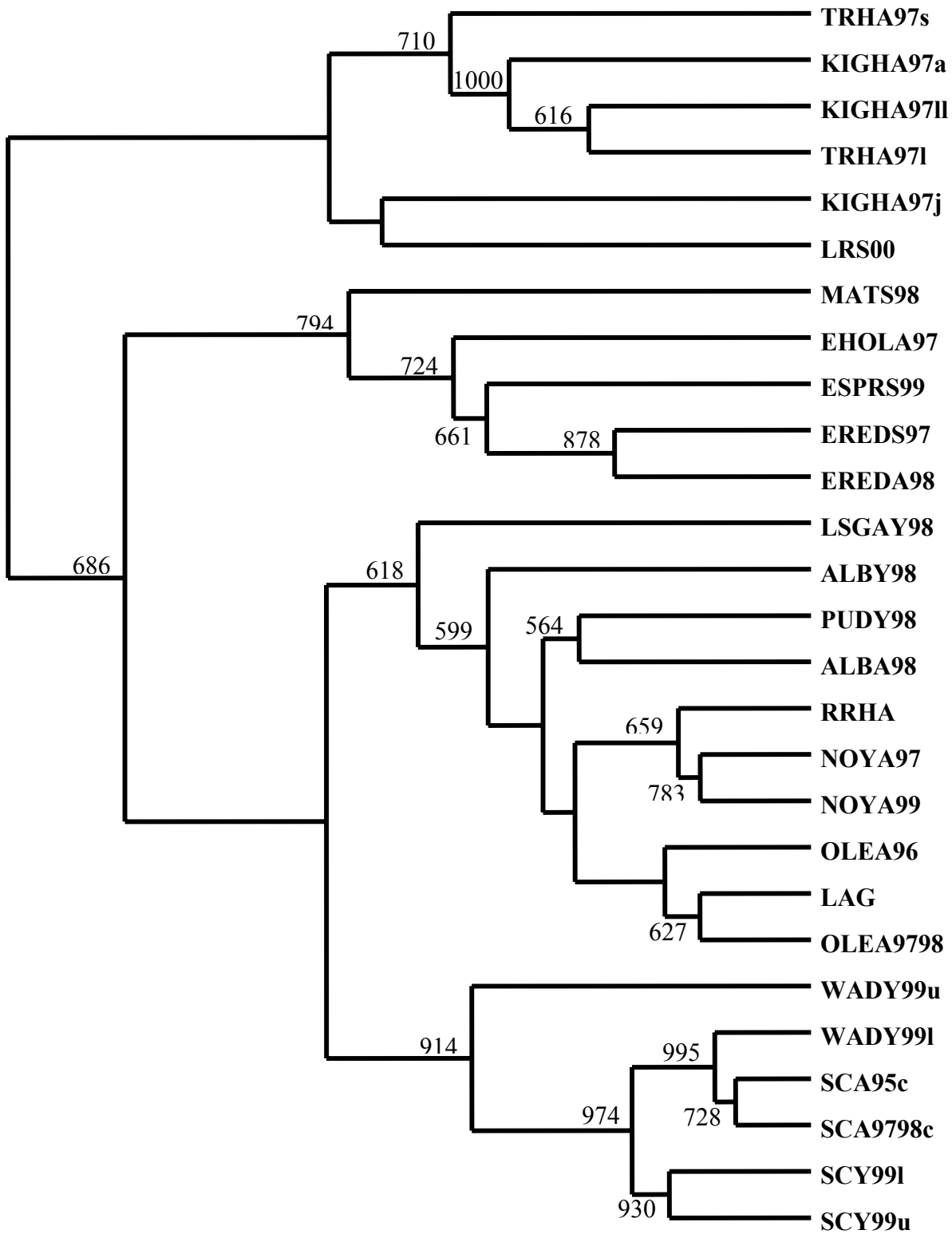

Fig. 8. An unrooted UPGMA phylogram, showing chord distances (Cavalli-Sforza and Edwards 1967) among 27 California coho salmon populations remaining after removal of Green Valley and Redwood Creek outliers on the tree in Fig. 8. Bootstrap values greater than 500 out of 1000 are shown.

Discussion

Progress towards research goals and deliverables

We contributed new knowledge relevant to all of the specific tasks in the scope for work:

1. We did determine relatedness in samples comprised of juveniles. Indeed, we went much further than that, adjusting most of these samples to correct them for family structure and to salvage them for use in describing the genetic diversity of coho salmon in Northern California.

2. We determined that temporal genetic variation among year classes is significant but smaller in magnitude than the geographical component of genetic structure.

3. We estimated significant genetic divergence among populations that was congruent with geographical distance and supportive of the present State of California ESU designations. We estimated that the effective breeding number for the Green Valley Creek population in 1998 was about 10, which raises concerns about the hatchery-based recovery program that is being based partially on captive broodstock obtained from this site.

4. We were unable to acquire historical samples to determine genetic change between historical and extant coho populations. Nevertheless, the phylogeographic structure of coho diversity suggests either that stock transfers have not erased genetic differences accumulated over evolutionary time or that the diversifying effects of genetic drift within relictual coho populations may be keeping pace with whatever homogenization has been or is being effected by hatchery practices.

5. We showed that independent environmental and biological data measured during the sampling process could be used to partition samples into subsamples that conformed better to random mating genetic equilibrium.

We elaborate on these points in the following sections.

Polymorphism of microsatellite DNA markers in coho salmon of California

We selected microsatellite DNA markers that had been developed for other species of Pacific salmon for use in the study of genetic diversity within and among coho salmon populations in California. These markers proved to be highly polymorphic, with average heterozygosities per individual ranging from 54% in a sample of juveniles from Waddell Creek to 80% in a sample of smolts from the Little River in Humboldt County. All markers are polymorphic in all populations, with the exception of *Ots-2*, which is fixed in the small sample of seven individuals collected from Green Valley Creek in 2000. The average number of alleles, which is highly dependent on sample size, ranges from 3.4 in this same Green Valley sample to 12.7 in the large pool of homogeneous samples from Lagunitas Creek. The polymorphism of the microsatellite DNA markers contrasts sharply with the low levels of protein polymorphism detected in these same coho salmon populations more than a decade ago by (Bartley et al 1992a), who reported polymorphism at only 23 of 45 loci (51%) and an average heterozygosity of only 2.7%.

The variability of these microsatellite markers makes possible the resolution of details concerning the genetics of coho salmon populations that were not possible to resolve by protein markers.

Departures from random mating equilibrium in California coho salmon populations

The distribution of genotypes within natural populations of Pacific salmon generally conform to those expected under random mating. This generalization is supported by thirty years of study of protein polymorphisms in these species (*e.g.* Bartley et al 1992b) and has been further substantiated in recent times by investigations of DNA polymorphisms (*e.g.* Banks et al 2000). Even though Bartley et al (1992a) found low variation throughout the region in protein markers, genotypic proportions at the few markers that were polymorphic did conform to those expected under random mating, and only 6.7% of the pairwise combinations of loci showed significant linkage disequilibrium.

In our study, we find widespread significant departures from random mating proportions of genotypes and more than 10% of pairwise combinations of loci showing linkage disequilibrium in nearly half of the samples formed after corrections for admixture and family structure. Part of this deviation could be attributable to residual family structure in some juvenile samples, despite our attempts to adjust for this. That family structure would be so much stronger in coho salmon populations than in samples of juvenile Chinook salmon that we were previously successful in adjusting (Banks et al 2000) suggests that the effective numbers of breeders may be quite small. Indeed, we estimate that the effective number of breeders in Green Valley Creek in 1998 may have been less than 10. Nevertheless, family structure is unlikely to explain departures from random mating genotypic proportions in adult populations, with the potential exception of small hatchery populations, such as the one in Scott Creek.

Part of the widespread deviations from random mating equilibria might be attributable to residual fine-scale Wahlund effects, *i.e.* deficiencies of heterozygotes owing to admixture in collections of individuals from populations that are genetically differentiated over small spatial scales. This seems unlikely to explain deviations in samples collected over small spatial or temporal scales, however. On the other hand, the size and significance of these departures, particularly in adult populations, suggests that these depressed populations may be experiencing inbreeding, owing to very small numbers of spawners. The finding of significant excesses of highly homozygous multi-loci genotypes in some adult populations is consistent with inbreeding. The implication of this finding is that inbreeding depression, owing to the deleterious effects of recessive lethal mutations that become homozygous upon inbreeding, just like these DNA markers have become homozygous, may be contributing to the decline in fitness of coho salmon populations in Central California.

Use of juvenile samples

In most juvenile samples, many pairs of individuals show statistically significant odds of being full brothers and sisters. Because such samples yield biased and inaccurate estimates of the genetic diversity in the adult spawning population, population geneticists in the past have avoided using juvenile samples. Nevertheless, the depressed state of coho salmon populations often precludes collections of sufficient numbers of adults. Juveniles, on the other hand, are more readily available in large numbers. Of the 57 collections available for this study, 27

comprised juveniles. To salvage these important samples for genetic analysis, we applied methods pioneered in our lab for adjusting samples for family structure to derive unbiased and accurate estimates of adult allele frequencies. Related individuals are either removed and replaced with reconstructed parents or simply removed from a sample, resulting in a sample that is smaller but usually closer to, if not in random mating equilibrium. Moreover, many these adjusted samples prove to be homogeneous with other samples from the same watershed, whereas the original sample was not. In the final phylogram used to infer the geographic distribution of genetic diversity in this study (Fig. 8), 11 of 27 populations are adjusted juvenile samples and two others are homogeneous pools that include adjusted juvenile samples. The substantial effort that juvenile samples require is repaid by the more robust inference about geographic pattern that is made possible by their use.

Temporal variation

Temporal samples or comparisons of year classes were available for seven sites: Klamath IGH, Noyo River, Russian River, Olema Creek, Lagunitas Creek, Redwood Creek (Marin Co.) and Scott Creek. Many temporal comparisons reveal significant variation. Jacks and adults were significantly different in the KIGHA samples. NOYA97 and NOYA99 were heterogeneous. The Russian River, Warm Springs Hatchery samples (RRHA95, RRHA96, RRHY97) were homogeneous but the Green Valley Creek samples were heterogeneous. The OLEA97 and OLEY98 samples were homogeneous but significantly different from OLEA96. Samples from four different years and several tributaries of Lagunitas Creek were homogeneous; only the LSGAY98 sample had to be excluded from the homogeneous LAG pool. The two samples from Redwood Creek could not be combined, even though they should represent samples from spawners (RWMA97) and offspring (RWMY98); however, these samples are outliers on the phylogram, which suggests that they are aberrant for some unknown reason. Finally, two of the Scott Creek adult samples were combinable but distinct from the third sample and from the partitioned sample of naturally spawned juveniles collected in 1999. Again, the striking deviations from random mating equilibria in these samples complicate the interpretation of temporal differences. Although temporal samples are often statistically heterogeneous, they do generally cluster closest on the phylograms, which suggests that temporal variation, though often significant is of smaller magnitude than the geographic component of genetic structure in these coho salmon populations.

Congruence of genetic diversity and geography

Bartley et al (1992a), using protein markers with low levels of polymorphism, found little congruence between genetic and geographic distances among coastal California populations of coho salmon, although they did find evidence of divergence on a larger geographic scale, between Oregon and California stocks. In our study of microsatellite DNA variation, we find genetic distances among coho samples correlating well with geographic distances among populations and strongly supporting the existing ESU designations. Given the long history of stock transfers within California and between California and other Pacific Northwest states, this congruence of genetics and geography is surprising. Two, not necessarily mutually exclusive hypotheses could explain the present spatial diversity of coho stocks in Northern California. Either the stock transfers have not "taken," owing to reduced fitness of salmon introduced via hatcheries, or the rate of population divergence has accelerated with the radical decline in the

abundance of coho salmon in the region, owing to an acceleration in genetic drift and a reduction in the absolute number of migrants between watersheds.

The implications of using Green Valley Creek coho salmon for recovery of Russian River stocks
Our finding of strong family structure in juvenile samples from Green Valley Creek has implications for the hatchery-based program aimed at recovering coho salmon populations in the Russian River watershed. Juveniles collected from Green Valley in 2001 are being reared at the Warm Springs Dam hatchery to serve as broodstock for hatchery supplementation. Because this population appears to be propagated by small numbers of breeders, perhaps as few as 10, it is quite likely that many of the juveniles collected from this creek are related to each other. Use of these fish as broodstock could accelerate inbreeding, leading to declines in population fitness and a decreasing chance of population recovery. In the 2001 annual progress report, we suggested that microsatellite genotyping could be used to help identify related broodstock and to minimize inbreeding. Our attempt to adjust for family structure based on seven microsatellite markers suggests that the reliable identification of relatives could prove very difficult unless based on a large number of DNA markers. Even if kinship could be reliably identified and inbreeding minimized, this small population appears to be anomalous and unrepresentative of the Central California ESU (see Figs. 6 and 7).

ASSESSING GENETIC VARIATION IN STEELHEAD POPULATIONS

Our scope of work listed the following objectives for steelhead: 1) to investigate the genetic consequences of migration barriers on resident populations, 2) to investigate the genetic relationship between residents and anadromous steelhead in the same watershed, 3) to investigate the genetic relationship between tributaries of the Russian River that have and have not received hatchery transplants, 4) to determine the genetic relationship of summer and winter steelhead in the Klamath and Eel rivers, which maintain large population sizes, and apply this to putative summer run stocks in the Russian River, 5) to assess whether there is evidence for widespread hatchery influence in ocean-going salmon throughout the Russian River watershed.

We began an archive of steelhead tissue samples for this research, but once the California coastal steelhead was listed federally as a threatened species, we did not have a permit to collect. Moreover, shortly after the initiation of this contract, Dr. Carlos Garza, a geneticist hired by the National Marine Fisheries Service, Santa Cruz laboratory in 2000, began a large survey of genetic variation in steelhead using microsatellite DNA markers. Rather than duplicate his effort, we focused on an alternative, though risky approach to finding markers in candidate genes for run timing differences, which was described in the 2001 annual report. This approach was discontinued after Carolyn Greig left the project for a position in Britain. The material developed by Carolyn was transferred to Dr. Michael Banks, who hopes to pursue this approach with Chinook salmon. No further effort on steelhead was made in the second year, as greater emphasis was placed on the objectives for coho and Chinook salmon.

STOCK ORIGIN ESTIMATES FOR CHINOOK JUVENILES CAPTURED IN THE RUSSIAN RIVER

This contract supported the development of baseline genetic data for Russian River Chinook salmon, permitting comparisons to Central Valley, Klamath, and Eel River stocks. The specific tasks in our scope for work were: 1) to establish a baseline of Chinook populations from Sonoma and Mendocino Counties and compare those populations to known stocks, 2) to determine the

relationship between Russian River and other coastal Chinook populations by including both extant and historical population samples from drainages such as the Eel River, 3) to continue to use and improve species identification tests developed in the first contract. Data relevant to the first two tasks is presented in this report. The species identification test, which was described in the 2001 annual progress report and by Greig et al (2002), did reveal the presence of Chinook salmon in Lagunitas Creek and did enable us to eliminate non-coho from three samples.

In a previous progress report (April 1999), we suggested that juvenile Chinook samples captured in the Russian River might not be descendants from Warm Spring Hatchery stock. We reassessed this result using seven microsatellite markers (*Ots*-2, 3, 9, 10, 104, 107 and *Oneμ* -13) and increased the sample number of both Russian River juveniles (n=78) and Warm Springs Hatchery sample sets. These results of this survey were presented in a July 2000 report, which was completed just at the beginning of this contract. Data from five river systems were analyzed: Klamath River, Trinity River, Warm Springs Hatchery (two sample sets derived from Eel River stocks), Russian River juveniles and Central Valley (winter, spring: Butte Creek, spring: Mill and Deer Creeks, fall and late fall). Genetic distance among sites show Russian River juveniles clustering with the Central Valley spring, fall and late fall populations rather than with either the two Warm Spring Hatchery populations or the Klamath/Trinity cluster. In the 2001 annual report, we cautioned that these results would have to be checked because a volunteer had initially scored the gels for the Russian River juvenile sample, and we had not yet tested and corrected for kin structure within this sample. A third problem was that the samples from the Warm Springs Hatchery showed significant departure from random mating genotypic proportions, the causes of which would need to be resolved, if possible, before their relationship to other Chinook stocks could be reliably ascertained.

Materials and Methods

We completed microsatellite analyses on 449 fish in order to assess the affinity of Russian River Chinook with other coastal Chinook populations, primarily from the Eel River (Table 9). For this report, we added 86 adults from nine mainstem Eel River samples collected by Scott Harris, CDFG. For the Russian River, we added 8 adults from Forsyth Creek and 82 smolts from Mirabel, collected by Harris and SCWA, respectively (Table 9). We compared these samples to samples of Chinook salmon from the Central Valley, which were studied by Banks et al. (2000). We also used data from samples of Chinook salmon collected in the Santa Clara Valley by the Santa Clara Valley Water District and from Chinook samples from the Klamath River, which were analyzed by Dr. Michael Banks (Oregon State University, personal communication).

DNA was extracted from samples using the Puregene™ DNA isolation kit (Gentra System), a superior extraction procedure to Chelex 100 (BioRad) particularly when extracting tissue from degraded carcasses. DNA extractions were performed using 96-well trays. We performed multiple extracts and amplifications when samples were not successfully typed.

