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Title: Patterns of Genetic Inheritance and Variation Through Ontogeny for Hatchery and Wild Stocks of Chinook Salmon

Abstract approved: Redacted for Privacy
Carl B. Schreck

Redacted for Privacy
Hiram W. Li

Although differences between selective pressures in hatcheries and streams have been theorized to cause genetic divergence between hatchery and wild salmonids, evidence of this is lacking. This study was initiated to document the presence or absence of genetic change in hatchery and wild stocks by characterizing genetic traits in fish of various life history stages within a single generation.

Nine biochemical traits (enzyme loci) and 12 meristic traits were characterized for adult fall chinook and one or more juvenile stages of their progeny of the 1984 brood year. Study groups consisted of hatchery-reared and naturally-reared subunits of populations in two tributaries of the lower Columbia River: Abernathy Creek and the Lewis River. Parents of both groups from Abernathy Creek were primarily of hatchery origin, whereas parents of both groups from the Lewis River were primarily of wild origin. The experimental design thus included reciprocal comparisons of hatchery- and wild-reared groups from each of two stocks: one that has been

propagated under hatchery conditions for at least five generations and one that has evolved in a stream environment.

Both biochemical and meristic traits varied among adult and juvenile stages within hatchery and wild groups. Changes in some of these traits appear to have been caused by natural selection. This was true even for Abernathy hatchery and Lewis wild groups, which have been in the same environment for many generations. The direction and/or degree of change in some biochemical and meristic traits differed between hatchery and wild groups from a given stream, suggesting that selective pressures of the hatchery and wild environments differed in those cases. However, it could not be determined from these data whether the observed divergence of traits reflects general differences in hatchery and stream environments, or if it reflects population-specific responses to site-specific environmental conditions. The extent to which patterns of genetic change within a single generation might vary among year classes or generations is likewise unknown.

Evidence of temporal changes in biochemical and meristic traits of hatchery and wild fish within a single generation has important implications regarding the use of those traits to characterize stocks. Assumptions of temporal stability of biochemical or meristic traits within or between year classes should be applied with caution. Sampling strategies of studies involving these characters should account for the possibility of temporal heterogeneity. Finally, these results suggest that workers using allozymes as genetic tags should test the assumption of selective neutrality of the particular allozyme markers being used.

Patterns of Genetic Inheritance and Variation Through Ontogeny for Hatchery and Wild Stocks of Chinook Salmon

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Patrick L. Hulett

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Professor of Fisheries in charge of major

Redacted for Privacy

Professor of Fisheries in charge of major

Redacted for Privacy

Head of Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

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INTRODUCTION

Background

Conservation of the genetic variation present in locally adapted populations of salmonids is important to insure the long-term viability of both hatchery and wild stocks (Smith and Chesser 1981; Altukhov and Salmenkova 1987). In recent years, hatchery stocks have become an increasingly predominant component of the production of steelhead and Pacific salmon (Light 1989; Miller et al. 1990). Accordingly, considerable concern has been expressed regarding the effects of hatchery supplementation on the genetic composition of wild populations of salmonids (Steward and Bjornn 1990). The genetic makeup of wild stocks may be compromised by interbreeding with hatchery fish that have a lower genetic capacity to survive and reproduce in the wild (Leider et al. 1990). It is therefore important to better understand the mechanisms responsible for genetic differences between hatchery and wild stocks, as this may help identify ways to minimize deleterious genetic interactions.

Differences between hatchery and wild stocks of salmonids have been cited for a variety of traits, including: morphology (Schreck et al. 1986; Taylor 1986); smolt outmigration rate (Hansen et al. 1984); age structure, smolt size, survival, and precocious male maturation (Piggins and Mills 1985); natural reproductive success (Chilcote et al. 1986; Leider et al. 1990); use of estuaries by juveniles (Levings et al. 1986; Macdonald et al. 1987); and juvenile

feeding behavior (Sosiak et al. 1979; O'Grady 1983; Johnsen and Ugedal 1986). Hynes et al. (1981) reviewed several traits reported to have been altered by hatchery propagation, including: increases in angling susceptability and in preference for surface water; and decreases in fright response, egg hatchability, fry survival, overall survival, growth rate, temperature and metabolite tolerance, stamina, age at maturity, longevity and variability of biochemical characters. However, the genetic basis of many of these traits is not well documented, with the notable exception of biochemical genetic variation.

Loss of biochemical genetic variability in hatchery stocks has often been attributed to inadequate broodstock size (Cross and King 1983; Stahl 1983; Vuorinen 1984). However, it has recently been shown that hatchery spawning practices can result in lost variation even when the number of broodstock used would be adequate for a randomly mating population (Gharrett and Shirley 1985; Simon et al. 1986; Withler 1988; Leary et al. 1989). Reduced genetic variation may be correlated with reduced growth and reproductive output (Gjerde et al. 1983; Kincaid 1983; Koljonen 1986), developmental stability (Leary et al. 1985a), developmental rate (Danzmann et al. 1986) and metabolic efficiency (Danzmann et al. 1987, 1988). Although hatchery stocks do not always possess less genetic variability than their wild counterparts (Thompson 1985; Schreck et al. 1986), they are often found to be genetically distinct from wild stocks in a given geographical region (Hjort and Schreck 1982; Krieg and Guyomard 1985; Schreck et al. 1986; Reisenbichler and Phelps 1987, 1989).

Hynes et al. (1981) classified genetically influenced changes in hatchery stocks, as being of two general types: 1) intentional changes via selective breeding for desired traits, and 2) unintentional changes, which the authors attributed largely to inbreeding. However, unintentional changes may result from other factors as well. Hatchery rearing conditions (to the extent that they differ from conditions in the wild) may also cause genetic changes as a result of selection for "optimal hatchery-type" fish (Helle 1981). In spite of theoretical expectations that differences in selective pressures of hatchery and wild environments might cause genetic divergence between hatchery and wild stocks, direct evidence of such genetic change is lacking.

Objectives

This study was initiated to document genetic change in hatchery and wild populations by comparing profiles of genetic traits characterized at various life history stages within a single generation, from parental adults through different juvenile stages of their progeny. As a secondary objective, I sought to identify the most probable causes of any changes observed, and possibly provide evidence for the influence of natural selection on the genetically controlled traits being studied. Finally, I hoped that through the comparison of changes in hatchery-reared fish with those of wild-reared fish of the same population, I could make reasonable inferences about the relative effects of selective pressures in hatchery and wild environments in shaping the genetic composition of a stock.

Clarification of Terms

As used here, "hatchery" fish are those that have been reared in a hatchery environment and "wild" fish are those that were spawned and reared in a natural stream environment, regardless of the ancestry of the fish. For the sake of convenience and brevity, I use the term "cohort" to refer to a given brood year of hatchery-reared or wild-reared fish of a particular stock.

My use of the term "natural selection" should also be clarified. In a strict sense, natural selection is a process whereby genetic changes are effected between generations, due to disproportionately higher production of viable offspring (i.e. those that survive to reproduce) by individuals with particular genetic compositions (i.e. those that produce the most "fit" phenotypes). That process may be subdivided into several components, which act on different portions of the life cycle. These components include: zygotic selection (zygote to mature adult), sexual selection (mature adult to mated pair), meiotic drive (mated pair to gamete), fecundity selection (mated pair to zygote) and gametic selection (gamete to zygote) (Hartl and Clark 1989). References to selection or natural selection with regards to the data of this report properly refer to some subset of these components (depending on the portion of the life cycle involved) rather than to the complete process.

METHODS

Study Populations

Populations not previously adapted to a specific environment may be more likely to undergo genetic change in response to that environment's selective pressures. My analysis therefore included characterization of a "stream-adapted" cohort reared in a hatchery environment and a "hatchery-adapted" cohort reared in a stream environment. The opportunity to do this existed for fall chinook (Oncorhynchus tshawytscha) populations in two lower Columbia River tributaries in southwest Washington: Lewis River (Columbia River mile 87.0) and Abernathy Creek (Columbia River mile 54.3).