Individuals were genotyped at up to 7 previously described unlinked microsatellite loci: *Ots-2*, *Ots-3*, *Ots-9*, and *Ots-10* (Banks et al.1999), *Ots-104* and *Ots-107* (Nelson and Beacham 1999), and *One-13* (Scribner et al. 1996). The forward PCR primer was labeled with a fluorescent phosphoamidite (HEX or fluorescein). PCR products were electrophoresed, 96 at the time with allelic controls, on a 45.0 cm wide by 22.5 cm high 8% denaturing polyacrylamide gel at 50 W

for 150 min. DNA fragments were visualized on the FMBIO® fluorescent imaging system (Hitachi Software Engineering America Ltd) and genotypes were scored with BIOIMAGE software. The data were double-checked for accuracy and independently verified by at least one other researcher. Individuals that did not produce repeatable genotypes and were difficult to score were not included in the analyses.

Table 9. List of Chinook tissue samples collected from the mainstem of the Eel river (Humboldt Co.), from the Russian River (Sonoma Co.) and from Lagunitas Creek (Marin Co.). Russian River-Warm Springs Hatchery samples originate from the Eel River[a] and the Van Arsdale[b] hatchery.

Watershed	Creek/Size Class	1997	1998	1999	2000	Total
Eel	RY/Adult	0	9*	0	0	9
	BA/Adult	0	17*	18*	0	35
	W/Adult	0	9*	0	0	9
	LV/Adult	0	9*	0	0	9
	S/Adult	0	7*	0	0	7
	T/Adult	0	0	5*	0	5
	O/Adult	0	0	6*	0	6
	BR/Adult	0	0	6*	0	6
Russian River	WS[a,b]/Adult	100[a], 94[b]	0	0	0	194
	F/Adult	0	0	8*		8
	M/Smolt	0	0	72[#]	82[#]	154
Lagunitas	Lag/Adults	0	0	0	7	7
Totals		194	51	115	89	449

Eel: (RY=Ryan; BA=Baechtal; W=Willits; LV=Long Valley; S=String; T=Tomki; O=Outlet; BR=Broaddus). Russian River: (WS=Warm Springs; F=Forsyth; M=Mirabel.
Collectors: * Harris, CDFG; [#]SCWA

We tested for deviations from Hardy-Weinberg (H-W) equilibrium within population, using GENEPOP version 3.3 (available at ftp://ftp.cefe.cnrs-mop.fr/genepop/). For linkage disequilibrium (LD), F_{IS} and F_{ST} tests we used the program GENETIX version 3.3 (available at http://www.univ-montp2.fr/~genetix/genetix.htm). The significance of F_{IS}, F_{ST} and LD ($\alpha = 0.05$) was determined by performing 500 permutations in GENETIX. We also tested genetic heterogeneity among populations from the Eel River and from the Russian River, and between Coastal populations including the Klamath River and between the Central Valley. We proceeded by measuring genetic distance between the largest homogeneous populations. The coastal populations included the Eel River, Russian River and Klamath River. The inland populations included five populations from the Central Valley (winter, spring from Butte Creek (BC), spring from Deer and Mill Creeks (DMC), fall, and late fall) and the Santa Clara Valley.

Cavalli-Sforza and Edwards (1967) (CSE) chord measures were calculated using GENDIST in the program PHYLIP (Felsenstein 1993) for data from five loci. Unweighted pair-group method

with arithmetic mean (UPGMA) or average distance trees (Sneath and Sokal 1973) were calculated using NEIGHBOR in PHYLIP. Bootstrap results for assessing the frequency of occurrence, and thus significance, of each tree cluster were attained using SEQBOOT and CONSENSE in PHYLIP with 1000 replicates. Trees were visualized using TREEVIEW (Page 1996).

Results

None of the nine Eel River samples deviates from H-W equilibrium or displays linkage disequilibrium (LD) (Table 10). In contrast, four of the five samples from the Russian River deviate from H-W equilibrium. Adult Russian River samples from the Warm Spring Hatchery deviate from H-W equilibrium at the $\alpha = 0.0001$ level and display high levels of LD, with 19 and 10 of 21 pairwise combinations of loci displaying significant nonrandom associations for samples A and B, respectively. Because these samples do not conform to the assumption

Table 10. Within-sample genetic diversity for 14 coastal Chinook salmon populations genotyped at seven loci (*Ots-2*, *Ots-3*, *Ots-9*, *Ots-10*, Banks et al. 1999; *Oneμ-13*, Scribner et al. 1996; *Ots-104* and *Ots-107*, Nelson and Beacham 1999).

Sample	Year	Collection Site	Life Stage	N	H_e	H_o	N_a	F_{IS}	$P_{H\text{-}W}$
E1	1998	Ryan	Adult	9	0.54	0.49	4.3	0.27	0.74
E2	1998	Baechtal	Adult	17	0.71	0.70	6.4	0.06	0.21
E3	1998	Willits	Adult	9	0.66	0.81	5.1	-0.15	0.96
E4	1998	Long Valley	Adult	9	0.65	0.69	4.6	0.04	0.88
E5	1998	String	Adult	7	0.56	0.65	3.6	-0.07	0.81
E6	1999	Baechtal	Adult	18	0.73	0.76	6.9	0.00	0.88
E7	1999	Tomki	Adult	5	0.50	0.61	2.9	0.07	0.53
E8	1999	Outlet	Adult	6	0.60	0.65	3.7	0.05	0.44
E9	1999	Broaddus	Adult	6	0.55	0.61	4.0	0.00	0.88
RR1	1997	Warm Springs [a]	Adult	100	0.70	0.62	6.0	0.12*	***
RR2	1997	Warm Springs [b]	Adult	94	0.74	0.61	8.6	0.18*	***
RR3	1999	Forsyth	Adult	8	0.66	0.76	4.2	-0.04	0.93
RR4	1999	Mirabel	Smolt	72	0.77	0.69	12.9	0.10*	*
RR5	2000	Mirabel	Smolt	82	0.75	0.69	10.7	0.09*	***
Lag1	2000	Lagunitas	Adult	7	0.48	0.51	3.2	0.08	0.81
Total				449					

Note: H_e, expected heterozygosity; H_o, observed heterozygosity; N_a, average number of alleles per locus; $P_{H\text{-}W\text{-}C}$, Hardy-Weinberg probability test – Fisher's exact method; significance, $P \leq 0.05$*; $P<0.001$***

required to perform tests of heterogeneity, both samples are omitted from further analyses. Russian River smolt samples, Mirabel 1999, and Mirabel 2000, deviate from H-W equilibrium at the $\alpha = 0.05$ and $\alpha = 0.0001$ levels, respectively. Neither of the smolt samples displays linkage disequilibrium, however. The deviation from H-W equilibrium for Mirabel 2000 is caused by loci *Ots-10* and *One-13*.

The first test of genetic heterogeneity is performed at the watershed level for the Russian River samples and for the Eel River samples. The Russian River samples include Mirabel 99 and '00 and Forsyth 1999 (Table 2). The range in pairwise comparisons of F_{ST} statistics for the three samples is –0.0064 to 0.0097. Forsyth is not significantly different from either Mirabel sample, but Mirabel 99 differs from Mirabel 2000 at the $\alpha = 0.05$ level. For the Russian River, Forsyth 1999 and Mirabel 2000 are combined to form a homogeneous group. For the nine Eel River samples, F_{ST} is 0.013, not statistically different from zero, so the nine samples are combined to form a homogeneous group.

The second test of genetic heterogeneity is performed among populations. The UPGMA phylogram derived from CSE, based on seven loci, shows that the coastal Chinook populations from the Eel River, Russian River and Klamath, cluster on one side of the tree while the inland

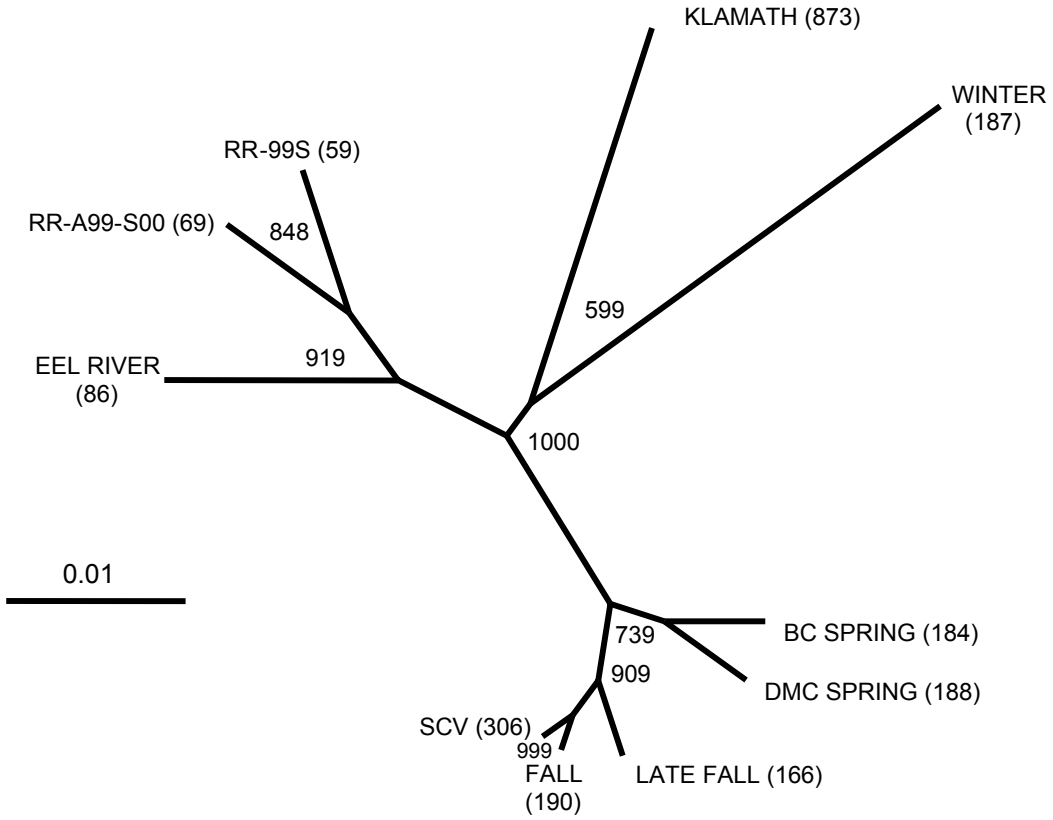

Fig. 9. A tree showing genetic relatedness based on CSE UPGMA from 7 microsatellite loci, with all Eel River samples combined. Russian River hatchery samples are excluded from analysis due to high linkage disequilibrium. Central Valley Chinook populations are from Banks et al. (2000; Table 1, fig. 4.) Numbers in parenthesis indicate sample sizes and numbers at the nodes indicate the number out of 1000 bootstrap simulations supporting a particular cluster. The scale indicates genetic distance.

Chinook populations from the Central Valley, Spring BS, Spring DC, Late Fall, Fall and the Santa Clara Valley, cluster on the other side of the tree (Fig. 9). This bifurcation is found in all 1000 trees made by bootstrapping loci. The Eel River and the Russian River cluster together, but the two populations are distinct from one another with a bootstrap value of 919. Although the

Russian River displays temporal structure, with a bootstrap value of 848, these samples are closer to each other than to samples from either the Eel or Klamath Rivers.

Discussion

Our final analysis of Chinook reveals quite a different picture than the preliminary results presented in previous reports. This change results primarily from the addition of new samples and careful scrutiny of the scoring of genotypes. No correction for family structure was needed, as the juvenile samples did not have high LD.

The major finding is that Chinook salmon in the Russian River are not closely related to Chinook salmon from either the Central Valley or the Eel River. A close relationship to Central Valley stocks might be postulated based on supposed straying of Central Valley hatchery stocks into the Russian River. Indeed, we see evidence of this in the very close affinity of Chinook in the Santa Clara Valley and Central Valley fall stocks (Fig. 9). On the other hand, owing to the diversion of Eel River water through Potter Valley into the Russian River, one might postulate a potential for enhanced gene flow between Russian River Chinook and Eel River Chinook mistakenly homing to this diverted Eel River water. This appears not to be the case. Chinook in the Russian River do appear to belong to a diverse set of coastal Chinook populations.

THE DEVELOPMENT AND MAINTENANCE OF ALTERNATIVE MALE-TYPES IN A POPULATION OF COHO SALMON.

Three adult phenotypes, 3-year-old "hooknosed" males, 3-year-old females and 2-year-old "jack" males represent coho salmon in California. During spawning, females defend an oviposition territory from other females. While brightly colored hooknosed males fight for access to females, jack males are much smaller and sneak into a female's oviposition site in order to attain egg fertilizations. Interestingly, large juvenile males are more likely to mature as jacks than are small juveniles (Gross, 1991). Additionally, there are at least three behavioral and morphological phenotypes reported in juveniles. These three phenotypes can be referred to as territorials, floaters and poolers (Puckett and Dill, 1985; Nielsen, 1992). Territorials are the largest; they hold and defend positions in the natal stream while floaters, the smallest, hold no permanent position and dash among the territorials. Poolers are intermediate in size and do not appear to defend territories. Territorials and floaters are found in areas of the creek that are marked by variation in water flow velocity, while poolers are found in areas that have little variation in water flow velocity, namely pools. No research has directly tied the observation of multiple juvenile behavioral phenotypes to the occurrence of alternative reproductive phenotypes in coho salmon. Dissertation research by graduate student Jason Watters examined the development, maintenance, and conservation significance of alternative male phenotypes in coho salmon (*Oncorhynchus kisutch*). The dissertation is being published in three separate articles, the abstracts of which follow.

Watters, J. V., S. C. Lema and G. A. Nevitt. 2002. Phenotype Management: A New Approach to Habitat Restoration. Biological Conservation, in press.
Abstract. The goal of habitat restoration is to provide environmental conditions that promote the maintenance and growth of target populations. But rarely is it considered how the allocation of resources influences the diversity of phenotypes in these populations. Here we present a framework for considering how habitat restoration can shape the development and expression of

phenotypes. We call this approach *phenotype management* as it entails restoring the resources in a habitat to manage phenotypic diversity. Phenotype management is achieved by manipulating the spatial and temporal distribution of resources to alter the degree of competition among individuals. Differences in competition, in turn, lead to changes in phenotypic and life history expression that affect population parameters including demography and effective population size (N_e). To illustrate how phenotype management can be applied, we explore how resource distributions shape variation in phenotypes in two imperiled fishes, Pacific salmon and desert pupfish. In both examples, modulating male reproductive phenotypes changes the allocation of reproductive success among population members to subsequently affect N_e. These examples further demonstrate that whether to increase or decrease phenotypic diversity depends on the primary conservation pressures faced by the species.

Waters, J. V., and G. A. Nevitt. Resource Clumping and Population Density Drive the Development of Alternative Phenotypes. Behavioral Ecology, in review.
Abstract. Though often eclipsed by genetic considerations, the environment plays a key role in directing development, and typically drives most individual variation within populations. Here, we describe how two simple parameters – resource clumping and population density – are likely to shape the distribution of growth rates in a population and affect the expression of alternative reproductive phenotypes. At low population densities, clumped resources increase the variance in growth while evenly spread resources decrease this variance. Increased population densities lead to a decrease in the mean growth rate, and eventually, in the case of clumped resources to a decreasing variance in growth. Thus, when phenotypic expression is contingent on growth rate, habitats with clumped resources are more likely to produce alternative phenotypes than habitats with evenly spread resources are and this is most probable at lower densities. We test these ideas empirically using a threatened species, coho salmon (*Oncorhynchus kisutch)* as a model system. Our results demonstrate that varying physical attributes of the rearing habitat dramatically influences the growth rates of juveniles and the subsequent expression of alternative male phenotypes at maturity.

Watters, J. V., and G. A. Nevitt. MS, in prep.
Abstract. Most studies of sexual selection investigate either intersexual or intrasexual interactions. Here I suggest that considering the simultaneous effects of inter- and intrasexual interactions will provide new insight into the evolution of mating systems and elaborate sexually selected traits. I present a model of mate choice in which females base their mating preferences on the heritable viability of males. In the model, male phenotypes are indicative of their viability. I show with the model that in order for males of lower fitness to attain reproductive success, they must contend free female mate choice by coercing females. Females and preferred high fitness males then must cooperate to facilitate mating and avoid the costs of coercion. When the costs of coercion are high relative to the benefits of cooperation, females may choose to mate with low fitness non-preferred males. In cases where coercion is common, cooperation to secure preferred matings may occur quickly. Indeed, I suggest that elaborate male traits are often those that are useful in coercion and that quick, efficient "sneak" mating may be a means to facilitate preferred matings. Where coercion cannot be avoided, I suggest that females may evolve life history characteristics that minimize the fitness costs of mating with non-preferred males.

This theory is the basis of the field observations that Watters has done on wild fish. Jason believes that hooknosed males are coercers and that jacks are cooperators. Jacks are much more likely to survive to maturity than hooknoses, so it is feasible that a female who mates with jacks can maximize her fitness, if the trait is not solely environmentally determined. These data being used to test this hypothesis are being analyzed.

DEVELOPMENT OF GEOGRAPHIC INFORMATION SYSTEMS

Overview

Researchers from REGIS (the Research Program in Environmental Planning and Geographic Information Systems, College of Environmental Design, UC Berkeley) in collaboration with researchers from Bodega Marine Laboratory (UC Davis) have developed a model for a web-based marine GIS (Geographic Information System) that focuses on coastal near-shore processes. The data layer inputs are marine physical attributes that have an impact on the health and survival of near-shore fisheries, such as coho salmon. The marine GIS is unique in that it incorporates data layers derived from near real-time data publicly available on the Internet. It is also the first GIS model to incorporate real-time ocean surface currents measurements derived from CODAR (Coastal Ocean Dynamics Application Radar) high frequency radar stations.

The URL for the working model of the GIS is **http://sonoma.regis.berkeley.edu/website/bml/salmon** (until 1/31/2003). We expect to move this site to **http://www.bml.ucdavis.edu** at some time in the near future.

Purpose

The intent of this project was not to compete with or duplicate the efforts of other projects but to explore ways to incorporate useful data pertaining to marine systems and fisheries in a relevant but novel GIS format. A number of excellent terrestrial databases have already been developed for salmon management such as the KRIS (Klamath River Information System) and RRIS (Russian River Information System) databases. Data pertaining to the marine environment are absent from these databases however. Marine physical factors have a significant impact on the health and productivity of fisheries. Salmon, for example, spend much of their lives in the ocean but resource managers have very little information about where these fish go between spawning events and what the conditions are that influence their growth and behavior. It is hoped that these terrestrial databases can eventually be combined with marine GIS databases in the future to provide a more complete picture of factors influencing commercial fisheries and the coastal environment.

Why a GIS mapserver?

Mapservers are a recent phenomenon and have evolved from the development of the Internet. In simple terms, they provide interactive map analyses and mapmaking capabilities to anyone with a computer connected to the web and running an up-to-date web browser. We chose to work with a web-based GIS rather than build a static GIS database because of its potential to deliver up-to-date information to broad audience in a timely manner. A minimal amount of GIS expertise is required to take advantage of this tool. The obvious benefit to resource managers, policy makers and educators is immediate access to current geographical data relevant to a particular problem, in this case fisheries management. Additions or updates to web-based

databases can be made quickly on a single computer, the mapserver, avoiding the need to mail digital media to users whenever database changes occur. A web-based GIS can also provide data from real-time sources as we have demonstrated with this project.

Software and Computing Platform Specifications

The Microsoft (tm) Windows 2000 Server platform was chosen because it was the easiest and most cost-effective platform on which to run mapserver software. A key goal was to keep all data and software geared to a commonly used computing platform so that our custom programming efforts could be shared with other website developers. Our GIS uses ESRI's (Earth Systems Research Institute) ArcIMS 3.1 software, an industry standard.

Details of the custom PERL programming scripts used to develop the unique features of this GIS are included as an appendix to this report. A Cdrom, containing the database files, ArcIMS directory structure, and scripting code is also included with this report.

Description

This project was not intended to be a comprehensive database or analytical tool but a model framework to help guide the development of marine GIS databases in the future. The sample data layers that we have used are completely functional and include examples of important physical attributes of the local coastal environment. Sea surface temperature, ocean surface currents, wave heights, stream flows, stream sediment loads and stream temperatures are all accessible through the data layers or live-linked URLs. The UC Berkeley REGIS group was conscripted for this project because of their demonstrated expertise with environmental GIS development and their experience with incorporating publicly accessible Internet databases into GIS format. They identified and incorporated four web accessible data sources into our GIS model for this project. These include ocean current measurements from BML CODAR installations, NOAA Data Buoy Center (NDBC) data for buoys along the central California coast, the USGS Stream Gauge database for the Sonoma and Marin County area and the California Data Exchange Center (CDEC) stream monitoring stations for the Sonoma and Marin County area.

Salmon Genetics Data

In our original request for supplementary funds to explore the use of GIS as a management tool we proposed to incorporate genetic (allelle-frequency) data as a test dataset. The spatial distribution of genetically differentiated groups of coho salmon was of interest to resource managers and a GIS was an obvious and appropriate tool to display these types of data. Unfortunately, these data came from numerous agencies and the formats and metadata collected were not consistent from agency to agency. Specifically the spatially explicit information needed (i.e. geographic coordinates) to code the genetic data for display was different for each sample set. Some samples were labeled with a generic stream name or watershed name, while others referenced local features such as road mileposts, access road gates. However, we were able to develop a sample data layer from one of the datasets with the requisite spatial information to illustrate how tabular data might be incorporated into a GIS. The Olema Creek data samples (provided by the National Park Service) were recorded using specific pre-determined river reaches defined by specific geographic coordinates. Using the ArcIMS browser, any location

along the creek can be selected with a click of the mouse. This activates a pop-up data table for that particular stream segment and lists the individual samples along with the corresponding metadata.

The allele-frequency data for the entire 1330-sample dataset (Appendix 1) were coded in a tabular format compatible with a GIS and it may be possible to retroactively add coordinate information to the data. However, the contributing agencies would need to invest a significant amount to standardize their data collection forms and agree on a protocol. Historic sample metadata would need to be appended with requisite spatial information and some of the samples may require input from the individuals who originally collected the samples. A few recommendations are made below that may help to make future data contributions more useful for display in map form and GIS analyses.

Recommendations

The primary limitation we encountered when attempting to visualize salmon genetics information in a GIS was the inconsistency or lack of spatial metadata provided with salmon fin clip samples. We recommend that future samples be provided with geographic coordinates obtained either from a handheld GPS (Global Positioning System) or USGS topographical map. Latitude and longitude (in decimal degrees) is the most flexible system for use with GIS software but UTM (North American Datum 1983) is also commonly used.

We also recommend that a standardized method of defining the stream reaches used by salmon be developed by the cooperating agencies. Aggregation of genetic samples to the reach level of resolution appears to be the optimal method based on the results of the salmon genetics study and demonstrated with the Olema Creek example. Point data (with geographic coordinates) provided with individual samples can always be aggregated into stream reaches at a later date, but in the long run it will be more cost effective to record the reach information at the time the sample is collected. A standardized data form agreed upon by all agencies would also be useful. This would minimize data input errors, when data are coded in digital format, and reduce costs.

We have demonstrated that a web-based GIS can be a useful method for timely dissemination of physical and ecological data pertaining to natural resource management of coastal marine systems. We have several suggestions that we can offer to assist with future development of interactive GIS as a marine resource management tool. First, the area covered by a fishery (such as salmon) is probably too large for a single GIS mapserver to handle in any great detail. We suggest that a coordinated effort be made to develop a standardized set of marine data layers to be archived and distributed at several regional or local level mapserver sites. A central computer hosting a more generalized data set could be established to field initial queries and then the query to the regional servers based on the level of detail required. We suggest that future GIS developers examine the marine data model currently being developed by ESRI and researchers at Oregon State University (http://www.esri.com/) as a starting point for marine data layer development. We have also identified several other for-fee data sources that would be very useful and are available as real-time data. These include polar orbiting AVHRR satellite imagery, which provides estimates of sea-surface temperature (SST) at 1km resolution, and SeaWIFs ocean color sensor data, which provide estimates of plankton content in coastal waters. Scripting to re-project these data for incorporation into a mapserver GIS are already available.

Other relevant data layers under development by the California Department of Fish and Game and available in the future include marine sanctuary and marine protected area boundaries and coastal kelp beds.

Additional CODAR sites should be added to real-time databases in the future in order to expand the coverage area for ocean surface current measurements. Currently there are twelve high frequency radar stations along the California coast covering roughly five percent of the California coastline. The southern most station is located on the Mexico border at Borderfield State Park and the northern-most is located at point St. George near Crescent City, California and operated by Oregon State University (OSU also operates five radars along the Oregon coast). The manufacturers (CODAR Ocean Sensors of Los Altos, California) have stated that there are a number of proposals in review that may help fund a coastal CODAR array covering the entire California coast. If these proposals are successful, arrangements should be made with the CODAR owner/operators for access to the real-time data for eventual inclusion into a mapserver.

Several large projects underway will develop observation systems that will eventually cover the entire coast of California. All of these data stations are designed to provide data in real-time and will be remotely accessible via the Internet. We expect that these could be easily adapted for use in a comprehensive marine GIS using the scripting methods developed by REGIS. A list of California coastal monitoring projects and contact information can be found at the NOAA website http://www.noaa.gov).

ACKNOWLEDGEMENTS

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APPENDIX 1. TABLE OF ALLELIC FREQUENCIES FOR 33 SAMPLES OF COHO SALMON IN CALIFORNIA

Allelic frequencies, expected and observed heterozygosities (*H exp.*, *H obs.*), Wright's inbreeding coefficient (F_{IS}), and its significance (*, P<0.05; **, P<0.01; ***, P<0.001), for seven microsatellite DNA markers in 33 samples of coho salmon populations in California.

	Southern Oregon / Northern California											South of San Francisco					
Ots-103	KIGHA97an	KIGHA97j	KIGHA97ll	TRHA97s	TRHA97l	LRS00	EHOLA97	EREDS97	EREDA98	ESPRS99	MATS98	WADY99l	WADY99u	SCA95c	SCA9798c	SCY99low	SCY99up
(N)	30	15	36	17	77	81	16	77	19	30	45	31	17	39	63	23	20
192	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
196	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
204	0.033	-	0.028	-	0.013	-	-	-	-	-	-	-	-	-	-	-	-
208	-	-	-	-	-	-	-	-	-	-	0.022	-	-	-	-	-	-
212	-	-	-	0.147	0.007	0.043	0.094	0.039	0.053	-	-	-	-	-	-	-	-
216	0.033	-	-	-	-	0.006	-	0.033	0.211	-	-	-	-	-	-	-	-
220	0.100	0.067	-	-	-	0.148	0.375	0.162	0.053	0.117	0.033	-	-	-	-	-	-
224	0.050	0.033	0.014	-	0.033	0.074	0.063	0.026	-	0.367	0.044	0.016	-	0.013	0.024	-	-
228	0.017	0.067	-	-	-	0.142	-	0.046	-	0.050	-	0.048	-	0.051	0.008	0.130	0.125
232	0.033	0.233	0.083	-	0.065	0.093	-	0.007	0.026	0.033	-	0.032	-	0.051	0.056	0.022	-
234	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
236	0.017	0.033	-	0.029	0.020	0.086	0.250	0.065	0.211	0.183	0.389	0.177	-	0.321	0.373	0.152	0.350
240	-	-	-	-	0.071	0.049	-	-	-	-	0.022	0.371	0.588	0.218	0.230	0.348	0.375
244	0.017	-	-	0.029	0.046	0.043	-	0.007	-	-	-	0.032	0.059	0.077	0.048	0.044	0.025
248	0.050	0.033	0.042	0.118	0.104	-	-	0.097	-	-	-	0.016	-	-	0.008	0.044	-
252	0.033	0.100	0.097	0.088	0.110	-	-	0.013	0.026	0.050	-	-	-	-	-	-	-
254	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
256	-	-	-	-	0.007	0.006	0.063	0.058	0.105	-	0.033	-	-	0.013	0.032	0.022	-
260	-	-	-	-	-	-	0.031	0.013	-	-	-	-	-	-	0.008	-	-
264	0.167	0.033	0.208	0.059	0.149	0.031	0.031	0.020	0.053	-	-	0.032	-	-	-	-	-
268	0.067	0.100	0.014	0.029	-	-	-	0.046	0.053	0.017	-	-	-	-	0.024	-	-
272	0.183	0.167	0.486	0.382	0.370	0.210	-	-	0.026	0.100	-	-	-	-	-	-	-
274	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
276	0.033	-	0.014	-	-	-	-	0.091	0.026	-	0.022	-	-	-	-	-	-
280	0.117	0.133	0.014	0.118	0.007	-	-	-	0.053	-	-	-	-	-	0.008	-	-
284	0.050	-	-	-	-	-	-	-	-	-	-	0.226	0.353	0.192	0.143	0.239	0.125
288	-	-	-	-	-	0.025	-	0.058	-	-	-	-	-	-	-	-	-
292	-	-	-	-	-	0.019	-	0.013	-	-	-	-	-	-	-	-	-
296	-	-	-	-	-	0.006	-	-	-	-	0.200	-	-	-	-	-	-
300	-	-	-	-	-	0.019	0.094	0.208	0.105	0.083	0.233	0.048	-	0.064	0.040	-	-
H exp.	0.897	0.867	0.701	0.791	0.805	0.884	0.770	0.893	0.873	0.795	0.749	0.772	0.526	0.797	0.778	0.777	0.705
H obs.	0.867	0.800	0.639	0.765	0.584	0.938	0.563	0.857	0.368	0.967	0.733	0.742	0.529	0.846	0.778	0.913	0.850
F_{IS}	0.050	0.111	0.102	0.063	0.280	-0.055	0.299	0.047	0.596	-0.200	0.032	0.055	0.024	-0.048	0.008	-0.154	-0.181
Sig.					***		*		***						*		

	Southern Oregon / Northern California											South of San Francisco					
Ots-2	KIGHA97an	KIGHA97j	KIGHA97ll	TRHA97s	TRHA97l	LRS00	EHOLA97	EREDS97	EREDA98	ESPRS99	MATS98	WADY99l	WADY99u	SCA95c	SCA9798c	SCY99low	SCY99up
(N)	30	15	36	17	77	81	16	76	20	30	46	31	17	39	65	23	20
176	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
178	0.050	0.133	0.014	-	0.026	0.136	-	0.053	0.050	-	0.076	-	-	-	-	-	
180	0.533	0.433	0.528	0.412	0.312	0.630	0.375	0.355	0.300	0.200	0.489	0.661	0.765	0.808	0.854	0.826	0.950
182	0.250	0.200	0.125	0.147	0.091	-	-	0.026	0.025	-	-	-	0.059	0.013	0.023	-	-
184	0.117	0.233	0.333	0.324	0.435	0.099	-	0.020	0.075	0.067	-	-	-	-	0.008	0.022	-
186	-	-	-	-	0.007	-	-	-	-	-	-	-	-	-	-	-	-
187	-	-	-	0.118	0.117	0.049	0.625	0.447	0.450	0.517	0.435	-	-	0.013	0.015	-	-
188	0.050	-	-	-	0.013	0.086	-	0.099	0.100	0.217	-	0.339	0.177	0.167	0.100	0.152	0.050
190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
192	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.634	0.700	0.595	0.690	0.691	0.566	0.469	0.660	0.689	0.642	0.566	0.448	0.381	0.320	0.260	0.294	0.095
H obs.	0.633	0.867	0.472	0.765	0.636	0.556	0.250	0.697	0.850	0.700	0.652	0.548	0.412	0.385	0.262	0.348	0.100
F_{IS}	0.019	-0.205	0.219	-0.078	0.085	0.024	0.492	-0.050	-0.210	-0.074	-0.142	-0.209	-0.052	-0.191	0.002	-0.162	-0.027
Sig.			*			*				*							

iso-Ots2	KIGHA97an	KIGHA97j	KIGHA97ll	TRHA97s	TRHA97l	LRS00	EHOLA97	EREDS97	EREDA98	ESPRS99	MATS98	WADY99l	WADY99u	SCA95c	SCA9798c	SCY99l	SCY99up
(N)	30	15	36	16	75	79	15	75	19	30	44	31	17	39	65	23	19
199	0.050	0.033	0.167	-	0.073	-	-	-	-	-	-	-	-	-	-	-	-
201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
203	-	-	-	0.031	0.013	0.006	-	-	-	-	-	-	-	-	-	-	-
205	0.350	0.200	0.208	0.375	0.280	0.260	0.400	0.573	0.658	0.417	0.239	0.613	0.529	0.718	0.462	0.609	0.737
207	0.017	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
209	0.017	-	0.014	-	-	-	-	-	-	-	-	-	-	-	-	-	-
211	-	-	-	-	-	0.038	0.033	0.007	-	-	-	-	-	-	-	0.044	-
213	0.050	0.067	0.153	0.063	0.147	0.006	-	0.100	-	0.133	0.023	0.016	-	0.039	0.085	0.022	-
215	-	0.067	0.042	0.063	-	0.032	0.167	0.173	0.158	0.100	0.284	0.065	-	0.064	0.062	-	-
217	0.117	0.100	0.069	0.031	0.080	0.070	0.033	-	0.026	-	0.114	0.016	-	-	0.015	-	-
219	0.017	-	0.028	0.125	0.053	-	-	-	0.105	-	0.011	0.016	-	-	-	-	-
221	0.067	0.067	0.069	0.094	0.027	0.146	0.067	0.120	-	0.133	0.227	0.032	-	0.039	0.015	-	0.053
223	0.033	0.233	0.014	-	0.040	0.177	-	0.013	0.026	0.083	-	0.016	-	0.039	0.069	-	-
225	0.033	0.067	0.083	0.063	0.107	-	-	-	-	-	0.046	-	-	-	-	-	-
227	0.017	0.067	-	0.031	0.053	0.101	0.300	0.013	0.026	0.133	0.057	0.226	0.471	0.103	0.292	0.326	0.211
229	-	-	0.014	0.094	0.093	0.101	-	-	-	-	-	-	-	-	-	-	-
231	0.067	0.100	0.028	0.031	0.007	0.025	-	-	-	-	-	-	-	-	-	-	-
233	0.017	-	-	-	-	0.006	-	-	-	-	-	-	-	-	-	-	-
241	-	-	-	-	-	0.006	-	-	-	-	-	-	-	-	-	-	-
245	-	-	-	-	-	0.006	-	-	-	-	-	-	-	-	-	-	-
247	-	-	0.014	-	-	0.006	-	-	-	-	-	-	-	-	-	-	-
249	0.050	-	0.042	-	0.013	0.013	-	-	-	-	-	-	-	-	-	-	-
251	0.050	-	-	-	0.007	-	-	-	-	-	-	-	-	-	-	-	-
253	-	-	0.014	-	0.007	-	-	-	-	-	-	-	-	-	-	-	-
257	-	-	0.014	-	-	-	-	-	-	-	-	-	-	-	-	-	-
260	0.050	-	0.028	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.839	0.862	0.882	0.811	0.860	0.851	0.716	0.616	0.529	0.756	0.792	0.567	0.498	0.466	0.685	0.521	0.410
H obs.	0.833	0.867	0.889	0.750	0.933	0.798	0.800	0.547	0.632	0.800	0.773	0.484	0.353	0.436	0.523	0.478	0.421
F_{IS}	0.024	0.029	0.006	0.107	-0.079	0.069	-0.084	0.120	-0.168	-0.041	0.036	0.163	0.319	0.076	0.244	0.104	0.000
Sig.		*	**		***	*		*				*			***		