The Lewis River supports a self-sustaining wild population of fall chinook salmon averaging about 12,500 spawners between 1964 and 1984 (Howell et al. 1985). The Washington Department of Fisheries (WDF) trapped a portion of this run at Merwin Dam (river mile 19.5) in the fall of 1984, spawned the fish, and reared their progeny at the two salmon hatcheries on the Lewis River: Speelyai Hatchery at river mile 29.5 and Lewis River Hatchery at river mile 15.5 (Figure 1a). The fall chinook were spawned at Speelyai Hatchery and reared there from September, 1984 to June, 1985, but completed their rearing (June to October, 1985) at Lewis River Hatchery. Fall chinook from these facilities have been released into the Lewis River intermittently since 1950, with no releases from 1965-1970 and 1972-1975. An average of about 284,300 fingerlings were released annually from 1976 to 1985, after which the program was terminated. Annual releases were substantially lower the last five years, averaging

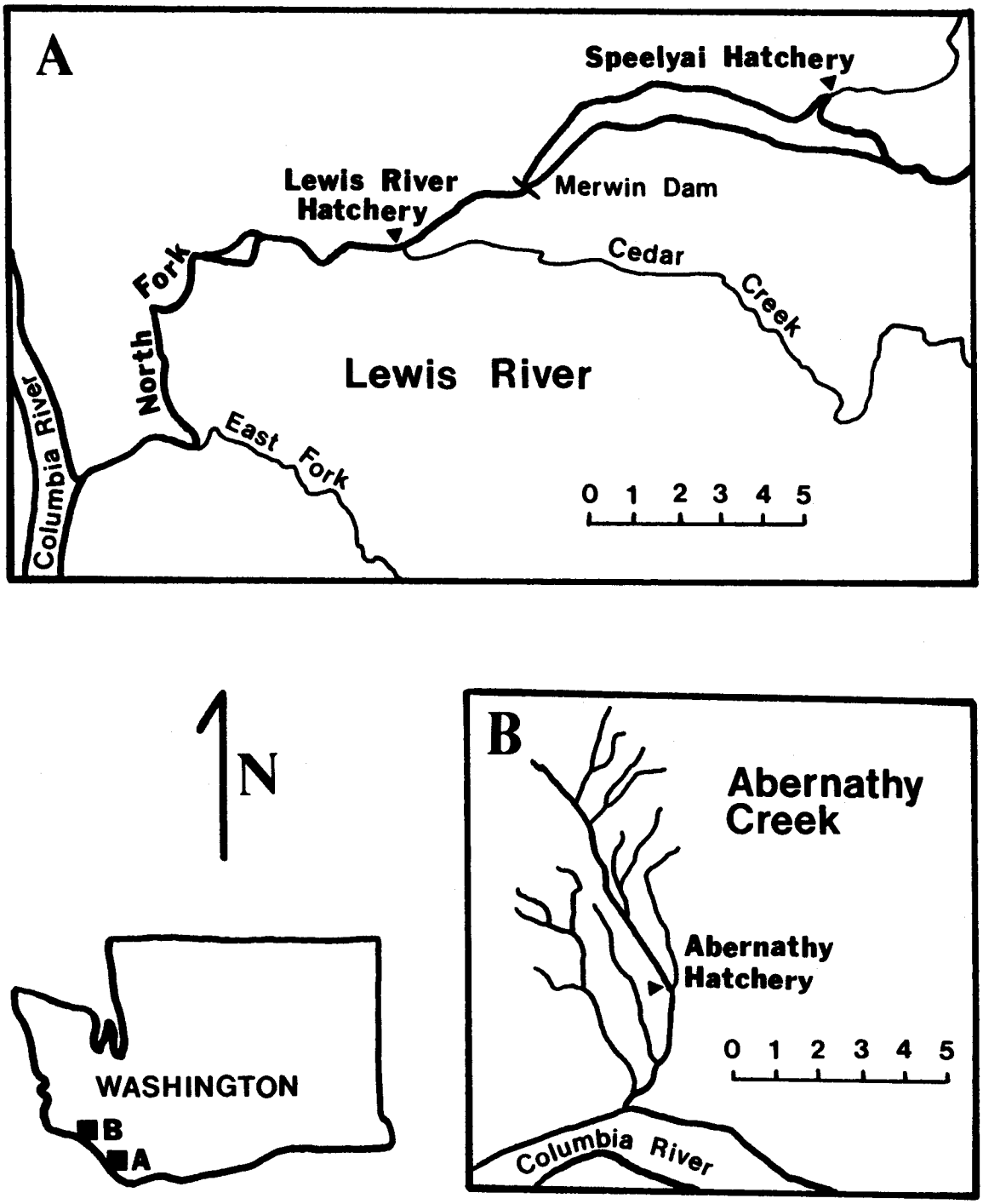

Figure 1. Study streams in southwest Washington: Lewis River (A) and Abernathy Creek (B).

about 199,100 fingerlings (WDF, unpublished data). Broodstock for the hatchery have been primarily of Lewis River wild origin (90%), with only a small contribution from Lewis River hatchery returns (3%) and strays from other lower Columbia hatcheries (7%), based on coded wire tag recoveries from the 1982-1985 return years (WDF, unpublished data). This situation provided the opportunity to monitor a presumably stream-adapted population under natural and artificial rearing conditions.

The U.S. Fish and Wildlife Service operates a hatchery (Abernathy Salmon Culture Technology Center, hereafter referred to as Abernathy hatchery) on Abernathy Creek (Figure 1b) that has produced returns to the hatchery averaging about 1640 adult fall chinook annually from 1964 to 1987. In addition, an average of 1600 fall chinook have attempted to spawn naturally in the limited spawning area available below the hatchery in Abernathy Creek from 1979-1987 (WDF, unpublished data). WDF biologists believe that returning hatchery adults accounted for much of that natural spawning. Some factors which support this contention are: (1) the time of natural spawning coincides with that of hatchery spawning, (2) much of the natural spawning occurs in the segment of Abernathy Creek immediately downstream of the hatchery, and (3) hatchery production far exceeds natural production so that hatchery adults far outnumber naturally produced adults returning to Abernathy Creek. Homing specificity and behavioral isolation mechanisms would likely not be fine-tuned enough to maintain discrete hatchery and wild populations under such conditions. The composite population would therefore be dominated by

the hatchery-reared component and would presumably be best adapted to hatchery rearing conditions. If so, this situation would provide the opportunity to monitor a hatchery-adapted population under natural and artificial rearing conditions. The assumption of hatchery adaptedness of the stream-reared fish was not critical to the study. However, the greater the degree of hatchery adaptedness (and assuming it is achieved at the expense of stream adaptedness) the more likely that selective pressures of the stream environment would cause genetic changes in the stream-reared cohort.

Genetic Characters

Two types of traits were chosen to serve as indicators of genetic change: electrophoretically detectable biochemical genetic characters and meristic characters. The biochemical genetic characters have distinct advantages over most genetic markers (Utter 1981): their variation is purely genetic, without confounding environmental effects; their variation is quantifiable because it reflects their simple Mendelian inheritance; and, their variation can be quantified for relatively large numbers of individuals with a moderate expenditure of effort. A disadvantage of using biochemical genetic characters as genetic markers is that the number of loci examined represents a very small portion of the total genome. Thus, inability to demonstrate genetic differences based on biochemical profiles does not preclude the possibility of genetic differences existing in some other portion of the genome. Clayton (1981) commented on the advantages of using both biochemical and standard taxonomic traits to broaden the scope of genetic characterizations.

With that in mind, meristic characters were included as genetic markers in this study. Meristic characters were chosen over other standard taxonomic traits because they are highly heritable (Leary et al. 1985b), because they are subject to environmental influence only at early stages of embryonic development (Taning 1952) and because they are relatively easy to characterize for a population.