	Southern Oregon / Northern California											South of San Francisco					
Ots-3	KIGHA97an	KIGHA97j	KIGHA97ll	TRHA97s	TRHA97l	LRS00	EHOLA97	EREDS97	EREDA98	ESPRS99	MATS98	WADY99l	WADY99u	SCA95c	SCA9798c	SCY99low	SCY99up
(N)	29	13	33	17	66	78	16	74	22	30	41	31	17	39	65	23	19
120	0.017	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
130	0.035	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
133	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
135	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
137	-	-	-	-	-	0.039	0.031	0.061	0.068	0.083	0.134	-	-	-	-	-	-
139	-	-	-	-	-	-	-	-	0.023	0.017	-	-	-	-	-	-	-
141	0.052	0.077	-	0.059	0.061	0.199	0.094	0.088	0.091	0.050	0.281	0.242	0.235	0.333	0.331	0.326	0.526
143	0.017	-	-	-	-	-	0.031	-	-	0.017	-	-	-	-	-	-	-
145	0.052	-	0.136	-	0.091	0.115	-	0.007	0.023	0.033	0.146	0.339	0.324	0.321	0.208	0.196	0.132
147	0.103	0.269	0.061	0.059	0.038	0.135	0.281	0.108	0.114	0.250	0.195	0.258	0.441	0.192	0.323	0.326	0.184
149	0.035	-	0.061	0.059	0.030	0.006	0.031	0.081	0.068	-	-	-	-	-	-	-	-
151	0.035	0.039	0.015	-	0.061	0.026	-	-	-	-	0.012	-	-	-	-	-	-
153	0.535	0.346	0.500	0.647	0.644	0.404	0.406	0.487	0.341	0.467	0.159	0.129	-	0.115	0.139	0.087	0.132
155	0.069	0.192	0.076	0.029	0.023	0.019	-	0.014	-	-	-	0.016	-	0.039	-	0.065	0.026
157	0.052	0.077	0.152	0.147	0.053	0.058	0.094	0.155	0.273	0.083	-	0.016	-	-	-	-	-
159	-	-	-	-	-	-	0.031	-	-	-	0.024	-	-	-	-	-	-
161	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
163	-	-	-	-	-	-	-	-	-	-	0.049	-	-	-	-	-	-
H exp.	0.687	0.757	0.695	0.548	0.564	0.760	0.734	0.709	0.778	0.702	0.816	0.743	0.645	0.734	0.724	0.737	0.654
H obs.	0.690	0.692	0.727	0.588	0.546	0.821	0.813	0.689	0.727	0.733	0.805	0.774	0.882	0.795	0.508	0.913	0.579
F_{IS}	0.013	0.126	-0.031	-0.042	0.041	-0.073	-0.074	0.035	0.088	-0.028	0.025	-0.026	-0.341	-0.069	0.306	-0.217	0.141
Sig.															***		

One-13	KIGHA97an	KIGHA97j	KIGHA97ll	TRHA97s	TRHA97l	LRS00	EHOLA97	EREDS97	EREDA98	ESPRS99	MATS98	WADY99l	WADY99u	SCA95c	SCA9798c	SCY99low	SCY99up
(N)	21	10	27	16	69	81	16	75	20	29	34	31	13	39	64	23	19
193	-	-	-	-	-	0.006	-	0.007	-	-	-	-	-	-	0.008	-	-
195	0.024	0.050	0.019	-	0.007	-	-	-	-	-	-	-	-	-	-	-	-
197	0.024	-	0.056	-	0.145	-	-	-	-	-	-	0.194	0.115	0.167	0.086	0.196	0.053
201	0.095	0.100	0.056	0.031	0.087	0.111	0.094	0.160	0.275	0.172	0.044	0.419	0.231	0.333	0.461	0.304	0.447
203	0.071	0.300	0.019	0.125	0.058	0.105	-	0.067	0.075	0.086	-	0.016	-	-	0.008	-	-
205	0.048	-	0.019	0.063	0.022	-	0.063	0.027	-	0.017	-	0.016	-	0.039	0.039	0.087	0.105
207	-	-	-	0.094	0.036	0.025	-	0.007	0.025	-	-	0.081	0.039	0.077	0.055	0.261	0.026
209	-	-	-	-	0.007	0.185	0.188	0.067	0.075	0.103	0.221	-	0.077	-	-	-	-
211	0.048	0.100	0.019	0.156	0.073	0.099	-	0.047	0.075	0.069	0.029	0.016	0.231	0.051	0.016	0.065	0.132
213	0.119	-	0.056	-	0.007	0.019	-	0.013	-	-	-	0.065	0.077	0.128	0.148	0.022	0.158
215	0.262	0.300	0.370	0.250	0.275	0.284	0.438	0.293	0.175	0.362	0.338	0.161	0.192	0.141	0.117	0.022	-
217	-	0.100	0.037	0.094	0.065	0.093	0.031	0.100	0.075	0.069	0.103	-	0.039	0.013	0.008	-	-
219	0.286	0.050	0.167	0.063	0.051	0.068	0.063	0.133	0.150	0.086	0.206	0.032	-	-	0.016	-	-
221	-	-	0.130	0.094	0.123	-	0.125	0.080	0.075	-	0.059	-	-	-	-	0.022	-
223	0.024	-	0.056	0.031	0.044	-	-	-	-	0.017	-	-	-	0.013	-	0.022	0.053
225	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.008	-	0.026
227	-	-	-	-	-	0.006	-	-	-	0.017	-	-	-	0.039	0.031	-	-
229	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
277	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.815	0.785	0.803	0.861	0.861	0.838	0.740	0.842	0.843	0.803	0.778	0.748	0.828	0.813	0.738	0.787	0.740
H obs.	0.762	0.800	0.593	0.938	0.884	0.877	0.875	0.867	0.950	0.931	0.794	0.839	0.923	0.897	0.672	0.913	0.737
F_{IS}	0.090	0.034	0.280	-0.056	-0.019	-0.040	-0.151	-0.022	-0.102	-0.142	-0.006	-0.105	-0.075	-0.091	0.098	-0.138	0.031
Sig.			*														

	Southern Oregon / Northern California											South of San Francisco					
P-53	KIGHA97an	KIGHA97j	KIGHA97ll	TRHA97s	TRHA97l	LRS00	EHOLA97	EREDS97	EREDA98	ESPRS99	MATS98	WADY99l	WADY99u	SCA95c	SCA9798c	SCY99low	SCY99up
(N)	29	15	36	16	77	81	14	77	20	29	47	31	17	37	65	23	20
150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
163	0.207	0.133	0.181	0.063	0.331	0.043	-	-	-	-	0.170	-	-	-	-	-	-
165	-	0.033	-	-	0.007	0.006	-	-	-	-	-	-	-	-	-	-	-
167	0.017	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
169	0.086	0.067	0.250	0.031	0.020	0.173	0.107	0.357	0.350	0.310	0.170	0.436	0.618	0.351	0.362	0.348	0.150
171	0.017	0.067	-	-	-	0.105	0.036	0.084	0.050	-	0.213	0.242	-	0.243	0.246	0.304	0.450
173	0.052	-	0.014	-	-	0.031	-	-	-	-	0.043	-	-	-	-	-	-
175	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
177	0.017	0.200	-	-	-	0.191	0.393	0.266	0.325	0.207	0.170	0.016	-	0.027	0.008	-	-
179	0.035	0.100	-	0.094	0.046	0.111	0.143	0.136	0.200	0.155	0.160	0.113	0.088	0.243	0.277	0.152	0.150
181	0.466	0.233	0.528	0.500	0.487	0.235	0.286	0.149	0.075	0.328	0.075	0.032	-	0.041	0.031	-	-
183	0.069	0.033	0.028	-	0.039	0.080	-	-	-	-	-	0.016	-	0.014	0.015	-	0.050
185	0.035	0.133	-	0.313	0.065	0.025	0.036	0.007	-	-	-	0.145	0.294	0.081	0.062	0.196	0.200
191	-	-	-	-	0.007	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.722	0.849	0.625	0.639	0.645	0.845	0.730	0.754	0.724	0.730	0.835	0.716	0.524	0.749	0.727	0.725	0.710
H obs.	0.690	0.800	0.722	0.500	0.584	0.877	0.643	0.779	0.650	0.690	0.787	0.807	0.647	0.730	0.446	0.652	0.850
F_{IS}	0.063	0.092	-0.141	0.248	0.100	-0.031	0.155	-0.028	0.127	0.072	0.068	-0.109	-0.205	0.040	0.393	0.122	-0.172
Sig.													*		***		

Oki-1	KIGHA97a	KIGHA97j	KIGHA97ll	TRHA97s	TRHA97l	LRS00	EHOLA97	EREDS97	EREDA98	ESPRS99	MATS98	WADY99l	WADY99u	SCA95c	SCA9798c	SCY99low	SCY99up
(N)	30	15	36	17	77	81	15	76	19	29	48	30	17	37	62	22	19
88	0.183	0.133	0.014	0.029	0.039	0.019	0.033	0.013	-	0.103	-	-	-	-	-	-	-
92	0.033	0.033	0.056	-	-	0.006	-	-	-	-	-	-	-	-	-	-	-
96	0.333	0.500	0.417	0.206	0.299	0.198	0.033	0.145	0.026	0.241	0.208	-	-	-	-	0.023	-
100	0.083	0.067	0.222	0.265	0.240	0.167	0.033	0.059	0.053	0.069	0.115	-	-	-	-	-	-
104	0.100	-	0.028	0.029	0.052	0.093	-	0.013	0.079	0.035	0.135	0.050	-	0.014	0.008	0.046	0.132
108	-	-	0.028	0.088	0.007	0.006	0.200	0.026	-	0.052	-	-	-	-	-	-	-
112	-	-	-	-	0.007	0.031	0.333	0.493	0.579	0.466	0.344	0.400	0.765	0.581	0.476	0.636	0.500
116	0.150	0.033	0.083	0.235	0.201	0.080	0.333	0.132	0.132	0.017	0.021	-	-	-	0.032	0.023	-
120	-	-	-	-	0.007	0.025	-	0.053	0.079	0.017	0.115	-	-	-	-	-	-
124	0.050	0.133	0.083	0.118	0.149	0.191	-	0.020	0.026	-	-	0.250	-	0.068	0.234	0.136	0.184
128	0.033	-	0.014	-	-	0.124	-	0.040	-	-	-	0.217	0.029	0.189	0.129	0.023	0.079
130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
132	-	-	-	-	-	0.019	-	-	-	-	-	0.083	0.206	0.149	0.105	0.114	0.105
136	-	-	-	-	-	-	0.033	0.007	0.026	-	-	-	-	-	0.016	-	-
140	-	-	-	0.029	-	0.043	-	-	-	-	0.063	-	-	-	-	-	-
144	0.033	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
148	-	0.067	0.056	-	-	-	-	-	-	-	-	-	-	-	-	-	-
152	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
160	-	0.033	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.810	0.702	0.755	0.808	0.786	0.862	0.733	0.709	0.630	0.705	0.790	0.721	0.372	0.600	0.690	0.560	0.681
H obs.	0.700	0.800	0.722	0.882	0.753	0.877	0.800	0.776	0.737	0.690	0.750	0.700	0.471	0.676	0.484	0.636	0.684
F_{IS}	0.152	-0.105	0.057	-0.062	0.048	-0.010	-0.057	-0.088	-0.143	0.039	0.061	0.046	-0.237	-0.113	0.306	-0.114	0.023
Sig.					*						*				**		

	Central California															
Ots-103	PUDY98	NOYA97	NOYA99	ALBA98	ALBY98	RRHA	RRGVY97	RRGVY98a	RRGVY98b	RRGVY00	LAG	LSGAY98	OLEA96	OLEA9798	RWMA97	RWMY98
(N)	44	42	40	21	16	64	8	25	39	6	128	17	58	106	11	23
192	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009	-	-
196	-	-	-	-	-	-	-	-	-	-	0.008	-	0.043	0.009	-	-
200	-	-	-	-	-	-	-	-	-	-	0.008	0.029	0.009	0.005	-	-
204	-	0.012	0.013	-	-	-	-	-	-	-	-	-	0.009	-	-	-
208	0.023	-	-	-	-	-	-	-	-	-	-	0.059	-	-	-	-
212	-	0.012	-	0.024	-	-	-	-	-	-	0.020	-	-	-	-	-
216	-	-	-	-	0.031	-	-	-	-	-	0.016	0.029	0.009	-	-	-
220	-	0.012	0.013	-	-	0.016	-	0.100	0.051	-	0.008	-	-	-	0.273	0.239
224	0.102	0.107	0.075	0.048	-	0.148	-	0.200	0.269	-	0.059	0.088	0.060	0.076	0.136	0.065
228	-	0.095	0.075	-	-	0.039	-	0.020	0.077	0.500	0.023	0.059	0.009	0.028	-	-
232	0.034	0.060	0.038	0.024	-	0.133	-	0.040	0.077	-	0.109	0.118	0.181	0.179	0.136	0.261
234	-	-	-	-	0.031	-	-	-	-	-	-	-	-	-	-	-
236	0.273	0.250	0.225	0.095	0.344	0.227	0.063	0.280	0.218	0.417	0.320	0.265	0.276	0.302	0.046	0.174
240	0.057	0.012	-	-	0.063	0.063	-	-	-	-	0.039	0.029	0.043	0.014	-	0.065
244	0.011	0.095	0.138	0.071	0.094	0.078	0.438	0.140	0.128	0.083	0.066	0.088	0.009	0.009	-	0.022
248	0.034	-	-	-	-	-	-	-	-	-	0.020	0.059	0.043	0.019	-	-
252	-	0.012	-	0.024	0.031	-	-	-	-	-	0.008	0.029	-	-	0.046	-
254	0.011	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
256	0.011	0.024	0.025	-	-	0.008	-	-	-	-	0.004	0.029	-	-	-	-
260	-	-	-	-	-	-	-	-	-	-	0.004	-	-	-	-	-
264	0.034	0.024	0.025	-	-	0.047	-	-	-	-	0.063	0.029	0.086	0.024	-	-
268	0.068	0.036	0.025	0.262	0.156	0.016	-	-	-	-	0.027	-	0.009	0.019	-	-
272	0.102	0.119	0.100	0.143	-	0.023	-	-	-	-	0.074	0.059	0.035	0.090	0.136	-
274	-	-	-	-	-	-	-	-	-	-	0.004	-	-	-	-	-
276	-	-	-	-	-	-	-	-	-	-	-	-	-	0.005	-	-
280	-	0.012	0.025	-	-	0.016	0.125	0.040	0.039	-	-	-	0.017	0.028	-	-
284	-	-	-	-	-	0.008	-	-	-	-	-	-	0.026	0.005	-	-
288	-	-	-	-	-	-	-	-	-	-	-	-	0.026	0.005	-	-
292	-	-	0.013	-	-	0.039	-	0.140	0.115	-	0.020	-	-	0.014	-	-
296	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
300	0.239	0.119	0.213	0.310	0.250	0.141	0.375	0.040	0.026	-	0.102	0.029	0.112	0.160	0.227	0.174
H exp.	0.836	0.873	0.860	0.797	0.779	0.873	0.648	0.827	0.834	0.569	0.853	0.881	0.859	0.834	0.814	0.805
H obs.	0.773	0.810	0.800	0.810	0.750	0.797	0.625	0.880	0.872	1.000	0.813	0.941	0.845	0.793	0.455	0.652
F_{IS}	0.087	0.084	0.082	0.009	0.070	0.095	0.103	-0.043	-0.033	-0.714	0.052	-0.039	0.025	0.054	0.479	0.211
Sig.	*		*												**	*

Central California

Ots-2	PUDY98	NOYA97	NOYA99	ALBA98	ALBY98	RRHA	RRGVY97	RRGVY98a	RRGVY98b	RRGVY00	LAG	LSGAY98	OLEA96	OLEA9798	RWMA97	RWMY98
(N)	44	43	40	19	16	64	7	25	39	7	135	17	67	105	15	24
176	-	-	-	-	-	-	-	-	-	-	0.022	-	-	0.005	0.033	0.021
178	0.023	0.058	0.100	0.026	-	0.063	-	-	-	-	0.026	-	-	0.014	-	-
180	0.671	0.802	0.625	0.763	0.531	0.688	0.929	0.740	0.603	1.000	0.563	0.647	0.754	0.667	0.800	0.646
182	0.057	0.023	0.050	0.053	0.125	0.016	-	-	-	-	0.096	0.029	0.067	0.043	-	-
184	0.011	0.023	0.100	-	0.031	0.102	-	0.120	0.141	-	0.044	0.059	0.015	0.048	0.033	-
186	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
187	0.011	-	-	0.026	0.031	0.031	-	0.020	0.039	-	0.093	0.147	0.067	0.081	-	0.313
188	0.193	0.093	0.113	0.132	0.281	0.102	0.071	0.120	0.218	-	0.156	0.059	0.097	0.143	0.133	0.021
190	0.034	-	0.013	-	-	-	-	-	-	-	-	0.029	-	-	-	-
192	-	-	-	-	-	-	-	-	-	-	-	0.029	-	-	-	-
H exp.	0.508	0.343	0.574	0.396	0.621	0.502	0.133	0.423	0.568	-	0.638	0.550	0.413	0.524	0.340	0.484
H obs.	0.409	0.372	0.600	0.474	0.563	0.500	0.143	0.480	0.667	-	0.585	0.471	0.373	0.514	0.333	0.500
F_{IS}	0.206	-0.073	-0.033	-0.170	0.126	0.011	0.000	-0.114	-0.161	-	0.086	0.174	0.104	0.024	0.054	-0.011
Sig.	*											*				

iso-Ots2	PUDY98	NOYA97	NOYA99	ALBA98	ALBY98	RRHA	RRGVY97	RRGVY98a	RRGVY98b	RRGVY00	LAG	LSGAY98	OLEA96	OLEA9798	RWMA97	RWMY98
(N)	43	43	39	18	16	65	7	25	39	8	128	17	9	103	13	23
199	-	-	-	-	-	0.008	-	-	-	-	-	-	-	-	-	-
201	0.012	-	-	-	-	-	-	-	-	-	0.004	0.029	-	-	-	-
203	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
205	0.477	0.267	0.154	0.333	0.469	0.285	0.500	0.620	0.500	-	0.473	0.353	0.611	0.515	0.423	0.544
207	-	-	0.013	-	-	0.008	-	-	-	-	0.016	-	-	-	-	-
209	-	-	-	0.028	0.031	-	-	-	-	-	-	-	-	-	-	-
211	0.128	0.116	0.205	0.028	0.156	0.123	-	0.060	0.051	-	0.051	0.177	-	0.024	0.077	0.044
213	-	-	0.013	-	-	0.039	0.214	0.020	0.128	0.250	0.016	-	-	0.049	-	-
215	0.116	0.198	0.218	0.250	0.063	0.154	0.286	-	-	-	0.203	0.147	0.222	0.223	0.039	-
217	-	0.047	0.192	-	-	0.069	-	-	-	-	0.066	0.059	-	0.015	0.039	-
219	-	-	-	0.028	-	-	-	-	-	-	-	-	-	-	-	-
221	0.035	0.058	0.026	0.056	0.063	0.046	-	-	0.039	-	0.020	0.029	0.056	0.053	-	-
223	-	-	-	-	-	0.008	-	0.020	0.064	0.313	0.016	-	-	0.005	-	-
225	-	0.035	0.051	-	-	0.008	-	-	-	-	0.023	-	-	-	0.077	-
227	0.233	0.198	0.103	0.278	0.219	0.169	-	0.100	0.218	0.313	0.106	0.206	0.111	0.117	0.346	0.413
229	-	0.012	-	-	-	0.008	-	-	-	-	-	-	-	-	-	-
231	-	0.047	0.013	-	-	0.054	-	-	-	0.125	0.004	-	-	-	-	-
233	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
241	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
245	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
247	-	-	-	-	-	0.023	-	0.180	-	-	0.004	-	-	-	-	-
249	-	0.023	0.013	-	-	-	-	-	-	-	-	-	-	-	-	-
251	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
253	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
257	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
260	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.687	0.827	0.835	0.744	0.699	0.839	0.622	0.569	0.678	0.727	0.716	0.775	0.562	0.666	0.686	0.532
H obs.	0.581	0.721	0.718	0.833	0.813	0.846	0.714	0.560	0.718	0.875	0.703	0.824	0.444	0.680	0.308	0.652
F_{IS}	0.166	0.140	0.153	-0.092	-0.130	0.000	-0.071	0.036	-0.046	-0.140	0.021	-0.032	0.264	-0.016	0.579	-0.204
Sig.															***	

Central California

Ots-3	PUDY98	NOYA97	NOYA99	ALBA98	ALBY98	RRHA	RRGVY97	RRGVY98a	RRGVY98b	RRGVY00	LAG	LSGAY98	OLEA96	OLEA9798	RWMA97	RWMY98
(N)	43	40	31	21	16	64	6	25	39	7	98	16	64	106	1	24
120	-	-	-	-	-	-	-	-	-	-	0.010	-	-	-	-	-
123	-	-	-	-	-	0.016	-	-	-	-	-	-	-	-	-	-
125	-	-	-	-	-	-	-	-	-	-	-	-	-	0.005	-	-
130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
133	0.012	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
135	-	-	-	-	-	-	-	-	-	-	-	0.031	-	-	-	-
137	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
139	0.012	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
141	0.140	0.063	0.048	0.214	0.125	0.094	0.083	0.060	0.039	-	0.179	0.313	0.117	0.231	-	0.250
143	-	-	-	-	-	-	-	-	0.013	-	-	-	-	-	-	-
145	0.326	0.175	0.290	0.143	0.250	0.305	0.667	0.460	0.436	0.500	0.311	0.313	0.352	0.283	-	0.125
147	0.116	0.200	0.307	0.214	0.125	0.180	0.250	-	-	-	0.260	0.125	0.273	0.354	-	0.313
149	0.012	-	-	-	-	0.023	-	-	-	-	-	-	0.039	0.009	-	-
151	0.070	0.038	0.032	0.143	-	-	-	-	-	-	0.026	0.063	0.023	0.005	0.500	0.125
153	0.314	0.488	0.307	0.238	0.375	0.305	-	0.400	0.385	0.357	0.199	0.125	0.164	0.085	0.500	0.042
155	-	-	-	-	-	0.008	-	-	-	-	0.005	-	-	-	-	0.083
157	-	0.038	0.016	0.048	0.031	0.047	-	0.080	0.128	0.143	0.005	0.031	0.031	0.028	-	0.021
159	-	-	-	-	0.094	0.016	-	-	-	-	-	-	-	-	-	0.042
161	-	-	-	-	-	0.008	-	-	-	-	0.005	-	-	-	-	-
163	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.757	0.685	0.724	0.808	0.756	0.770	0.486	0.618	0.644	0.602	0.763	0.768	0.758	0.733	0.500	0.798
H obs.	0.837	0.600	0.710	0.857	0.875	0.813	0.500	0.600	0.718	1.000	0.816	0.875	0.609	0.726	1.000	0.875
F_{IS}	-0.094	0.137	0.036	-0.036	-0.126	-0.047	0.062	0.050	-0.102	-0.615	-0.065	-0.108	0.204	0.014	-	-0.076
Sig.		**											***	*		

One-13	PUDY98	NOYA97	NOYA99	ALBA98	ALBY98	RRHA	RRGVY97	RRGVY98a	RRGVY98b	RRGVY00	LAG	LSGAY98	OLEA96	OLEA9798	RWMA97	RWMY98
(N)	40	41	31	21	15	64	4	25	39	8	114	15	57	105	2	21
193	0.013	0.024	0.016	-	-	0.039	-	-	-	-	0.013	-	0.009	0.019	-	0.048
195	-	-	-	0.048	-	0.016	-	-	-	-	0.013	-	-	0.005	-	-
197	0.050	0.122	0.081	0.071	0.133	0.047	-	0.160	0.282	0.188	0.057	0.067	-	0.024	-	-
201	0.275	0.378	0.307	0.143	0.267	0.289	0.500	0.260	0.218	0.188	0.276	0.400	0.123	0.271	-	0.714
203	-	-	0.016	-	-	0.008	-	-	0.013	-	0.031	-	0.044	0.014	0.500	0.048
205	0.075	-	0.032	-	0.033	0.023	0.125	-	-	-	0.044	-	0.079	0.014	0.250	-
207	0.038	-	0.032	0.071	0.100	0.023	-	-	-	-	0.022	0.033	-	0.019	-	0.024
209	0.163	0.012	-	0.071	-	-	-	0.060	0.090	0.188	0.040	-	-	0.010	0.250	-
211	-	0.012	-	0.095	0.033	0.047	-	-	0.013	-	0.022	0.067	0.035	0.119	-	-
213	0.125	0.012	0.081	0.143	0.167	0.086	-	0.060	0.039	-	0.088	0.100	0.132	0.081	-	-
215	0.125	0.134	0.113	0.095	0.167	0.102	0.125	0.100	0.039	-	0.118	0.067	0.175	0.110	-	-
217	0.063	0.110	0.194	0.095	-	0.117	0.125	0.040	0.051	-	0.105	0.033	0.149	0.076	-	0.024
219	0.050	0.134	0.016	0.119	0.067	0.086	0.125	0.240	0.205	0.438	0.044	-	0.035	0.129	-	0.024
221	-	-	0.016	-	-	0.008	-	-	-	-	0.048	0.033	0.097	0.019	-	0.048
223	-	0.024	0.016	-	-	0.047	-	-	-	-	0.018	-	0.009	-	-	-
225	-	-	-	-	-	-	-	-	-	-	0.004	-	0.018	-	-	-
227	0.025	0.037	0.081	0.048	0.033	0.063	-	0.080	0.051	-	0.057	0.200	0.061	0.076	-	0.071
229	-	-	-	-	-	-	-	-	-	-	-	-	0.035	0.010	-	-
277	-	-	-	-	-	-	-	-	-	-	-	-	-	0.005	-	-
H exp.	0.850	0.791	0.833	0.898	0.838	0.864	0.688	0.824	0.814	0.703	0.874	0.773	0.889	0.863	0.625	0.476
H obs.	0.800	0.732	0.774	0.810	0.933	0.891	1.000	0.720	0.872	0.750	0.842	0.867	0.790	0.867	1.000	0.476
F_{IS}	0.071	0.087	0.087	0.123	-0.080	-0.023	-0.333	0.146	-0.058	0.000	0.041	-0.087	0.121	0.001	-0.333	0.024
Sig.				***				*			*		*			

P-53	PUDY98	NOYA97	NOYA99	ALBA98	ALBY98	RRHA	RRGVY97	RRGVY98a	RRGVY98b	RRGVY00	LAG	LSGAY98	OLEA96	OLEA9798	RWMA97	RWMY98
(N)	43	41	39	21	16	64	6	25	39	8	115	16	68	102	15	23
150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.022
161	0.023	-	-	-	0.031	-	-	-	-	-	-	-	0.007	-	-	-
163	-	-	-	-	-	-	-	-	-	-	0.017	-	0.022	0.025	0.100	-
165	-	-	-	-	-	0.016	-	-	-	-	0.004	-	-	-	-	-
167	-	-	-	-	-	-	-	-	-	-	-	0.219	-	0.010	-	-
169	0.326	0.293	0.244	0.262	0.375	0.172	0.250	0.120	0.141	-	0.291	0.188	0.257	0.324	0.233	0.130
171	0.163	0.073	0.103	0.048	0.094	0.188	-	-	-	0.188	0.170	-	0.029	0.074	0.133	0.196
173	-	-	-	-	-	0.023	-	0.120	0.141	-	-	-	0.015	-	-	-
175	-	-	-	-	-	-	-	-	-	-	0.004	0.031	-	-	-	-
177	0.081	0.037	-	0.048	0.031	0.023	-	0.020	0.077	0.313	0.044	0.375	0.088	0.069	0.100	0.044
179	0.244	0.232	0.346	0.405	0.125	0.258	0.333	0.100	0.077	0.188	0.261	-	0.199	0.304	0.367	0.609
181	-	0.098	0.090	0.119	0.188	0.086	0.250	0.500	0.449	0.188	0.113	0.156	0.360	0.181	-	-
183	0.058	0.110	0.051	0.048	0.031	0.063	0.083	0.100	0.077	-	0.030	0.031	0.007	-	0.067	-
185	0.105	0.159	0.167	0.071	0.125	0.172	0.083	0.040	0.039	0.125	0.065	-	0.015	0.015	-	-
191	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.786	0.807	0.772	0.742	0.781	0.827	0.750	0.699	0.740	0.781	0.798	0.750	0.755	0.759	0.769	0.572
H obs.	0.814	0.854	0.795	0.905	0.625	0.781	1.000	0.800	0.718	1.000	0.791	0.625	0.779	0.735	0.667	0.609
F_{IS}	-0.023	-0.045	-0.017	-0.197	0.231	0.063	-0.250	-0.124	0.042	-0.217	0.013	0.198	-0.025	0.036	0.167	-0.042
Sig.					*								*	*		

Oki-1	PUDY98	NOYA97	NOYA99	ALBA98	ALBY98	RRHA	RRGVY97	RRGVY98a	RRGVY98b	RRGVY00	LAG	LSGAY98	OLEA96	OLEA9798	RWMA97	RWMY98
(N)	39	42	42	20	16	62	5	25	39	6	101	16	64	106	13	23
88	-	-	-	-	-	-	-	-	-	-	0.020	-	-	0.005	0.039	-
92	0.064	-	-	-	-	0.016	-	0.260	0.205	-	0.005	-	-	0.014	0.039	0.152
96	0.039	0.083	0.095	0.025	-	0.210	0.200	0.160	0.167	-	0.064	0.125	0.031	0.047	0.192	0.087
100	0.013	0.060	0.107	0.025	0.094	0.105	-	0.080	0.090	0.167	0.109	0.031	0.063	0.028	0.154	-
104	-	0.024	0.012	0.025	0.031	0.024	-	-	-	-	0.045	-	0.063	0.076	-	0.044
108	-	-	-	-	0.094	-	-	-	-	-	-	-	-	-	-	-
112	0.205	0.107	0.143	0.275	0.063	0.153	-	0.080	0.205	-	0.228	0.188	0.359	0.269	0.192	0.196
116	0.039	0.131	0.143	0.075	0.188	0.097	0.200	0.200	0.180	0.250	0.064	0.156	0.023	0.071	-	-
120	-	-	-	-	-	-	-	-	-	-	-	-	0.039	0.009	-	-
124	0.077	0.107	0.214	0.150	0.063	0.081	0.300	0.080	0.064	-	0.114	0.094	0.180	0.264	0.192	0.304
128	0.154	0.333	0.179	0.150	0.313	0.210	-	0.120	0.051	0.333	0.233	0.313	0.180	0.146	0.154	0.109
130	-	-	-	-	-	-	-	-	0.013	-	-	-	-	-	-	-
132	0.115	0.155	0.083	0.250	0.094	0.081	0.300	0.020	-	-	0.079	0.031	0.047	0.038	0.039	0.109
136	0.295	-	0.024	0.025	0.063	0.024	-	-	0.026	0.250	0.030	0.063	0.016	0.033	-	-
140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
144	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
148	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
152	-	-	-	-	-	-	-	-	-	-	0.010	-	-	-	-	-
160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H exp.	0.821	0.814	0.853	0.809	0.828	0.854	0.740	0.833	0.840	0.736	0.851	0.813	0.793	0.820	0.837	0.813
H obs.	0.744	0.786	0.714	0.700	0.625	0.807	0.600	0.880	0.872	0.833	0.891	1.000	0.734	0.802	1.000	0.826
F_{IS}	0.107	0.047	0.174	0.160	0.275	0.064	0.294	-0.036	-0.025	-0.042	-0.042	-0.200	0.082	0.027	-0.156	0.006
Sig.		*	*		**		**			*		*	*	*		

APPENDIX 2. UCD-BML-SCWA ARCIMS GIS PROJECT

The enclosed CDrom contains sample data, programming code and scripted batch files, for the ArcIMS website: http://sonoma.regis.berkeley.edu/website/bml/salmon. With the exception of the ESRI software and the Windows 2000 Server operating system, all software is available free of charge from the sources listed below. Information about ArcIMS licensing can be obtained from the URL http://www.esri.com. ESRI's ArcView and ArcGIS software packages were also used for pre-processing the geographic data.

We expect and encourage ArcIMS developers to incorporate, borrow and/or modify the methods described here if they are found to be useful.

NOTE: This site may be moved and linked to the following URL at sometime in the near future: http://www.bml.ucdavis.edu

Computing Environment and System Requirements

The software needed to install and run this site includes:

- ESRI ArcIMS 3.1
- Microsoft IIS-Internet Information Server (or Apache 2.0.4 webserver)
- Jakarta-Tomcat 4.0 servlet engine (http://jakarta.apache.org/)
- ActiveState ActivePerl 5.6, PERL programming tools (http://www.activestate.com/)
- gen2shp.exe (a software utility to create a shapefile(tm) from a text file). "gen2shp.exe" is a third-party GNU general public license utility. Un-compiled C source code and additional information can be found at the URL http://www.intevation.de/~jan/gen2shp
- jsImagePlayer (a software utility available at http://sgi.felk.cvut.cz/~xholecko/)

The ArcIMS 3.1 GIS data and processing scripts were designed to run on a Windows 2000 or XP server. Contact ESRI for questions regarding installations using alternative operating systems.