For genetically controlled traits to be useful indicators of genetic change resulting from differential selective pressures in hatchery and stream environments, those traits must have bearing on individual fitness or be genetically linked to traits that do. Whether biochemical traits are subject to natural selection or are selectively neutral (see Kimura 1979) has been a subject of controversy. However, evidence is accumulating which suggests that some biochemical traits are at least linked to fitness-related traits, if not directly affecting fitness themselves (Tsuyuki and Williscroft 1977; Klar et al. 1979a,b; Redding and Schreck 1979; Koehn et al. 1980; Samollow 1980; Northcote and Kelso 1981; Allendorf et al. 1983; Berry and Hudy 1983; Gartner-Kepkay et al. 1983; Samollow and Soulé 1983; Smith et al. 1983; Gauldie 1984; Hilbish and Koehn 1985; Philipp et al. 1985; Chilcote et al. 1986; Torrissen 1987; Dempson et al. 1988; Ferguson et al. 1988; Pemberton et al. 1988; plus others reviewed by Hartl and Clark 1989). Selective advantage of variation in meristic characters has been shown for: vertebrae in the threespine stickleback, Gasterosteus aculeatus (Swain and Lindsey 1984) and in the peamouth, Mylocheilus caurinus (Swain 1988); dorsal spines in the fourspine stickleback, Apeltes

quadracus (Blouw and Hagen 1984a-d, 1987); and the pelvic skeleton and associated spines in the brook stickleback, Culaea inconstans (Reist 1980a,b, 1981, 1983). The relative lack of evidence for adaptive significance in meristic variation (in comparison to the more extensive list above for biochemical variation) may simply reflect the infrequency of studies adequately designed to test for it (Pemberton et al. 1988).

Study Design

I collected samples of adult chinook returning in the fall of 1984 and of different juvenile stages of their progeny (1984 brood year) from each of four cohorts: Lewis hatchery, Lewis wild, Abernathy hatchery, and Abernathy wild. I compared genetic profiles of biochemical and meristic traits between adult and juvenile life stages sampled within each cohort to determine whether the means of any of those traits had changed during the ontogeny of the cohort. Adult genetic profiles were considered to be the starting point and smolt profiles to be the endpoint for genetic comparisons within each cohort. As used here, "smolt" represents the general stage of juvenile development just preceding oceanward migration, and does not necessarily infer that those fish have undergone the physiological process of smoltification. Likewise, "fry" represents a general stage following emergence and initiation of feeding and may include fish of about 35-50 mm in fork length.

Sample Collection

To insure representation of the entire run, I collected adult samples throughout the spawning season, roughly in proportion to

their temporal abundance. Samples of wild adults were collected from spawner carcasses in the Lewis River (25 October - 13 December, 1984) and in Abernathy Creek (18 September - 13 October, 1984). For electrophoresis, I collected tissue samples primarily from "fresh" carcasses to minimize enzyme degradation. Characteristics of "fresh" carcasses included: pink to red colored gill lamellae, clear or only slightly clouded eyes, a rigid body and firm body musculature. Relatively few fresh carcasses were located in Abernathy Creek, despite thorough surveys on five sampling trips during the 1984 spawning season. Low numbers of adults returning and the flushing action of fall freshets both contributed to the small numbers of fresh carcasses located in Abernathy Creek.

I sampled hatchery adults at Abernathy hatchery (18 September - 12 October, 1984) and at Speelyai Hatchery (22 October, 1984) immediately after adults were killed and spawned. Samples were obtained on only one day at Speelyai Hatchery because the spawning season ended unexpectedly abruptly (prior to the peak of wild spawning activity) after only 58 females had been spawned. At that time, my sampling efforts were focused on locating fresh carcasses of wild spawners in the Lewis River. As a result, only 15 adults were sampled for biochemical traits, and none for meristic traits, at Speelyai Hatchery in 1984. I collected a sample of adults from Speelyai Hatchery for biochemical characterization in 1985 (October 16 and November 1) to supplement the scant data obtained from hatchery spawners in 1984. However, I did not substitute the 1985 sample for the 1984 sample during data analyses, because the effects

of between-year variation could not be ascertained.

Tissue samples for hatchery and wild adults consisted of about 1 cm^3 each of heart, white (cheek) muscle and liver tissues, and about 0.5 ml of vitreous humor drawn from the eye using a hypodermic syringe with an 18 gauge needle. I transported adults used for meristic analysis to the laboratory (where they were stored frozen), except for some carcasses from Abernathy Creek which I examined on site.

Juvenile chinook from Abernathy Creek were collected using electroshocking and seining techniques. Juvenile chinook were surprisingly scarce in Abernathy Creek. None were collected that exceeded 45 mm in fork length, despite extensive sampling on 11 dates between 19 January and 1 July, 1985. Larger juveniles apparently leave Abernathy Creek to rear in the Columbia River, although the possibility that they remain in the creek but have extremely poor survival cannot be ruled out. Samples of juvenile chinook from the Lewis River were collected by seining with the assistance of WDF biologists on seven approximately evenly spaced dates between late March and late August of 1985. Unlike the situation at Abernathy Creek, catch per effort was very high on the Lewis River and there was a great range in the size and stage of development of the juvenile chinook caught on each of the sampling trips. Collections consisted of random samples of each catch, because samples obtained by targeting particular developmental stages (e.g. fry or smolts) on successive sampling trips would not represent the progeny of the entire run. To facilitate analysis of meristic and biochemical

characters by developmental stage, I measured the fork length of each fish, and used it as a rough index of developmental stage. I then subsampled fish from the field collections to form samples of "fry" (46-50 mm, $\bar{x}$=47.4 mm) and "smolts" (60-103 mm, $\bar{x}$=69.6 mm) for the Lewis wild cohort.

I used a dipnet to collect samples of fry and smolts from several locations in each raceway or pond at the Lewis and Abernathy hatcheries. In addition, a sample of dead parr were collected at Abernathy hatchery during a period of unusually high mortality (e.g. 13% died in one raceway during February). The dead parr were collected specifically to test whether the elevated level of mortality that occurred during that event was random with respect to the biochemical genetic characters used in this study. Because the mortality was determined to be random, the parr were used as a representative sample of the cohort (i.e. the survivors) at that intermediate juvenile stage.

I froze adult tissue samples and all juvenile samples on dry ice immediately upon collection and stored them at -15° C until electrophoretic and meristic analysis could be done. The sample sizes for biochemical and meristic analyses are shown in Table 1.

Biochemical Characterization

I dissected the liver, one or both eyes and 1 cm^3 of anterior epaxial white muscle tissue from juveniles just prior to electrophoretic analysis. For small fry (<40 mm), I used all skeletal muscle after removing the head, tail, viscera and skin. Tissue samples were combined with 2-4 drops of distilled water or

Table 1. Sample sizes of fall chinook used for electrophoretic and meristic analysis by population, cohort and life stage.

Population Cohort	Stage	Type of analysis	
		Electrophoresis	Meristics
Lewis			
Wild	Adult	187	34
	Fry	200	-
	Smolt	194	20
Hatchery	Adult	15	0
	Fry	100	-
	Smolt	131	20
	1985 Adult	105	-
Abernathy			
Wild	Adult	26	15
	Fry	124	20
Hatchery	Adult	120	25
	Fry	200	-
	Parr	100	-
	Smolt	200	20

tissue buffer (0.0002 M pyridoxal-5-phosphate, 0.05 M Pipes® biological buffer, 0.05% Triton X-100®, pH 6.8) and centrifuged at 1500 x g for 10 minutes at 2° C. Starch-gel electrophoresis methodology followed that of May (1975) and May et al. (1979), with protein-specific staining procedures similar to those of Aebersold et al. (1987). Initially, 20 enzyme systems were examined at 31 loci (Appendix 1). From those, I chose 9 loci that were polymorphic in these populations and were resolved in most of the sample groups to use in the analysis (Table 2). Enzyme nomenclature follows the system described by Shacklee et al. (1990).

Because the adult and smolt samples represent the chronological end points of the cohorts, those samples were analyzed first. Comparisons between those two stages should best reflect any changes in the biochemical genetic profile within a cohort. Fry samples were analyzed only at the loci for which differences had been observed between adults and smolts of a given cohort, except that I analyzed Abernathy wild fry at all loci since no smolts were collected.

Meristic Characterization

Counts of the 12 following meristic characters were made from samples of adults and smolts (fry in the case of Abernathy wild): scales in the lateral series and scales above the lateral line; rays of the left pectoral, left pelvic, dorsal, anal and caudal fins; left brachiostegal rays; upper, lower and total (upper plus lower) gill rakers of the first left gill arch; and vertebrae. Counting procedures followed those of Hubbs and Lagler (1957). Rudimentary rays and gill rakers were included in the counts, except that only

Table 2. Enzyme name, Enzyme Commission (E.C.) number, abbreviation, and mobility of variant alleles (percent mobility of common allele) for nine polymorphic loci of chinook salmon. Peptide substrates used to resolve peptidases are given in parentheses.