Sample Data Layers Enclosed

Samples of the following data layers and the ArcIMS directory structure are included on the enclosed data disk. Many of the raster layers (e.g. 1m DOQQs and DRGs) on the ArcIMS server are very large files. DOQQs for 7.5 minute quads are ~ 160MB in raw form, for example. Smaller subset samples of each data type were included in order to fit a representative sample on a single CDrom. Data layers used in the GIS include:

Live Data

- CODAR ocean surface current measurements (1,200 sq. mile coverage off Marin and Sonoma Counties)
- NOAA Data Buoy Center buoy locations and live-links for central and northern California
- CDEC (California Data Exchange Center) stream monitoring and real-time live-links for the Sonoma and Marin County area
- USGS stream flow gauge locations and real time live-links for the Sonoma and Marin County area

Marine/Stream

- Bathymetry-(10m contours) for northern California coast from 10-600m
- California watershed boundaries, three levels of aggregation
- Marin County streams
- Olema Creek segmented layer with linked sample data (provided as proof-of-concept)

Political/Governmental

- County boundaries and names
- Major roads

Raster/Image Data

- USGS DOQQ samples at 1m and 2m resolution (Digital orthographic quarter-quads, i.e. geo-referenced aerial photographs)
- USGS DRG samples at 1:24K, 1:100K, 1:250K depending on level of zoom (Digital Raster Graphics, i.e. digital topographic maps)

- Shaded relief layer

Salmon Related

- Coho salmon hatchery locations for northern California
- Coho salmon ESU (for Central California)
- Lagunitas Creek coho spawning sites (J. Watters study)

Description of Custom PERL Scripts and CODAR Data Processing

The following scripts are used for processing the real-time CODAR data:

- *codar.bat*
- *ftp_codar_win2k.pl*
- *process_codar_win2k.pl*
- *gen2shp.exe*
- *cleanup_codar_win2k.pl*

Hourly real-time Total Vector Files are incorporated as data layers into the GIS browser. The 24-hour animation tool is linked to the metadata panel on the bottom of the GIS browser window. The direct link to the CODAR animation tool is: http://sonoma.regis.berkeley.edu/website/bml/codar/animation/jplay.html

CODAR data is processed as follows:
The batch file *codar.bat* runs every hour as a Windows 2000 "scheduled task". This requires PERL (ActiveState) with CPAN PERL Modules: Net::FTP, Time:ParseDate, Date::Manip and the GNU licensed utility *gen2shp.exe* to be installed.

The batch file first executes *ftp_codar_win2k.pl* which ftp's the BML CODAR server, figures out what the 24 most recent CODAR files are and downloads those that are not already on the ArcIMS server. It then determines the most recent Total Vector File and jpeg picture file and downloads those that are not already on the local system (this is a check against those periods when the FTP server is down, otherwise only the latest file would be needed). It then determines the name of the most recent jpg and TVF files, and copies these to files named *codar_tvf_latest.txt* and codar_jpg_latest.jpg. It also makes the **codar.js** file, which is used in the ArcIMS layer list to correctly label the shapefile(tm) with the current date and time.
(Note: CODAR servers are not publicly accessible and require that an ftp account be established for successful login).

The batch file then executes *process_codar_win2k.pl* that converts the CODAR ASCII Total Vector File to an arcgenerate text file (a proprietary format compatible with ESRI GIS software). The execution of *gen2shp.exe* creates the CODAR vector shapefile(tm) layer in the GIS.

The final batch process is execution of *cleanup_codar_win2k.pl* which removes temporary files and files used for creation of the preceding sample. This script creates sorted lists of the latest CODAR jpg and TVF files on the file system, uses the copy command to create the CODAR animation jpg files from the 24 most recent jpg files (named codar1.jpg to codar24.jpg) and then deletes all but the 24 most recent CODAR jpg and Total Vector Files.

The CODAR animation is displayed using a simple Javascript(tm) tool, *jsImagePlayer*, so that it can be run in most web browsers without the user needing to download a plug-in.

Description and Processing of Live-link Data Sources

The following text describes how the live web-based data sources were incorporated into the ArcIMS site:

Example 1:
NOAA Data Buoy Center (NDBC)
URL http://www.ndbc.noaa.gov/

The NDBC develops, operates, and maintains a network of buoy and C-MAN stations.

All stations measure wind speed, direction, and gust; barometric pressure; and air temperature. In addition, all buoy stations, and some C-MAN stations, measure sea surface temperature, salinity,
wave heights and periods. See the website for more information.

Processing Steps:

1. Go to NDBC web site
2. Use buoy station map to identify name, location, and id of all buoys in northern California (north of Monterey Bay).
3. Input this info into a text file in the following format:

```
---------------------------------------------------------------------------
LATITUDE,LONGITUDE,ID,NAME,DESC
41.85,-124.38,46027,ST Georges,Northern CA NOAA National Data Buoy Station
40.72,-124.52,46022,Eel River,Northern CA NOAA National Data Buoy Station
---------------------------------------------------------------------------
```

4. Convert the list of stations to a shapefile in ArcView
5. Using the arcIMS hyperLink functionality (implemented in the arcIMSparam.js file) use the Station ID field to link station points in ArcIMS to data page on the CDEC website, like so:

```
hyperLinkLayers[2] = "NOAA NDBC Stations";
hyperLinkFields[2] = "ID";
hyperLinkPrefix[2] = "http://www.ndbc.noaa.gov/station_page.phtml?$station=";
```

6. The hyperlink information is then used to launch related real-time data web pages on the NDBC website (e.g. http://www.ndbc.noaa.gov/station_page.phtml?$station=46026)

Last updated 09/13/02

Example 2:

California DWR Data Exchange Center (CDEC) River Stage Data
URL is http://cdec.water.ca.gov
Attributes: stream flow, precipitation, humidity air temperature, river stage

Processing steps:

1. Go to CDEC Web site
2. Go to List of all Real-Time Reporting Stations, Sorted By Station Name (our list represents those stations last UPDATED: 05/03/2002).
http://cdec.water.ca.gov/misc/realStations.html
3. Save the list of stations as a textfile
4. Subselect only the stations in the following 4 counties:
Marin, Napa, Sonoma, Mendicino.
5. Reformat as textfile in MS Excel as comma delimited text file, with field names, like this:

```
-----------------------------------------------------------------
Station ,ID ,Elev ,Latitude ,Longitude,County ,River Basin
ARROYO CORTE MADERA MILL VALLEY,ACM,3,37.898,-122.535,MARIN,SF BAY
-----------------------------------------------------------------
```

6. Convert the list of stations to a shapefile in ArcView
7. Using the arcIMS hyperLink functionality (implemented in the arcIMSparam.js file) use the Station ID field to link station points in ArcIMS to data page on the CDEC website, like so:

```
hyperLinkLayers[1] = "CDEC River Stage Data";
hyperLinkFields[1] = "ID";
hyperLinkPrefix[1] = "http://cdec.water.ca.gov/cgi-progs/plotReal?staid=";
```

(e.g. http://cdec.water.ca.gov/cgi-progs/plotReal?staid=ACM)

Last updated 09/13/02 by Patty Frontiera

Example 3:

USGS Stream Flow Data

URL http://waterdata.usgs.gov/ca/nwis/
Attributes: stream flow, water temperature, suspended sediments, river stage

The USGS NWIS Webdata website contains location and general information about ground water, surface water, and meteorological sites, including realtime data on current conditions transmitted from selected surface-water, ground-water, and water-quality sites.

Our shapefile of CA NWIS real-time sites on the BML-Salmon Site, represents those sites identified on the NWIS Webdata site on: 2002-07-24 14:05:51 EDT

Processing Steps:

1. Go to USGS NWIS webdata web site for California
http://waterdata.usgs.gov/ca/nwis/
2. Go to Real-time Data > Build table (http://waterdata.usgs.gov/ca/nwis/current)
3. Select "county" as the only site selection criteria and then click "submit"
(http://waterdata.usgs.gov/ca/nwis/current?search_criteria=county_cd&submitted_form=introduction)
4. On next form:
   a) under the Select Sites section, select four counties (Marin, Mendocino, Napa, Sonoma)
   b) Then, under "Choose Output Format" section, select "Site-description information displayed in Tab-separated format"
   c) Under this, select the following fields: Agency, Site identification number, Site name, Decimal latitude, Decimal longitude, County code
5. Save the resultant web page as a textfile.
6. Reformat this textfile in Excel as comma delimitted text file, for example:

```
---------------------------------------------------------------------
SITENO,SITEID,STATION,LAT,LONG,CO_CODE
1,11460400,LAGUNITAS C A SP TAYLOR STATE PK CA,38.0269,-122.7353,41
2,11460600,LAGUNITAS C NR PT REYES STATION CA,38.0803,-122.7833,41
3,11460750,WALKER C NR MARSHALL CA,38.1758,-122.8172,41
4,11462500,RUSSIAN R NR HOPLAND CA,39.0267,-123.1294,45
---------------------------------------------------------------------
```

7. Convert the list of stations to a shapefile in ArcView
8. Using the arcIMS hyperLink functionality (implemented in the arcIMSparam.js file) use the Station ID field to link station points in ArcIMS to data page on the CDEC website, like so:
hyperLinkLayers[0] = "USGS Streamflow Data";
hyperLinkFields[0] = "SITEID";
hyperLinkPrefix[0] = "http://waterdata.usgs.gov/ca/nwis/uv/?site_no=";
hyperLinkSuffix[0] = "&agency_cd=USGS";
9. The hyperlink information is then used to launch related real-time data web pages on the NDBC website, Example:
http://www.ndbc.noaa.gov/station_page.phtml?$station=4602

Projection Information

The shapefile created by the above process is in unprojected, geographic coordinates. However, the data served by ArcIMS for this project is in UTM Zone 10, NAD83. To resolve this, ArcIMS is used to reproject the shapefile on the file to UTM. Since it is such a small file, there is negligible performance issue.

Additional Information

See the file **codar_readme2.txt** on the CDrom for more details on the scripts used to process the codar data and installation notes. For information regarding the development of this site, contact Patty Frontiera at pattyf@regis.berkeley.edu or visit http://www.regis.berkeley.edu. For information on CODAR Ocean Sensors email support@codaros.com or visit http://www.codaros.com.

APPENDIX 3. RESPONSE TO REVIEWERS' COMMENTS

SCWA has taken the unusual step of soliciting three outside reviews of the final report for this contract, one from an academic researcher and two from private consultants. On the whole, these reviews laud the report as containing "very valuable research," "impressive effort," and "extremely useful information for resource managers."

Our colleague, Dr. Bernie May, has provided the most useful technical comments and suggestions. We incorporate most of Dr. May's specific suggestions (#s 4, 6-9, 11-12, 14-16, 20, 23) in this version of the final report. Other suggestions (3, 18, 19) will be addressed in the manuscript that will ultimately be submitted to a peer-reviewed journal. We did not incorporate the following specific suggestions for the reasons given:

1. The title of the contract used the word biodiversity, of which genetic diversity is an element.

2. We discussed several tasks, which did not yield results and were discontinued, in the 2001 annual report and in the main body of the final report. We chose to highlight the successful elements of the project in the Summary.

5. Objective 4 is simply re-stated from the original contract.

10. Moving Fig. 1 and Table 2 to the page following their first mention would make the <u>Statistical methods</u> section disjointed.

13. Bonferroni correction does not apply in this instance, since we are not performing multiple tests of the same underlying hypothesis across iterations.

17. The collection date of the smolts is the biological datum, related to different times of migration, as demonstrated by the genetic heterogeneity of the later outmigrants.

21. The Green Valley 1998 collections comprise a few large families (n=25, 15) and have relatively few unrelated individuals, so that the estimated N_b is less than N. In other juvenile samples, the smaller family sizes and relatively larger numbers of unrelated individuals, each one of which requires two parents, make $N_b > N$. In our judgment, based on experience with similar calculations for the Sacramento River winter Chinook salmon, the number and sizes of temporal samples would not support estimation of temporal variance with any precision.

22. We based our statement about the CCC and SSF ESUs on the significance of the nodes that unite all populations within these groups (nodes with bootstrap values of 618 and 914 in Fig. 8), which we interpret as support for the monophyly of the CCC and especially the SSF clades. The reviewer is questioning the significance of these groups based on the lack of support for the next deeper node in the tree. The ESU concept, however, is a statement about the similarity of populations within the unit, not a statement about the relative affinities of the ESUs to each other. Moreover, the lack of bootstrap support for the deeper node dividing CCC and SSF is caused, not by affinities of CCC and SSF populations but by spurious affinities of some CCC populations with some populations in the southern group of the SO/NC ESU.

The review from Chris Beasley et al., FishPro, deals mainly with methodological issues. The review often questions the biological relevance of the data and is especially concerned with sampling. We note that our study did concern threatened salmonid populations that are in low abundance. Still, by organizing and coordinating a large network of collectors from throughout the northern California region (this was accomplished primarily in the parent project to this contract), we were extraordinarily successful in sampling the small populations and ESUs of interest. We agree that sample size is an important consideration in a population genetic analysis, which is why the report devotes considerable attention to this issue. We give an especially detailed accounting of how coho salmon samples (listed in Table 2) are split, combined, or adjusted for kinship for further population genetic analyses. The objective of these procedures was to increase sample sizes for populations within and between drainages. That this was achieved is illustrated by comparing the distributions of sample sizes in the collections and the two subsequent data sets (Fig. A.3.1). A shift to the right in the modal sample size and a reduction in the number of samples having 10 or fewer fish are evident comparing the collections (blue bars) to the progressively adjusted data sets (yellow, then red bars).

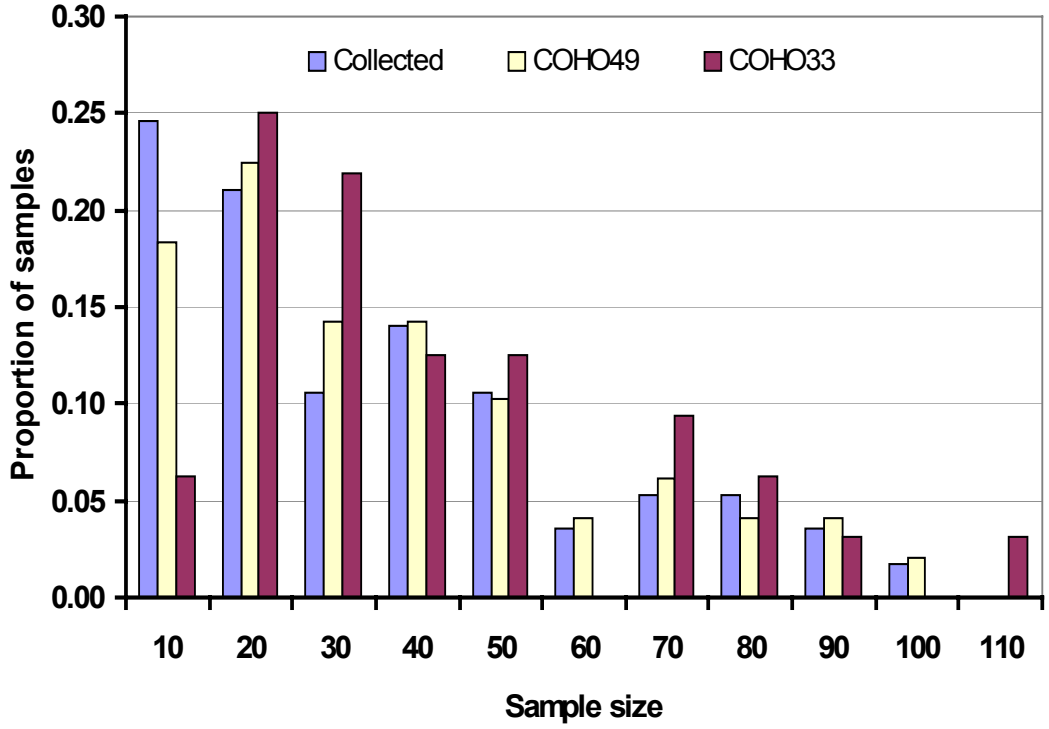

Fig. 1. Distribution of sample sizes in samples of coho salmon, as collected (57 collections, Table 2), after subdividing or dropping certain samples (COHO49, Table 3), and after further corrections for kinship and pooling of homogeneous samples within drainages (COHO33, Table 7; Lagunitas Creek [N=140] omitted).

We note that sample sizes of 20-50 individuals are typical for population genetic studies. As there are two alleles per individual, this number of individuals provides a sample of 40 to 100 alleles per sample, yielding sampling variances for an allele at a frequency of 0.2 between 0.004 and 0.0016, respectively. Finally, unbiased estimates of allelic frequencies can be obtained as long as sampling is done randomly with respect to age class and genotype. With the juvenile

samples, we show how to deal with non-random sampling of age-classes. Moreover, testing for departures from random mating expectations provides a compelling rationale for splitting or combining samples that is based on the extensive evidence that H-W equilibrium is observed in most natural populations of Pacific salmon.

In the statistical evaluation, the FishPro review seeks more rationale for the selection of markers, a discussion of the assumptions of SIBLINGS, and suggests the use of the program MIGRATE to determine effective population sizes and migration rates. As 57 of the 69 markers were eliminated either because they did not work or were not variable (Table 1), we were not compelled to give an elaborate rationale for rejecting those markers. We did select seven markers that seemed to show differences among California samples and rejected five that did not show promise for revealing population structure. The rationale and assumptions for the SIBLINGS program are elaborated in the Banks et al. 2000 reference cited. Estimation of migration rates and effective population sizes in natural populations is fraught with pitfalls. Assumptions underlying the classical method based on the relationship of $F_{ST} = 1/(4Nm + 1)$ are likely to be violated in the highly perturbed coho salmon populations of northern California (Whitlock and McCaulley 1999). MIGRATE makes fewer assumptions but requires data for effective convergence of the likelihood simulation and cannot yet account for variation among subpopulations in effective size and growth rates.

FishPro's biological evaluation raises three issues, the effect of overlapping generations on temporal genetic variance, straying as a potential explanation of linkage disequilibrium in certain samples, and biological justification for binning population samples. Overlapping generations do confound the estimation of temporal genetic variance, though in a more complicated manner than the review suggests. Allele-frequency change between consecutive years overestimates mean temporal genetic variance in the population (Jorde and Ryman 1995), even when mean generational time is determined by a dominant year-class, as it is in coho salmon and the Sacramento River winter Chinook salmon (Churikov, Sabatino, Rashbrook, and Hedgecock, MS in preparation). Again, we did not attempt to estimate temporal genetic variance in any formal manner, because temporal sampling was not adequate. The comments about straying appear to confuse congruence between geographic and genetic distance with disequilibrium in juvenile samples, such those from Green Valley. Straying or admixture of adult stocks would not produce non-equilibrium proportions of genotypes in a cohort of progeny if mating among adults were at random. A Wahlund effect in a juvenile sample would only result from the admixture of progeny produced by different spawning populations, which seems unlikely in the case of the small Green Valley population but likely in other cases (*e.g.* WADY99, SCY99). Moreover, admixture would not produce significant kinship among pairs of individuals. To explain the congruence of geographic and genetic distance among samples, we hypothesize (not assume) that anthropogenic mixing has been ineffective and we propose an alternative explanation, as well. Finally, we partition samples when they show evidence of non-equilibrium genotypic proportions, when we have independent information, such as collection date or size, with which to partition them, and when the resulting sub-samples show equilibrium genotypic proportions. Likewise, we bin samples only when differences in allele frequencies among subpopulations are not significant and the resulting pooled sample conforms to random mating equilibrium proportions. With some samples (*e.g.* ESPRS99, MATS), we split and subsequently bin to differentiate the two causes of non-equilibrium. The justification for these manipulations is

genetic not ecological, but the results do suggest possible biological explanations (*i.e.* that fry emerging at different times or smolts emigrating from estuaries at different times come from genetically divergent populations of adults).

The FishPro review appears to agree with most of the interpretation of the coho results. We do think that Green Valley juveniles are a poor choice of brood stock for a hatchery restoration effort on the Russian River because they come from a very small number of adults and because Green Valley samples, even after adjustment for kinship, do not cluster with other CCC populations (Fig. 7). The congruence of geographic and genetic distance revealed by microsatellite DNA markers is not contradicted by the lack of single nucleotide polymorphisms in the coding genes examined by Kate Bucklin, since the mechanisms and rates of mutation in these two kinds of DNA are quite different.

FishPro raises similar concerns with respect to the Chinook salmon portion of the report. The Forsyth sample of eight adults was too small to stand on its own, so we pooled it with the nearest sample with which it was homogeneous, Mirabel 2000. Genetic distances among Russian River samples are less than 0.01, much smaller than the average distances of about 0.25 between samples from the Russian and Eel Rivers. The alternative pooling of Forsyth with the Mirabel 1999 sample would not alter this outcome. Likewise, temporal variation within the Eel River cannot explain the large divergence between Eel and Russian River samples. The 1998 and 1999 adults in the Eel River are homogeneous, even though temporal change between adjacent years should overestimate temporal variance in the population as a whole. We agree that temporal variation may be larger in the Russian River population than in the Eel River population, but the temporal variation that we have observed in the Russian River is much less than the spatial variation between drainages, a typical result in salmon population genetic studies. Contrary to the opinion expressed by this review, we feel that the samples of Chinook salmon analyzed in this study are sufficient to reach the conclusion that the Russian River stock has not been influenced by either Central Valley or Eel River stocks.

We are pleased that the third review by Ruth Sundermeyer, ENTRIX, Inc., finds that we have fulfilled our contractual obligations and contributed meaningful data. We respond briefly to some of her specific comments.

We do not agree that our summary statements or results "sum up to 'We don't know the effect of stock transfers.'" The congruence of geographical and genetic distance is difficult to explain in the face of concerted efforts to transfer stocks among drainages, basins, and states. Generally, concordance of geography and genetics is achieved over evolutionary time scales. Whatever stock transfers have occurred (and we agree that a detailed account of these would be helpful were it available), they have not erased the phylogeographic pattern. We do entertain the not mutually exclusive hypothesis that drift in small populations may have kept pace with anthropogenic homogenization, but we believe that this explanation is not as likely as the first.

Whether divergence among populations at microsatellite DNA markers reflects the forces of natural selection or random genetic drift in a network of incompletely isolated populations cannot be determined in most population genetic studies and may not be necessary to resolving population units for conservation. We believe that our results support the ESUs identified by the

California ESA, which recognizes a subdivision between the CCC and SSF units. A deviation from H-W that disappears upon partitioning of a sample according to independent information is a clear reflection of population subdivision whether this is caused by adaptive divergence or drift.

We welcome the information regarding the history of the Waddell Creek and Scott Creek coho salmon populations, which may provide insight into their genetic affinities. It is unfortunate that this information has not been published in peer-reviewed journals.

Reference

Jorde, P. E., and N. Ryman. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. Genetics 139:1077-1090.

APPENDIX 4. REVIEWS

Reviews of the penultimate draft of the final report that were solicited by SCWA are attached. The three reviews are from:

Dr. Bernie May, UC Davis
Chris Beasley, FishPRo,
Ruth Sundermeyer, Entrix

Review of Final Report "Documenting Biodiversity of Coastal Salmon (*Oncorhyncus* spp.) in Northern California by Hedgecock et al. Dec. 2002

by Bernie May (Jan. 10, 2003)

UCDavis

General Comments

1. Above all this report describes very valuable research in salmonid genetics, with special relevance to those of Northern California. SCWA clearly received their money's worth over the past five years from the efforts of the Hedgecock laboratory. The comments detailed below are queries and suggestions for the overall improvement of this final report.

2. In general the report is very clear and well organized, although the text features (capital letters, boldface, underlining, italics, etc.) used to indicate hierarchy of headings and subheadings can be confusing. Hierarchical numbering would improve the readability of the text and use of the TOC.

3. Good explanations of basic genetic principles like H-W equilibrium and F_{ST} are given and used effectively throughout the report. If any of this material is still unclear to SCWA, I encourage them to ask for further clarification. This is a great opportunity to get genetic concepts explained.

4. The use of their computer program Kinship to remove the bias of related individuals from juvenile samples is well explained. Removing this bias is a significant contribution by this group of investigators to population genetic studies of any organisms.

5. The report does an excellent job of addressing the objectives outlined in the SOW with a few exceptions. The section on "Alternative male-types" was underdeveloped. The attachment of three vague abstracts provides limited useful descriptions of the experiments and results. In the objectives for the coho study it is stated that protein-encoding gene and mtDNA markers will be developed as well as microsatellite loci; why this was not done should be mentioned more extensively. Also in the coho objectives, it was stated that estimates of migration rates among and effective population sizes of spawning runs would be given, although only the effective population size of the green

Valley Creek samples are presented. Some of these issues were resolved in the 2001 report, but probably should be repeated in this Final report since they are pertinent to the SOW.

6. A final overall section on "Future Needs" or "Recommendations" should probably be added that are directed towar5ds SCWA and not simply scientific interest.

Specific Comments

TITLE:

1. While "biodiversity" was used in the SOW, the results described are primarily about salmonid "genetic diversity".

SUMMARY:

2. Steelhead work is mentioned in the introductory sentence but not discussed further in the summary. The discontinuance of the several portions of the SOW (e.g., steelhead work, historical samples, mtDNA, phylogeny) should be mentioned in the summary.
3. Possible admixtures are offered as an explanation for the deviations from random mating equilibrium. How do you distinguish the effects of admixtures from the effects of stock transfers?
4. "Diversifying effects of genetic drift" (bottom of 1st full paragraph on page 3) – awkward wording since you associate genetic drift with a loss of heterozygosity. Perhaps 3 and 4 could be addressed in the discussion of genetic principles.

POPULATION GENETICS OF COASTAL CALIFORNIA COHO SALMON POPULATIONS:

Introduction

5. In the list of objectives, number 4 would be better split into two objectives.
6. One of the conclusions of the report is that the genetic data corroborates the previously established ESUs for Coho the U.S. Pacific coast. While it is certainly beyond the scope of the report to describe how the ESUs were established in the first place, it would be

nice to see a reference to the document (possibly a Federal Register) that describes the ESUs for Coho. Additionally a map showing the geographical distribution of the ESUs would be helpful.

Materials and methods

7. Page 5 fix wording so as not to use "$(p+q)^2=Np^2+2Npq+Nq^2$" which is not mathematically correct.
8. Pg. 6- last paragraph; the following sentence describing F_{ST} could be more clear. "The genetic correlation between gametes drawn from different demes or subpopulations, with respect to the allelic frequencies in the total population, is given by F_{ST}, the ratio of the variance of allelic frequencies among subpopulations to the maximum." Perhaps the above sentence would be clearer if the term "maximum" were replaced w/ the phrase "variance in allelic frequencies among all subpoulations".
9. Fig. 1 legend should indicate that sample sizes are found in Table 2.
10. Fig. 1 and Table 2 should be included in the text after page 12 (the page where they are first mentioned).
11. Page 8 under "Microsatellite DNA markers": The text states that there were 67 microsatellites for testing, while the table refers to 69.
12. Top of page 13: remove the parentheses around the decimal values… otherwise, it appears that you're multiplying. Try "1/10 or 0.1"
13. In determining significance of F_{ST} values multiple tests were done iteratively by testing a group of populations, removing the most divergent one, and then retesting the group until none of the remaining populations were significantly different. Some discussion of adjusting P values (i.e. Bonferroni corrections) for this kind of testing should be included, since adjustments would greatly effect which populations are excluded and included from the final set.
14. Define LOD abbreviation on page 14.
15. Page 15, KIGHA description: N=15, not 13.
16. Page 16, ESPRS99: Should the populations be 68-92 and 96-110?

17. Page 17, MATS: What biological data supports the initial division of the samples collected 5/7-5/11 and the samples collected 5/12-5/16?

18. Page 18, RRGV98b: It might be of concern that some of the same individuals from 98a may be represented in the 98b population. Was a test for homogeneity of these two populations performed? It's interesting that both 98a and 98b have the same proportion of loci pairs showing significant associations.

Results

19. Color coding (or alternate acronyms) of populations by ESU/river in Figs. 6-8 would help the reader see the geographic relationships presented.

20. Table 3: Does NA signify no amplification?

21. An estimate of the number of breeders was done for the Green Valley samples. Why wasn't this done for any other populations to see if genetic drift can explain the random mating deviations? Alternatively, why were the temporal variation data not used to estimate N_e?

22. Page 30, last paragraph: It is stated that the phylogenetic trees correspond to the designated ESUs. However, the CC and SSF clusters is not supported by the bootstrap analysis. Therefore, I am unclear how the distinction between those 2 ESUs can be confirmed.

23. Pg. 30- I believe they mean Fig. 8 and not Fig. 9 in the last paragraph on this page.

Discussion

24. Under <u>Departures from random mating equilibrium in CA coho salmon populations</u>, third paragraph, it is suggested that the Wahlund effect probably doesn't explain the observed deviations from random mating equilibria due to small spatial and temporal scales involved. Give examples and references that show temporal and spatial homogeneity in other salmonids populations (some researchers have shown significant heterogeneity on small spatial scales (i.e. within tributaries) for east coast U.S. Atlantic salmon).

25. Under Departures from random mating equilibrium in CA coho salmon populations, fifth paragraph, some examples with references regarding temporal genetic heterogeneity would be helpful.

STOCK ORIGIN ESTIMATES FOR CHINOOK JUVENILES CAPTURED IN THE RUSSIAN RIVER

Results

26. Results are written in the present tense, while results of the coho work were presented in past tense.
27. Page 40, Results: Table 10 should be referenced, not Table 2.
28. Since there are no historical samples of Russian River fish, derivation of the current Russian river fish seems unknown. The possibility for inclusion of some hatchery component would still seem feasible. It would seem that more coastal chinook populations should be included in an analysis before any conclusions are drawn.

DEVELOPMENT OF GEOGRAPHIC INFORMATION SYSTEMS

29. Testing a GIS mapserver was interesting. However, I was unable to use the web site at all. Even trying to retrieve a document gave the response "The document that you have requested is currently being processed or updated. Please check back later." Use of these technologies to map genetic data with biogeography will certainly extend our understanding of the effects of environmental variation on the numbers of salmonids and consequently genetic variation. The discussion is clear and extensive about the value of these tools and ways they can be developed and extended. However, this is a general scientific discussion. Specifically, what should SCWA do now? Should they be involved? How should they be involved? Who will maintain the existing mapserver?

Final Note: This is an excellent example of a report from a university investigator to a funding agency. It is unusual to have these reports reviewed. That being said, such reviews should be common occurrences.

Review of Hedgecock et al. 2002:

Documenting Biodiversity of Coastal Salmon (*Oncorhyncus* spp.) in Northern California, **Final Report, December 2002**

Prepared by:
FishPro, a division of HDR
Chris Beasley, principal author

PART I: COHO SALMON ANALYSES

Introduction

In general the researchers have made an impressive effort to elucidate the relationship among coho salmon spawning aggregates in California. That being said, a more detailed account of sampling effort is desperately needed. Throughout the report, the authors note that only juvenile samples were available from some spawning aggregates, and in many cases, the total sample size of those juvenile groups was small. In order to determine whether the results of the analyses are biologically meaningful, the reader must know if sample sizes and sampled life history stages were limited by sampling effort or actual abundance. For example, were temporal replicates from spawning aggregates not available because no one attempted to collect them, or because fish were not encountered after exhaustive sample efforts? Given this limitation, the only meaningful comments that I can make regarding this research are methodology based.

I have grouped comments into three categories: statistical, biological, and interpretation of results.

Statistical Evaluation

The researchers have employed several methods to statistically compensate for small sample sizes and less than ideal distributions of sampled life history stages. However, there are some details that could be profitably expanded upon. For example, a general discussion of marker selection, the assumptions of some of the methods, as well as the potential for utilizing alternative analyses should be included.

The researchers screened some 67 microsatellites from which they selected seven for use in their study of coho salmon. This represents a substantial effort, however it is not entirely clear why some loci were excluded, or why only seven loci were selected. I am not suggesting that the selected loci were insufficient, only that some discussion of the statistical value of the selected loci as well as the tradeoff between using highly polymorphic markers versus markers with less polymorphism is warranted. Was the selection of markers tailored to the methods that the authors intended to use, or were analysis methods selected *post hoc*? In general however, I agree that the selected markers exhibit a degree of polymorphism well matched to the sample sizes that were available, with the possible exception of *Ots-103*, for which the number of alleles is larger than the sample size for some groups.

The use of the programs KINSHIP and SIBLINGS allowed for a much more statistically rigorous examination of the genetic relationships of individuals within subgroups, and hence

allowed for potentially more robust tests of differentiation/relatedness of subgroups and spawning aggregates. However, a discussion of the assumptions required by SIBLINGS, as well as the potential biases introduced by the use of this program is lacking.

Beyond the use of the program SIBLINGS, the analysis of the resulting hypothetical parental and unrelated samples is straightforward and sufficient for the purpose of the report. However, there are some additional analytical tools that might aid in interpretation of the results. For example, the program MIGRATE (Beerli and Felsenstein 2001) could be used as an alternative method for computation of effective population sizes, and additionally could be used to estimate rates of migration between spawning aggregates. For many of the key questions it might be useful to use a number of methods, each with different assumptions, to calculate the quantity of interest. Doing so would erase any doubt that a given result is method dependent and indicate whether results are robust regardless of the methodology used and the assumptions required (and potentially violated).

Biological Evaluation

Some basic life-history characteristics of coho could be discussed in more detail. For example, throughout their range, coho exhibit a relatively fixed three-year life cycle. So, one might expect greater temporal variation between repeated samples from a coho spawning aggregate than for other Pacific salmonids (e.g., chinook salmon) for which overlapping generations might have the effect of decreasing temporal variation within a spawning aggregate. While this feature of coho salmon life history is probably not an adequate explanation for the F_{ST} values observed between temporal samples from some of the spawning aggregates, it could explain at least a portion of that variation. This is particularly true for the "jack" versus adult samples analyzed from the Trinity River Hatchery.

Regarding the congruence of geography and genetics, the authors should consider a discussion of documented straying among coho spawning aggregates from the Pacific Northwest (rather than dismissing the potential contribution of strays to a Wahlund effect – the stated alternative to inbreeding as an explanation for high rates of observed linkage disequilibrium). This is a particularly important point for Green Valley samples, given the history of different broodstock sources potentially introduced to this area from hatchery programs. Additional ancillary data suggest that the assumption that decreased fitness of hatchery stocks may have precluded their contribution to natural production may be faulty. For example, in the Columbia River Basin, reintroductions of coho salmon in the upper Columbia and Snake Rivers, using downriver hatchery stocks, has been enormously successful, suggesting one or more of several alternatives; that coho exhibit a remarkable degree of plasticity, are not as prone to large fitness differentials resulting from local adaptation, and/or are not as greatly effected by hatchery rearing as other species of Pacific salmon. However, these data are available only in the form of "white" papers, and hence are not part of the accessible pool of peer-reviewed literature.