Enzyme name	E.C. number	Locus abbreviation	Variant mobilities
Alcohol dehydrogenase	1.1.1.1	*ADH**	-52
Aconitate hydratase	4.2.1.3	*sAH-1**	86, 116
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*sIDHP-3,4**	127, 74
Malate dehydrogenase	1.1.1.37	*sMDH-3,4**	121, 70
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	109, 95
Dipeptidase (glycyl-L-leucine)	3.4.-.-	*PEPA-1**	90
Tripeptide aminopeptidase (DL-leucylglyclyglycine)	3.4.-.-	*PEPB-1**	130
Phosphoglycerate kinase	2.7.2.3	*PGK-2**	90
Superoxide dismutase	1.15.1.1	*sSOD-1**	-260

the primary rays were counted on the caudal fin. Juveniles were examined using a dissecting microscope after the fin rays, branchiostegal rays and gill rakers were highlighted with alizarin red solution and the scales with malachite green solution. I counted vertebrae of smolts from x-ray plates and counted vertebrae of Abernathy wild fry after they had been cleared and stained using the methodology of Potthoff (1984). Vertebrae of adults were counted after removing the flesh from the left side of the body.

I did not count scales on wild adults or fry from Abernathy Creek because of the descaled condition of the available adult carcasses and because of the small size of the fry. I also omitted gill raker counts for Abernathy Creek fry to avoid potential bias caused by differences between fry and adults in the development and visibility of rudimentary gill rakers.

Because incubation temperature can influence meristic counts in salmonids (Mottley 1934, 1937; Taning 1952; Seymour 1959; Kwain 1975), data on incubation temperatures were collected for each cohort to determine the potential influence of this variable on observed differences in meristic counts within a cohort or between hatchery and wild cohorts of the same stream. Daily mean water temperatures for the two hatchery cohorts were calculated from records at Abernathy hatchery and Speelyai Hatchery. Records from Lewis River Hatchery were used for incubation temperature of the Lewis River wild cohort. The hatchery records were considered to reasonably approximate Lewis River incubation temperatures because the temperature was recorded from an inflow pipe of Lewis River water and

because the hatchery is located at the downstream end of the principal spawning area of the river (Howell et al. 1985). Similar data were not available for Abernathy Creek from September through December 1984, because well water, rather than creek water, was used at Abernathy hatchery during that period. Therefore, daily mean temperatures for Abernathy Creek during that period were estimated from the regression of daily means for Abernathy Creek (from Abernathy hatchery records) on those for the nearby Elokomin River (from Elokomin Salmon Hatchery records) for the periods of January through May 1984 and January through October 1985. Point estimates were made from the regression equation: $y = 1.073x + 0.554$, where "y" is the estimated daily mean temperature of Abernathy Creek and "x" is the daily mean temperature of the Elokomin River ($n = 434$, $r^2 = 0.930$, $P<0.0001$).

Standard periods of development for temperature comparisons between hatchery and wild cohorts were defined from the periods of greatest temperature influence (critical periods) identified by Taning (1952) for vertebrae, anal rays, dorsal rays and pectoral rays of Salmo trutta. The critical periods for S. trutta were calibrated to chinook development rates by the formula: $y = 512x / 400$, where "y" is the upper or lower end of the critical period for chinook, "x" is the upper or lower end of the critical period for S. trutta, 512 is the number of Celcius temperature units (CTU, the sum of daily mean temperatures above 0° C) required to hatch chinook (based on Abernathy hatchery chinook at 11.9° C) and 400 is the number of CTUs required to hatch S. trutta (Taning 1952). The starting point

(0 CTU) for the calculation of critical periods was taken as the date on which 50% of the adults had spawned. This date was determined from Abernathy hatchery records for the Abernathy hatchery and wild cohorts (coincidental spawn timing), from Speelyai hatchery records for the Lewis hatchery cohort and from the relative abundance of fresh carcasses encountered during tissue sampling efforts for the Lewis wild cohort. Although fresh carcass abundance is admittedly a crude reflection of spawn timing, the date (November 15) obtained by this method agrees within 2 days of the mean peak spawner count observed by WDF biologists from 1964 to 1982 (Howell et al. 1985).

Data Analysis

A method was developed to evaluate the possible effects of bias in the determination of enzyme banding patterns (scoring bias) on the genotype and allele frequencies for sample sets in which banding patterns for some fish were not identifiable. This evaluation was considered prudent given that the resolution of enzyme banding patterns representing genotypes was variable among sample sets and among loci, and that failure to identify the banding patterns of individual samples on a gel may not be random with respect to genotypes. The method used to evaluate potential scoring bias effects was based on the following assumptions:

(1) On average, heterozygote banding patterns for single locus monomeric or dimeric enzymes are twice as likely to be unidentified on a gel as homozygote patterns.

(2) The ratio of particular genotypes within heterozygote or homozygote classes is not affected by variable enzyme resolution, and is therefore the same among both identified and unidentified samples. (3) Samples that are unidentified on 10 or more consecutive gel spaces are the result of factors unrelated to banding patterns representing particular genotypes and can therefore be excluded from consideration in the evaluation of scoring bias.

The first assumption is based on the expected relative intensities of bands in patterns representing various genotypes (Solazzi 1977). The darkest band in a heterozygote pattern is expected to be one half as intense as that of a homozygote pattern for single locus monomeric or dimeric enzyme systems. Thus, the ratio of heterozygotes to homozygotes among unidentified samples may be expected to be twice that of the full sample set (identified plus unidentified samples). In practice, the "bias factor" (against heterozygotes) of 2.0 was also applied for two duplicate-loci, dimeric enzyme systems, *sIDHP-3,4** and *sMDH-3,4**. This was considered a conservative approach (over-estimating the potential effects of scoring bias) for those two enzyme systems, since the theoretical bias factor would be between 1.8 and 2.0, depending on whether one or two doses of the variant allele were expressed.

A first approximation of the predicted genotype counts among unidentified samples (excluding those in 10 or more consecutive gel spaces) was estimated from the counts observed for the identified samples using the following relationships:

(1) $HTx_u = [2HT/(2HT + HM)](HTx/HT)(N_u)$

where, HTx_u = number of unidentified heterozygotes of genotype "x",

HT = the number of identified heterozygotes,

HM = the number of identified homozygotes,

HTx = the number of identified heterozygotes of genotype "x",

and N_u = the number of unidentified samples, excluding those grouped in 10 or more consecutive gel spaces; and

(2) $HMx_u = [HM/(2HT + HM)](HMx/HM)(N_u)$

where, HMx_u = the number of unidentified homozygotes of genotype "x",

HMx = the number of unidentified homozygotes of genotype "x",

and HM, HT and N_u are defined as above.

The estimated counts for unidentified samples were then added to the observed counts for identified samples to give an estimate of total (observed + unidentified) genotype counts. However, this method underestimates the proportion of heterozygotes among the unidentified samples, because the ratio of heterozygotes to homozygotes among unidentified samples is calculated as twice that among the identified samples (which may be biased) rather than twice that among the full sample set. To correct for this, the procedure just described was repeated iteratively, replacing the "identified" counts in equations 1 and 2 with the total (identified plus unidentified) counts from the previous iteration until a stable genotype count was achieved (usually less than 10 iterations). Equations 1 and 2 thus become:

(3) $HTx_{u(n)} = [HT_{(n-1)}/(2HT_{(n-1)} + HM_{(n-1)})](HTx_{(n-1)}/HT_{(n-1)})(N_u)$

where, $HTx_{u(n)}$ = the number of unidentified heterozygotes of genotype "x" for the nth iteration,

$HT_{(n-1)}$ = the total (identified + unidentified) number of heterozygotes from the previous iteration,

$HM_{(n-1)}$ = the total number of homozygotes from the previous iteration,

$HTx_{(n-1)}$ = the total number of heterozygotes of genotype "x" from the previous iteration, and

N_u = the number of unidentified samples, excluding those grouped in 10 or more consecutive gel spaces; and

(4) $HMx_{u(n)} = [HM_{(n-1)}/(2HT_{(n-1)} + HM_{(n-1)})](HMx_{(n-1)}/HM_{(n-1)})(N_u)$

where, $HMx_{u(n)}$ = the number of unidentified homozygotes of genotype "x" for the nth iteration,

$HMx_{(n-1)}$ = the total number of homozygotes of genotype "x" from the previous iteration, and

$HM_{(n-1)}$, $HT_{(n-1)}$ and N_u are defined as above.

This procedure was used to evaluate potential scoring bias for all sample sets in which more than 2% of the banding patterns were unidentified (excluding those in 10 or more consecutive gel spaces). However, because the assumed relationships between identified and unidentified genotype ratios are based on untested theory, the "bias-corrected" estimates of allele and genotype frequencies were not used to replace the "observed" data in the analyses. Rather, this procedure was used to identify cases in which scoring bias could potentially have affected the "observed" data.

I calculated confidence limits for allele frequencies by interpolating from tables of confidence limits for percentages based on the binomial distribution (Rohlf and Sokal 1981).

Log likelihood ratio analyses (Sokal and Rohlf 1981) were used to identify significant differences in enzyme frequencies among life stages within and between hatchery and wild cohorts from the same stream. The test statistic (G) was calculated at each locus as $G = 2\sum^{c}(f_i[\ln(f_i/\hat{f}_i)])$, where "$f_i$" and "$\hat{f}_i$" are observed and expected frequencies, respectively, for the "ith" class (allele) and "c" is the number of classes (alleles) for the locus being tested. The distribution of the test statistic approximates the chi-squared distribution and critical values can be read from a table of critical values of the chi-squared distribution using (c - 1) degrees of freedom. The correction factor suggested by Williams (1976) was used to to improve the fit to the chi-squared distribution for pairwise tests. I also used log likelihood ratio analysis to test for goodness of fit of observed genotype frequencies to Hardy-Weinberg expectations for each single-locus enzyme system. I could not test the fit to Hardy-Weinberg expectations for the duplicate-loci enzyme systems, *sIDHP-3,4** and *sMDH-3,4**, because allozyme variation could not be attributed to a particular locus. The test statistic was calculated as before, except that the classes represented genotypes and the degrees of freedom were the number of genotypes minus the number of alleles. I used hierarchical log likelihood analyses (Sokal and Rohlf 1981) to test for heterogenity within cohorts and between hatchery and wild cohorts at those loci in which one or more

pairwise test was significant ($P<0.05$). The null hypotheses tested by this procedure were that samples were homogenous: (1) within a hatchery or wild cohort, (2) between a hatchery and wild cohort, and (3) overall (i.e. within Abernathy or Lewis populations). A significant test at the within-cohort level indicates a significant difference among the stages sampled for that cohort. Significant variation at the between-cohort level indicates that, collectively, the samples of the hatchery cohort differ from those of equivalent stages sampled from the wild cohort. Significant total (overall) variation indicates that the entire set of samples (hatchery and wild) for a given stream are unlikely to have been drawn from one homogenous population.

Partial fitness values of genotypes were calculated for cases in which allele frequencies differed among stages sampled within a cohort and selection was not ruled out as the causal mechanism. In most cases, the partial fitness values of the common homozygote and heterozygote genotypes (W_{11} and W_{12}, respectively) relative to that of the alternate homozygote (W_{22}=1.00) were calculated by the following relationships (modified from Redfield 1974):

(5) $W_{11} = (Obs._{11}/Exp._{11})(Exp._{22}/Obs._{22})$

(6) $W_{12} = (Obs._{12}/Exp._{12})(Exp._{22}/Obs._{22})$

where the "Obs." and "Exp." terms are the observed and expected frequencies for the common homozygote (subscript 11), heterozygote (subscript 12) and alternate homozygote (subscript 22) genotypes. However, in some cases alternate homozygote genotypes were too few in number to reliably use that genotype as a reference point. In such

cases, the partial fitness of the common homozygote (W_{11}) relative to that of the heterozygote (W_{12}=1.00) was calculated by:

(7) $W_{11} = (Obs._{11}/Exp._{11})(Exp._{12}/Obs._{12})$

where the terms are defined as above.

In calculating partial fitness values between adult and juvenile stages, the expected genotype frequencies were the Hardy-Weinberg expectations of the adult sample. For comparisons between juvenile stages, the expected values were the sample genotype frequencies of the earlier juvenile stage. In all cases, the observed genotype frequencies were those from the sample collected last.

If two variant alleles were present, they were counted as one allele in calculating partial fitness values. The second variant was too rare (Appendices 5 and 6) to reliably calculate partial fitness values for their genotypes separately. Partial fitness values for duplicate loci (*sIDHP-3,4** and *sMDH-3,4**) were calculated assuming the variation was equally split between the two loci. Calculations made assuming the variation was expressed at only one locus were found to affect partial fitness estimates less than 2%.

I used one-way analysis of variance (ANOVA) to test for significant variation in meristic traits among life stages within a cohort. When an ANOVA was significant, I used the LSD test (Snedecor and Cochran 1980) to determine which samples differed from others.

RESULTS

Biochemical Traits

There appeared to be little potential for scoring bias (as previously defined) to substantially influence the allele frequencies in a vast majority of the 44 cases examined. The absolute difference between the observed allele frequencies and those adjusted for scoring bias was less than 0.020 in all but six cases, and approached significance ($P<0.10$) in only two cases (Table 3). Further, the affect of scoring bias could potentially have contributed to a significant difference in allele frequencies for a pairwise comparison in only one of those cases (Abernathy hatchery fry at the *ADH** locus, to be discussed later). Therefore, apart from the noted exception, the results for biochemical traits are assumed not to have been appreciably affected by scoring bias.

Abernathy Groups

The various stages sampled from the Abernathy hatchery and wild cohorts were not homogenous with respect to biochemical characters. Significant variation was detected at two of the four loci for which heterogeneity was tested among Abernathy samples (Table 4). Enzyme allele frequencies differed among hatchery and wild samples for 12 (16%) of 74 pairwise comparisons (Appendix 2) involving five of the nine loci analyzed (Figure 2). Four of the significant differences were between samples from different stages of the hatchery population and eight were between hatchery and wild samples.

Table 3. The number and percent of samples for which electrophoretic banding patterns were unidentified (N_u), sample size (N) and probability (P) of a greater difference by chance in the observed (Obs.) and scoring bias-adjusted (Adj.) allele frequencies for cases in which the frequency difference was greater than 0.02.

Locus	Sample[a]	N_u		N	Allele 1	Allele 2	Allele 3	P
*ADH**	AH Fry	66 (44%)	Obs.:	84	0.946	0.054		0.063
			Adj.:	150	0.898	0.102		
*sAH-1**	LH Adult	6 (40%)	Obs.:	9	0.778	0.222		0.756
			Adj.:	15	0.738	0.262		
*sAH-1**	LW Adult	42 (23%)	Obs.:	144	0.823	0.170	0.007	0.776
			Adj.:	186	0.801	0.191	0.008	
*PGK-2**	AH Smolt	56 (56%)	Obs.:	44	0.920	0.080		0.014
			Adj.:	100	0.812	0.188		
*PGK-2**	AW Fry	24 (31%)	Obs.:	53	0.830	0.170		0.618
			Adj.:	77	0.806	0.194		
*PGK-2**	LW Fry	46 (46%)	Obs.:	54	0.694	0.306		0.418
			Adj.:	100	0.649	0.351		

[a] LH = Lewis hatchery, LW = Lewis wild, AH = Abernathy hatchery and AW = Abernathy wild

Table 4. Test statistics and degrees of freedom (in parentheses) for hierarchical log likelihood ratio tests of sample heterogeneity within and between hatchery and wild cohorts. Significance levels are: P<0.05 (*), P<0.01 (**) and P<0.001 (***).

Population / Locus	Source of variation: Within cohorts: Hatchery	Within cohorts: Wild	Within cohorts: Total	Between[1] cohorts	Total
Abernathy					
sAH-1	0.10 (1)	0.00 (0)	0.10 (1)	-	6.47* (2)
sIDHP-3,4	6.34 (4)	3.13 (2)	9.46 (6)	0.00 (1)	13.10 (8)
sMDH-3,4	11.57** (3)	2.40 (1)	13.97** (4)	0.88 (1)	15.27** (5)
PEPB-1	0.66 (3)	2.05 (1)	2.71 (4)	2.95 (1)	7.01 (5)
Lewis					
ADH	0.00 (0)	2.23 (1)	2.23 (1)	4.88* (1)	10.62** (2)
sIDHP-3,4	0.79 (2)	9.74** (2)	10.53* (4)	5.64 (2)	16.17* (6)
sMDH-3,4	2.11 (2)	1.94 (1)	4.04 (3)	2.02 (1)	8.45 (4)
PEPA-1	7.18* (2)	6.92* (2)	14.11** (4)	0.19 (1)	14.29* (5)
PGK-2	7.36** (1)	0.20 (1)	7.56* (2)	0.62 (1)	8.18* (3)
sSOD-1	0.41 (2)	4.84 (2)	5.25 (4)	14.70*** (1)	19.95** (5)

[1] Between-cohort tests exclude stages for which data were not available for both cohorts. A dash indicates data from equivalent stages were not available in both cohorts.