Finally, in an attempt to minimize linkage disequilibrium, the researchers decomposed sample groups into a number of subgroups, some of which were clearly justifiable, but others that are biologically questionable. For example, separating "jack" coho from other adult coho at the Trinity River Hatchery has a biological basis, given that they are of different cohorts, and hence

arise from temporally isolated parental populations. Alternatively, it is not at all clear that binning samples based on capture date from the lower South Fork trap on the Little River is biologically justifiable (it may be, but some justification is required). The same is true for the ESPRS99 sample group (which was subdivided based on a size gap from92 to 96 mm, which could potentially be explained by a few days difference in emergence and growth) and the MATS sample group (subdivided based on apparently arbitrary binning of capture dates). Again, there could be credible biological justification for these subdivisions, but it is not provided.

Interpretation of the Results

Overall, it is my opinion that the researchers present an unbiased interpretation of the results, although the management ramifications of their interpretations could be discussed in greater detail. I agree that the data indicate that the Eel, Russian (Green Valley), and Noyo River samples all exhibit high within group relatedness (e.g., high probability of inbreeding), and that this interpretation is a more plausible, and better supported, explanation for observed linkage disequilibrium than the Wahlund effect. In general, sample groups exhibited high levels of relatedness, however the authors' assertion that inbreeding depression may be contributing to the decline of the species cannot be directly addressed with these data without ancillary information.

I agree with the authors that the Green Valley coho spawning aggregate exhibits a high degree of relatedness, thus the authors rightfully express concern regarding a program that derives broodstock solely from a (apparently) highly inbred population. However, I am confused by the parting statement that "this small population appears to be anomalous and unrepresentative of the Central California ESU." What is intended by this comment? Does its distinctiveness make this spawning aggregate critical for conservation, or are the authors suggesting that recovery efforts for this "anomalous" population should be abandoned?

Conclusions

The stated objectives for this research included: 1) to determine relatedness in samples comprised of juveniles; 2) to determine temporal genetic variation within year classes; 3) to estimate genetic divergence among and effective population sizes of spawning runs; 4) to determine genetic change between historical and extant coho populations; and 5) to relate the genetic diversity of California coho populations to environmental and biological factors being measured in the sampling process. In general, objectives one, two, and three were satisfied, with the exception that available samples limits my faith in the interpretation of results, and with the exception that effective population size was dealt with explicitly only for the Green Valley sample group. Objective four was not addressed, and could not be addressed with the samples that were analyzed. Objective five also was not addressed, but should be given that the interpretation of results relies on the ability of the samples to represent biological reality.

Some puzzling statements were included in the report. For example, the authors suggest that the results support existing ESU designations, but this assertion appears inconsistent with the research completed by Kate Bucklin that found "very little" variation at the nucleotide level. Perhaps more detail could explain the potential discrepancy.

Finally, while it is arguably outside the scope of the contracted work, the authors undoubtedly are in the best position to suggest management alternatives from a genetic standpoint. While management decisions for California coho cannot, and should not, be based solely on genetic considerations, the researchers could provide more directed management guidance. A statement relaying the faith that the authors place in the results would be helpful.

PART II: STEELHEAD ANALYSES

Apparently none of the objectives for steelhead were completed. In addition, the proposed research into candidate genes controlling run timing, while interesting, would have been unlikely to address all listed study objectives.

PART III: CHINOOK SALMON

Introduction

Similar to the review of coho analyses, I have structured comments regarding the analyses pertaining to chinook salmon as statistical, biological, and interpretation. Also, as with the coho analyses, it is unclear why sample sizes and replication of sample groups is less than desirable. Two of the largest sample groups (Warm Springs Hatchery adults samples from 1997) were excluded from analyses based on high rates of observed linkage disequilibria. Are the authors comfortable that the samples made available for analysis adequately represent genetic variation, and hence can be used as a basis for biologically meaningful management decisions?

Statistical

The analyses employed by the researchers to address genetic variation and differentiation among the sampled groups of chinook salmon would be acceptable if sample sizes and temporal replicates were available. However the sample sizes available, and the temporal distribution of those samples decreases my faith in the resulting analyses (see "biological" comments). The researchers emphasize that the resolved Russian River samples cluster together (bootstrap value of 848/1000), but are "distinct" from Eel River samples (bootstrap value of 919/1000), this is a problematic statement for two reasons.

First, given that the Forsyth 1999 sample group was not significantly distinguishable from either Russian River sample group it is not clear why the Forsyth samples were grouped with the Mirabel 2000 samples (why not group them with the Mirabel 1999 samples, or better yet treat them independently?). In this case it might be useful to review the sources of genetic sampling error that might obscure comparisons. Within the Russian River samples for example, eight adult samples from 1999 were grouped with 82 juvenile samples from 2000. We might expect that such a grouping would potentially increase genetic distance between the 1999 Mirabel juvenile sample and the combined 1998 Forsyth adult/2000 Mirabel juvenile sample. In essence, temporal variation might be introduced by the grouping of juvenile and adult samples, as well as grouping samples from different geographic locations (Mirabel versus Forsyth). Although both sources of variation were shown to be insignificant when treated independently, it is possible

that the respective errors, when treated concurrently by grouping samples, could contribute to the perceived distinctiveness in further tests (e.g., Mirabel 2000/Forsyth 1999 versus Mirabel 1999). Unless there is a good reason to group the Mirabel 2000 and Forsyth 1999 sample, I would recommend treating them separately.

Second, adults from the Eel River are being compared to juveniles (and a few adults) from the Russian River. Aside from the fact that the authors suggest this is less than ideal situation; at least in regards to the coho analyses, there are reasons to believe such a comparison for these samples is problematic. Foremost, there is potential for temporal variation to obscure the relationship between Eel River adults and Russian River juveniles. Eel River adults likely arose from spawning in 1993 through 1996, while Russian River juveniles likely arose from spawning in 1998 and 1999. Up to six years of genetic drift (likely exacerbated by small and declining population size) separates these sample groups. While the Eel River spawning aggregates appear to be temporally stable, as evidenced by non-significant differentiation between adults sampled in 1998 and 1999, such stability is apparently not exhibited by Russian River spawning aggregates, as evidenced by significant temporal variation between smolts sampled from Mirabel in 1999 and 2000. How much of the perceived differentiation between these groups could be assigned to temporal variation?

In short, the researchers likely embarked on a series of exploratory analyses that led them to group samples for the final analyses presented in this report. For the reader, it might be useful to show the results of these analyses, or to describe them in more detail. Finally, I would recommend that that the authors present a Cavalli-Sforza and Edwards UPGMA dendrogram that shows the genetic relationship of ungrouped samples. If the overall result (differentiation between Eel and Russian River samples) is supported by analysis of ungrouped samples, interpretation would be more straightforward.

Biological

Regardless of statistical methods employed, the utility of genetic analyses relies on how well samples represent a population of interest. It is not at all clear that the chinook salmon samples used in these analyses are capable of expressing the range of temporal and geographic variation that is exhibited within and among the sampled spawning aggregates. As mentioned above, the most biologically troubling aspect of this analysis is the comparison of temporally distant samples from the Eel and Russian Rivers.

Interpretation

The major finding of this research is that chinook salmon collected in the Eel River appear to be distinct from chinook salmon collected in the Russian River. Whether this result is biologically meaningful remains questionable.

Conclusion

This report was intended to address two tasks: 1) establishment of a genetic baseline for chinook salmon populations from Mendocino and Sonoma counties, and comparison of those spawning

aggregates to known spawning aggregates and 2) to determine the relationship between Russian River and other coastal chinook spawning aggregates including both extant and historical population samples from drainages such as the Eel River. The second task was apparently not addressed by this research, and the first task, while underway, in my opinion is incomplete. It would be helpful if the authors provided a definition for what constitutes a "genetic baseline." My definition, which is only one interpretation, would be a series of samples which when analyzed would yield an estimate of the temporal and geographic variation exhibited by chinook spawning aggregates within a watershed of interest. To construct such a baseline would require temporally repeated samples (ideally taken annually for an entire generation) from geographic locations within a watershed known to support spawning. Comparing such baselines from a number of watersheds would allow a robust construction of the relationships between stocks on a larger geographic scale. Data presented in this report are therefore a good start to such a baseline, but fall short of my definition. As such, the conclusions reported by the researchers are potentially effected by some unknown degree by temporal and/or geographic variation that may not have been measured.

OVERALL CONCLUSIONS

The objective of this review was to determine whether contractual obligations were satisfied by this research. With regard to coho salmon, I would say that the researchers have satisfied their obligation, with the caveats mentioned below. Steelhead objectives have not been achieved, at least as presented here, nor have the objectives for chinook salmon been achieved. Each of the previous statements, however must be bounded by the caveat that a laboratory can only analyze the samples that are received. In general, this research, and the conclusions that can be drawn from the analyses, is severely limited by sample availability.

Aside from sampling considerations, this report would greatly benefit from increased detail regarding the researchers faith in the results. Are the authors convinced that the results of their analyses are biologically meaningful, or do they feel that the results are constrained by sample availability to a degree that limits their utility for management? Given that the researchers are in arguably the best position to provide management guidance from a genetic perspective, it would be useful (and a departure from the ordinary) for them to include a section detailing their respective opinions regarding management.

Finally, the GIS applications as well as the research into alternative male coho phenotypes was not linked to specific contractual obligations – despite the fact that both of these data types could be potentially provide guidance to data analysis.

Literature Cited

Beerli, P. and J. Felsenstein (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by
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Review of Hedgecock *et al*. 2002 Documenting Biodiversity of Coastal Salmon (*Oncorhynchus* spp.) in Northern California

By Ruth Sundermeyer, ENTRIX, Inc., January 2003

Summary

This review of Hedgecock *et al*. 2002 includes comments on the report's scientific merit, methodology, fulfillment of contract terms and conditions, and literature review.

Hedgecock *et al*. 2002 provides extremely useful information for resource managers as they make decisions in resource and recovery planning. Furthermore, the study is written in such a way that should make it easier for managers and biologists who are not geneticists to not only understand the results of the study, but to understand how this information can and can not be used. It will be important for people with local knowledge within their watershed to help interpret the genetic information presented so that informed management decisions can be made. One of the strongest features of the study is the statistical tools developed to address factors that are important to consider when interpreting genetic results, in particular, correction of juvenile samples for sibling relationships. When working with threatened or endangered species, assumptions of hypothetical models or statistical analysis packages can be difficult to meet, and the researchers on this study have made important contributions to the resolution of some of these issues.

Fullfillment of Tasks in the Contract

The major objective of the contract, to describe the genetic diversity of the coho salmon populations along the central and northern coast of California, was fullfilled. Specific tasks that were fullfilled included 1) to determine relatedness in samples comprised of juveniles, 2) to determine temporal genetic variation among year classes and 5) to relate the genetic diversity of California coho populations to environmental and biological factors being measured in the sampling process. Task 3) to estimate genetic divergence among and effective population sizes of spawning runs, was mostly completed, but effective population sizes of spawning runs were not determined for all populations for which this information might be useful. Task 4) to determine genetic change between historical and extant coho populations to assess influence of hatchery plantings and reductions in abundance, is a difficult task that may never be completely resolved. Furthermore, an historical collection free of hatchery influence would be difficult to compile, given the extensive and incompletely documented stocking history.

Tasks in the Chinook portion of the contract were fulfilled.

Specific Comments to Text

Page 3, Summary, end of 2nd paragraph.

"*The congruence of genetic and geographic distance is surprising in light of the history of coho stock transfers within California and between California and other Pacific Coast states*."

However,

"*Stock transfers appear to have left no genetic mark on extant populations. Alternatively, or in addition to stock transfers, the diversifying effect of genetic drift within the relict coho populations of California may be keeping pace with whatever homogenization has been or is being affected by hatchery practices*."

These two sentences sum up to 'We don't know the effect of stock transfers.' The analysis presented in this report does an excellent job of outlining existing population structure, but without an accurate, historical baseline and a more through review of past hatchery practices, analysis of effects of stock transfer remains speculative. It is hoped that resource managers

familiar with local stocking histories and local ecological factors will be able to apply their knowledge to help refine the interpretation of the findings presented in this study.

Page 4 Last paragraph.

This is a good summary to help show how F_{ST} can be interpreted. It might be helpful to add a point about local adaptation as a potential factor for diversity between populations. An artificially induced deficiency of heterozygotes is a different kind of management problem than genetic structure and amount of genetic diversity influenced by natural selection, which may have a direct effect on fitness of the population. Furthermore, population structure that is based on natural selection can change if the adaptive landscape changes, such as large changes in weather patterns associated with extremely wet years and El Nino events, and may also cause changes in H-W and F_{ST}. Although this is a difficult question to answer, conceptually it may be important to when trying to make management decisions based on genetic analysis. A through understanding of historical hatchery practices and local ecological data are essential, especially when one is trying to identify remnant "natural" populations for protection.

It can be difficult to know at what scale management of differentiated population units should occur – one can micromanage populations on too fine a scale within a watershed, or manage on a scale that does not protect local adaptations.

Page 14.

"*Wahlund effect in the original sample would be evidenced by non-significant departures from H-W within subsamples, but significant F_{ST} among subsamples*."

Are there no other possibilities besides an artificially induced Wahlund effect? Subsamples collected from different sites within a watershed – couldn't they be in H-W equilibrium and have significant F_{ST} if admixture occurred with a change in the adaptive landscape other than artificial stocking?

It should be noted that the analysis on subgroups within samples based on information such as year class, size, and geographic information is information that is not routinely presented in studies of this kind, but can be very useful to interpret the population structures observed. It may also help identify admixture due to hatchery influences.

Page 19 Waddell and Scott Creeks

Waddell and Scott creeks provide an interesting case study because there is a long-term juvenile abundance data set for Scott and Waddell creeks spanning almost a decade, as well as relevant ecological information and stocking history that help explain population trends (Smith 2002).

WADY99: "*Samples originating from RM 4.7 were heterogeneous to both RM 3.1 and 3.9 and were removed (WADY99up)*."

SCY99: *Removal of Upper Fork and RM 4.9 samples (SCY99up) yields a homogenous population (SCY99low) with a substantial number of siblings.*

Big Creek hatchery (from the Scott Creek watershed) coho fry were planted in lower Waddell in 1996, (perhaps progeny of SCA95), but not in upper Waddell. Juvenile fish from the 1996 year class in Waddell would have spawned the 1999 year class, including WADY99up and WADY99low, while juvenile fish from the strong Scott Creek 1996 year class would have contributed to the SCA98 and SCY99 samples. This hatchery stocking might help explain why WADY99low is more closely related to SCA9798 (F_{ST} 0.019), SCA95 (F_{ST} 0.014) and SCY99low (F_{ST} 0.017) but WADY99up is more distant to these same populations (F_{ST} 0.076, 0.074, and 0.041 respectively). It also appears that SCY99up is more closely related to SCY99low (F_{ST} 0.024) than to WADY99up (F_{ST} 0.120).

The survival of naturally spawned juvenile fish is more certain in some sections of Waddell and Scott creeks than in others depending on winter storms and summer flow (Smith 2002) and it would be interesting to see if the population structure documented in this study persists. The 1993, 1996, 1999 and 2002 year class set (a set that spans the three year life history of coho) is currently the only viable set in Waddell (Smith 1999, 2002), which makes the WADY99 populations (and 2002 juveniles) important. Smith 2002 describes the status of coho in several streams in the south of San Francisco group in detail.

Page 36 Temporal Variation

Although temporal samples are available for seven sites, additional information is needed to interpret some of these data.

In Scott Creek, presumably the SCA95 population contributed to the SCA98 population. In 1993, a strong juvenile year class was documented (Smith 2002) and precocial females from that year were raised in the hatchery to supplement weak year classes. This suggests that the Scott Creek populations examined in this study may be a better case study for artificially induced year-to-year variation than for natural temporal variation. Furthermore, the persistence of weak year classes could contribute to the genetic variability between year classes, and tests for inbreeding would be helpful.

It would be helpful to examine hatchery stocking and natural factors affecting population trends in other watersheds as well. For example, hatchery planting may contribute to homogeneity of samples between locations and years, such as is found in Lagunitas. The surprising heterogeneity between the Redwood Creek samples (RWMA97 and offspring RWMY98), as well as the fact that they are outliers is a mystery that draws curiosity.

Page 42 Discussion on Chinook results

"Chinook in the Russian River do appear to belong to a diverse set of coastal chinook populations."

Although it is useful to know that Russian River chinook are not closely related to Central Valley or the Eel River chinook, the relationship to other populations in this ESU can not be determined without comparison to additional data, such as hatchery populations from which extensive stocking in the Russian River has occurred. In any case, this may be a difficult question to answer, given the long history of stock transfers in this basin.

References

Smith, J.J. 2002. Distribution and abundance of juvenile coho and steelhead in Gazos, Waddell and Scott creeks in 2002. Department of Biological Sciences, San Jose State University, San Jose, CA.

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