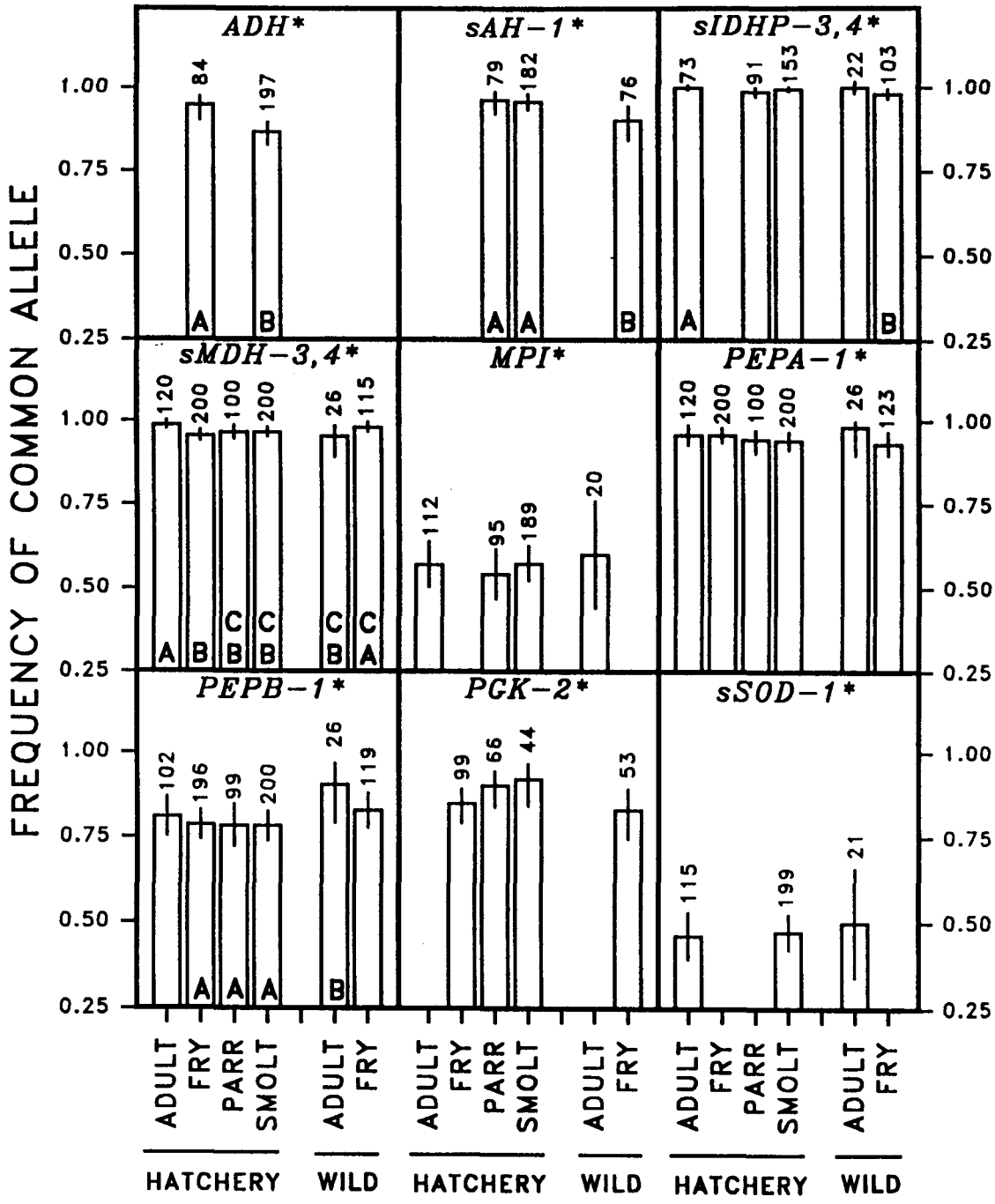

Figure 2. Allozyme frequencies with 95% confidence intervals and sample sizes at nine loci for Abernathy hatchery and wild cohorts of fall chinook from adults through various juvenile stages of their progeny (1984 brood). At each locus, labeled bars that do not share a common letter (A, B, or C) differ ($P<0.05$) from one another. Unlettered bars do not differ ($P>0.05$) from any of the bars at that locus.

Within-Hatchery Variation

Within the Abernathy hatchery cohort, the frequency of *sMDH-3,4*100* was greater in the parental adults than in their fry (P=0.0008), parr (P=0.027) and smolt (P=0.011) offspring (Figure 2), resulting in significant heterogeneity within the cohort at that locus (Table 4). Also, fry had a greater frequency of *ADH*-100* than did smolts (P=0.003) (Figure 2), but this may have been caused by scoring bias against *ADH** heterozygotes in the fry (Table 3). The *ADH** allele frequencies of smolts did not differ (P=0.211) from those of fry when the latter were adjusted for scoring bias (Table 3). The allele frequencies among Abernathy hatchery samples were similar (P>0.05) at the remaining seven loci (Figure 2).

Within-Wild Variation

Abernathy wild adults and fry did not differ (P>0.05) at any of the four loci for which comparisons were possible, but the small sample size for the adults precluded detection of differences of small or moderate magnitudes.

Hatchery vs. Wild Variation

Lack of major differences between allele frequencies of Abernathy hatchery and wild adults suggest genetic homogeneity of the hatchery and wild populations. Although the adults differed (P=0.041) at *sMDH-3,4**, they were similar (P>0.10) at five other loci (Figure 2). The magnitude of difference observed at *sMDH-3,4** is within that expected by chance given that six loci were tested simultaneously for those samples (Cooper 1968). But as previously noted, small to moderate differences would likely not be detected

given the small sample size of the wild adults.

There were significant differences between other Abernathy hatchery and wild samples at four loci. Wild fry had a lower *sAH-1*100* frequency than did hatchery parr (P=0.033) and smolts (P=0.024) (Figure 2). Wild fry also differed from hatchery adults at *sIDHP-3,4** (P=0.022) and from hatchery fry at *sMDH-3,4** (P=0.015) (Figure 2). Collectively, however, the hatchery adult and fry samples did not differ (P=0.348) from the wild adult and fry samples at *sMDH-3,4** (Table 4). Although the *PEPB-1*100* frequency was higher in wild adults than in hatchery fry (P=0.033), parr (P=0.037) or smolts (P=0.029) (Figure 2), heterogeneity between hatchery and wild samples of adults and fry was not significant (P=0.086) (Table 4). Total heterogeneity among all Abernathy samples at *PEPB-1** was also not significant (P=0.220) (Table 4), suggesting that those samples could have been drawn by chance from a single homogenous population.

Hardy-Weinberg Departures

Of 28 cases tested (Appendix 3), genotype frequencies for Abernathy hatchery and wild samples differed (P<0.05) from Hardy-Weinberg expectations at a particular locus in only three cases (Table 5). In all three cases, the departure involved a deficiency of heterozygotes. Only the departures for hatchery adults at *MPI** and for wild fry at *PGK-2** were significant (P<0.05/n) when accounting for the total of "n" loci that were tested simultaneously for those samples (Cooper 1968). None of these departures from Hardy-Weinberg expectations involved loci for which Abernathy hatchery or wild samples differed significantly from one another.

Table 5. Comparison of the number of genotypes observed (obs) and those expected (exp) from Hardy-Weinberg proportions for the samples (H = hatchery, W = wild) and loci in which the observed and expected values differed significantly (P<0.05). Asterisks denote significance of log likelihood ratio test statistic (G) at the appropriate degrees of freedom (df): *, P<0.05; **, P<0.01; and ***, P<0.001. Adult[85] samples are from 1985 spawners (others are from 1984, see text).

Population Sample	Locus		Genotype 1/1	1/2	2/2	1/3	2/3	3/3	G (df)
Abernathy									
H adult	*MPI*	obs:	45	37	29	1	0	0	12.55**
		exp:	36.6	54.3	20.1	0.6	0.4	0.0	(3)
H parr	*PEPA-1*	obs:	91	7	2				5.45*
		exp:	89.3	10.4	0.3				(1)
W fry	*PGK-2*	obs:	40	8	5				9.12**
		exp:	36.5	14.9	1.5				(1)
Lewis									
H fry	*PEPB-1*	obs:	84	11	3				5.42*
		exp:	81.7	15.5	0.7				(1)
H fry	*sSOD-1*	obs:	24	27	22				4.98*
		exp:	19.3	36.5	17.2				(1)
H smolt	*ADH*	obs:	115	13	3				5.59*
		exp:	112.7	17.6	0.7				(1)
H adult[85]	*MPI*	obs:	24	27	33	1	3	0	11.19*
		exp:	16.4	41.5	26.2	1.7	2.2	0.0	(3)
H adult[85]	*PGK-2*	obs:	60	21	11				10.91***
		exp:	54.0	33.0	5.0				(1)
W adult	*MPI*	obs:	20	23	28	1	1	0	8.58*
		exp:	14.0	35.1	21.9	0.9	1.1	0.0	(3)
W adult	*sSOD-1*	obs:	65	45	26				10.45**
		exp:	56.3	62.4	17.3				(1)

Lewis Groups

Samples from the Lewis hatchery and wild cohorts were also heterogenous with respect to biochemical traits. In fact, more so than the Abernathy samples. Five of six loci tested for heterogeneity among samples revealed significant ($P<0.05$) total variation (Table 4), rejecting the null hypothesis that the samples were drawn from one homogeneous population. Allele frequencies differed ($P<0.05$) in 25 (24%) of 105 pairwise comparisons (Appendix 4) involving six of the nine loci analyzed (Figure 3). Of the 25 comparisons in which allele frequencies differed, 2 were between stages within the hatchery cohort, 3 were between various stages of the 1984 hatchery cohort and the parents of the 1985 hatchery cohort, 3 were between stages within the wild cohort and 17 were between hatchery and wild samples.

Within-Hatchery Variation

Heterogeneity among Lewis hatchery samples was significant ($P<0.05$) at two loci, *PEPA-1** and *PGK-2** (Table 4), suggesting that allele frequencies at those loci shifted between stages of the hatchery cohort. Hatchery adults (from 1984) had a higher frequency of *PEPA-1*100* than did their smolt progeny ($P=0.016$). The *PEPA-1*100* frequency of hatchery fry was intermediate to, but not significantly different from, that of the adult ($P=0.061$) and smolt ($P=0.176$) samples (Figure 3). However, heterogeneity (i.e. within-cohort variation) was significant ($P=0.028$) among the three hatchery samples at that locus (Table 4). Also, the adults sampled in 1984 had a higher frequency of *PEPA-1*100* than those sampled in 1985

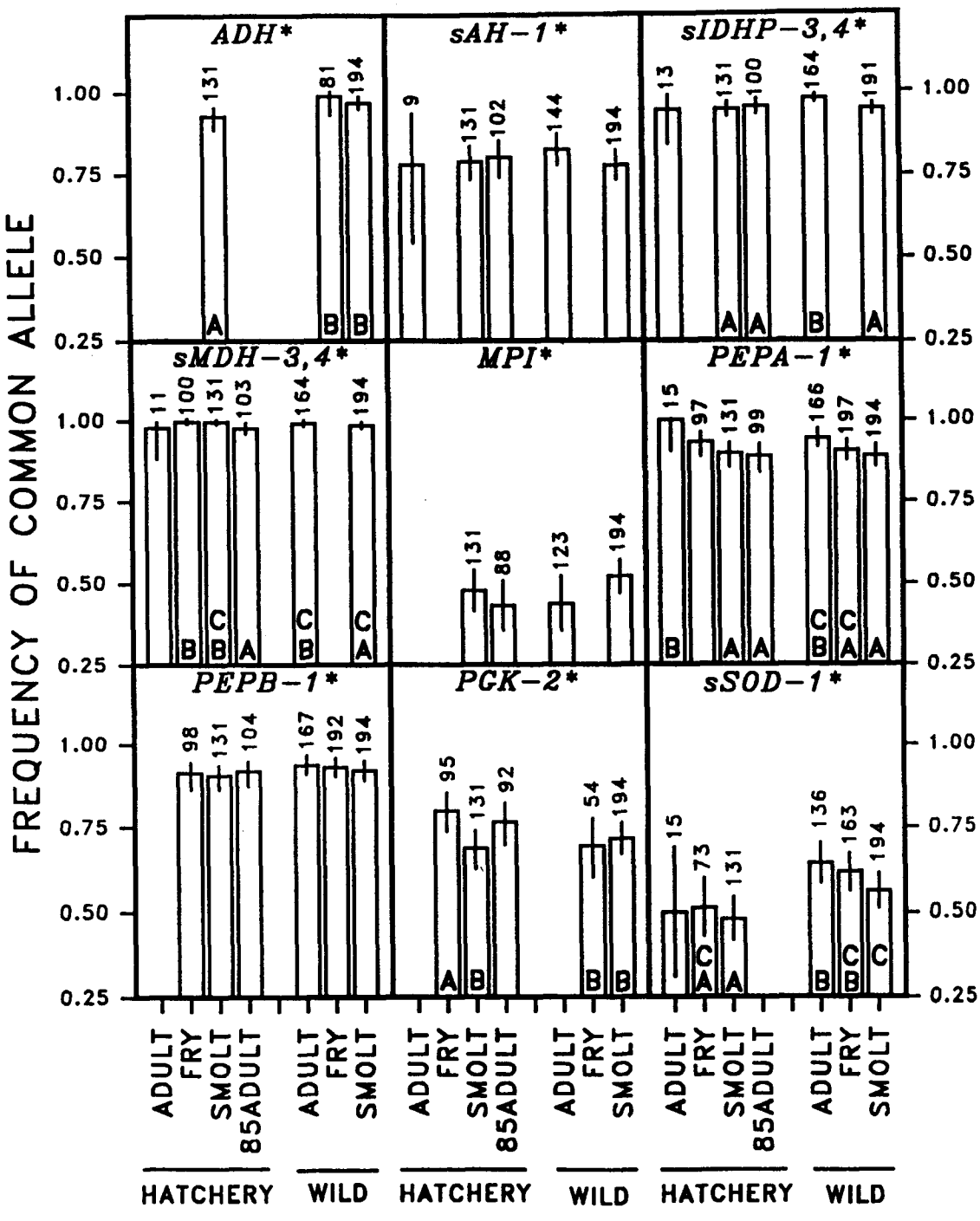

Figure 3. Allozyme frequencies with 95% confidence intervals and sample sizes at nine loci for Lewis hatchery and wild cohorts of fall chinook from adults through various juvenile stages of their progeny (1984 brood; 85ADULTS = parents of 1985). At each locus, labeled bars that do not share a common letter (A, B, or C) differ ($P<0.05$) from one another. Unlettered bars do not differ ($P>0.05$) from any of the bars at that locus.

(P=0.013), indicating significant between-year variation at that locus. It should be noted that the *PEPA-1*100* frequency of 1.000 from the 1984 adults that were sampled (n=15) underestimated the frequency in the hatchery spawners overall, as evidenced by the detection of *PEPA-1*90* alleles in their fry and smolt offspring. However, this does not necessarily invalidate the conclusion that the *PEPA-1*100* frequency of the 1984 adults differed from that of their smolt progeny or from that of the 1985 adults.

Lewis hatchery fry had a higher frequency of *PGK-2*100* than did smolts (P=0.007) from the same cohort (Figure 3), reflecting an increase in the frequency of *PGK-2*90* between the fry and smolt stages. *PGK-2** was not resolved from the adults sampled in 1984. The frequency of *PGK-2*100* for the 1985 adults were similar to that of the 1984 brood fry (P=0.431). However, this information cannot be used to infer a relationship between the *PGK-2** allele frequencies of adults and their fry progeny because the extent of between-year variation (i.e. 1984 adults vs. 1985 adults) is not known.

The hatchery adults sampled in 1985 also differed from the 1984 brood fry (P=0.005) and smolts (P=0.006) at the *sMDH-3,4** locus (Figure 3). Although the *sMDH-3,4*100* frequency of the 1984 adults was similar to that of the 1985 adults, the 1984 adults did not differ from either the fry (P=0.290) or smolts (P=0.330) at that locus, due to the small sample size of the 1984 adults. It is therefore unclear whether the observed differences represent variation between adult return years or a decrease in the *sMDH-3,4*121* frequency between adults and their juvenile offspring.

Allele frequencies were similar among Lewis hatchery samples at *sAH-1**, *sIDHP-3,4**, *MPI**, *sSOD-1** and *PEPB-1** (Figure 3).

Within-Wild Variation

Heterogeneity among Lewis wild samples was significant at two loci, *PEPA-1** (P=0.031) and *sIDHP-3,4** (P=0.008), and approached significance (P=0.089) at a third locus, *sSOD-1** (Table 4). Lewis wild adults had a higher *PEPA-1*100* frequency than did their smolt offspring (P=0.010). As with the hatchery samples, the wild fry were intermediate to, but not significantly different from, the adults (P=0.063) and smolts (P=0.431) at *PEPA-1** (Figure 3). Wild adults also had a greater frequency of the common allele than did their smolt offspring (P=0.009) at *sIDHP-3,4** (Figure 3). Failure to resolve *sIDHP-3,4** from wild fry precluded a more precise identification of the time frame of this allele frequency shift than between adults and their smolt progeny. Similarly, wild adults and smolts differed (P=0.036) in their frequencies of *sSOD-1** alleles (Figure 3), whereas fry were intermediate to (but not significantly different from) adults (P=0.498) and smolts (P=0.139). Heterogeneity test results (within-wild variation, Table 4) suggest that the *sSOD-1** frequencies of the three samples could have been drawn from one homogenous population by chance (P=0.089). However, the heterogeneity test does not take into account the ontogenetic relationship (i.e. time series nature) of the samples. Allele frequencies were similar (P>0.05) among Lewis wild samples at *sAH-1**, *ADH**, *sMDH-3,4**, *MPI**, *PGK-2** and *PEPB-1** (Figure 3).

Hatchery vs. Wild Variation

There was significant heterogeneity between Lewis hatchery and wild cohorts at two loci: *ADH** and *sSOD-1** (Table 4). However, inconclusiveness of the comparisons between the hatchery and wild adult samples precluded determination of whether the observed differences reflect differential shifting of frequencies within each cohort or differences in the initial (i.e. adult) allele frequencies of each cohort. The hatchery smolts had a lower frequency of the *ADH*-100* allele than did either the wild fry (P=0.003) or wild smolts (P=0.027) (Figure 3). Hatchery adults, fry and smolts each had a lower *sSOD-1*-100* frequency than did their wild counterparts at the same stage (Figure 3). Although the differences were significant only at the fry (P=0.037) and smolt (P=0.042) stages, collectively, heterogeneity at the *sSOD-1** locus was highly significant (P=0.0001) between hatchery and wild samples (Table 4).

In contrast, although hatchery and wild samples differed ($P<0.05$) among various stages at *sIDHP-3,4**, *sMDH-3,4**, *PEPA-1** and *PGK-2** (Figure 3), collectively, the hatchery and wild samples were not heterogenous ($P>0.05$) at any of those four loci (Table 4). Furthermore, Lewis hatchery and wild adults were similar ($P>0.05$) at all five loci tested. However, failure to reject the null hypothesis of homogeneity between adults is not surprising given the very small sample size of the hatchery adults. Hatchery adults sampled in 1985 differed (P=0.028) from the wild adults sampled in 1984 at the *PEPA-1** locus. However, it is unclear whether that reflects variation between return years or between hatchery and wild segments

of the population. No differences were detected between any hatchery and wild samples at *sAH-1**, *MPI** or *PEPB-1** (Figure 3).

Hardy-Weinberg Departures

The observed frequencies of heterozygotes were lower ($P<0.05$) than expected under Hardy-Weinberg conditions in seven cases regarding Lewis hatchery and wild samples (Table 5). However, the departures were of a magnitude beyond that expected by chance when testing multiple loci simultaneously (Cooper 1968) only in the 1985 hatchery adults at *PGK-2** ($P=0.001$) and in wild adults at *sSOD-1** ($P=0.001$). Scoring bias, as previously defined, cannot account for the magnitude of heterozygote deficiency observed in either case.

Missing Data

Analyses of biochemical traits were hindered by missing data and small sample sizes. In some cases this was caused by the collection of low numbers of fish at certain stages (e.g. Lewis hatchery adults and Abernathy wild adults). However, in many more cases it resulted from an inability to identify allelic phenotypes at certain loci due to poor resolution of protein banding patterns. For example, I failed to resolve the *ADH** locus for any adult liver samples because that enzyme exhibits low activity in adult chinook. Resolution was also poor at other loci that are expressed primarily in liver tissue, such as *sAH-1**, *sIDHP-3,4** and *sSOD-1**. This was particulary true of fry, which often lacked sufficient liver tissue. I also failed to resolve allelic banding patterns of *MPI** and *PGK-2** from some or all of the individuals in several samples, presumably due to degradation of enzymes during tissue storage. Other loci, such as *GPI-3**, *GR**

and *TPI-4**, were excluded from final analysis because they were infrequently resolved. Complete tables of the allele frequencies for all loci examined are presented in Appendix 5 for Abernathy samples and in Appendix 6 for Lewis samples.

Meristic Traits

Certain meristic traits differed significantly among samples within the Abernathy sample group (pectoral fin rays), the Lewis sample group (scales above the lateral line and gill rakers) or both sample groups (anal fin rays and vertebrae). Mean numbers of lateral series scales, pelvic fin rays, dorsal fin rays, caudal fin rays and branchiostegal rays did not vary significantly among samples within either group (Table 6). The number of primary caudal fin rays was nearly invariant at 19 rays, except in one Abernathy hatchery adult with 18 rays and one Abernathy Creek fry with 20 rays.

Abernathy Groups

ANOVA Results

The significant variation in numbers of pectoral fin rays, anal fin rays and branchiostegal rays among Abernathy samples (Table 6) stems largely from differences between adults and juveniles, both within and between the hatchery and wild cohorts (Figure 4). On average, Abernathy hatchery adults had more pectoral fin rays ($P<0.0005$) and fewer vertebrae ($P<0.05$) than did their smolt offspring. Likewise, Abernathy wild adults had more pectoral fin rays ($P<0.05$) and fewer anal fin rays ($P<0.005$) and vertebrae ($P<0.00005$) on average than did their fry offspring. Regarding comparisons of hatchery vs. wild samples, four of six significant

Table 6. F-ratio, numerator and denominator degrees of freedom (v_1,v_2) and probability (P) of a greater F-ratio by chance from analysis of variance of meristic characters of fall chinook from Abernathy Creek and Lewis River. Asterisks indicate significant variation among samples: * = P<0.05; ** = P<0.01; and *** = P<0.001.

	Abernathy cohorts		Lewis cohorts	
Character	F-ratio (v_1,v_2)	P	F-ratio (v_1,v_2)	P
Lateral series scales	2.62 (1,38)	0.114	0.76 (2,68)	0.473
Scales above lateral line	0.20 (1,39)	0.570	5.06 (2,69)	0.009 **
Pectoral fin rays	7.54 (3,80)	0.0002***	0.10 (2,71)	0.907
Pelvic fin rays	0.53 (3,80)	0.665	0.95 (2,71)	0.392
Dorsal fin rays	0.72 (3,80)	0.544	2.25 (2,71)	0.112
Anal fin rays	4.04 (3,80)	0.010 **	3.71 (2,70)	0.030 *
Branchiostegal rays	1.87 (3,78)	0.141	0.14 (2,71)	0.866
Upper gill rakers	1.44 (2,59)	0.246	7.79 (2,71)	0.0009***
Lower gill rakers	0.15 (2,57)	0.862	7.60 (2,71)	0.001 **
Total gill rakers	0.25 (2,57)	0.780	10.49 (2,71)	0.0001***
Vertebrae	40.00 (3,71)	<0.00005***	7.58 (2,71)	0.001 **

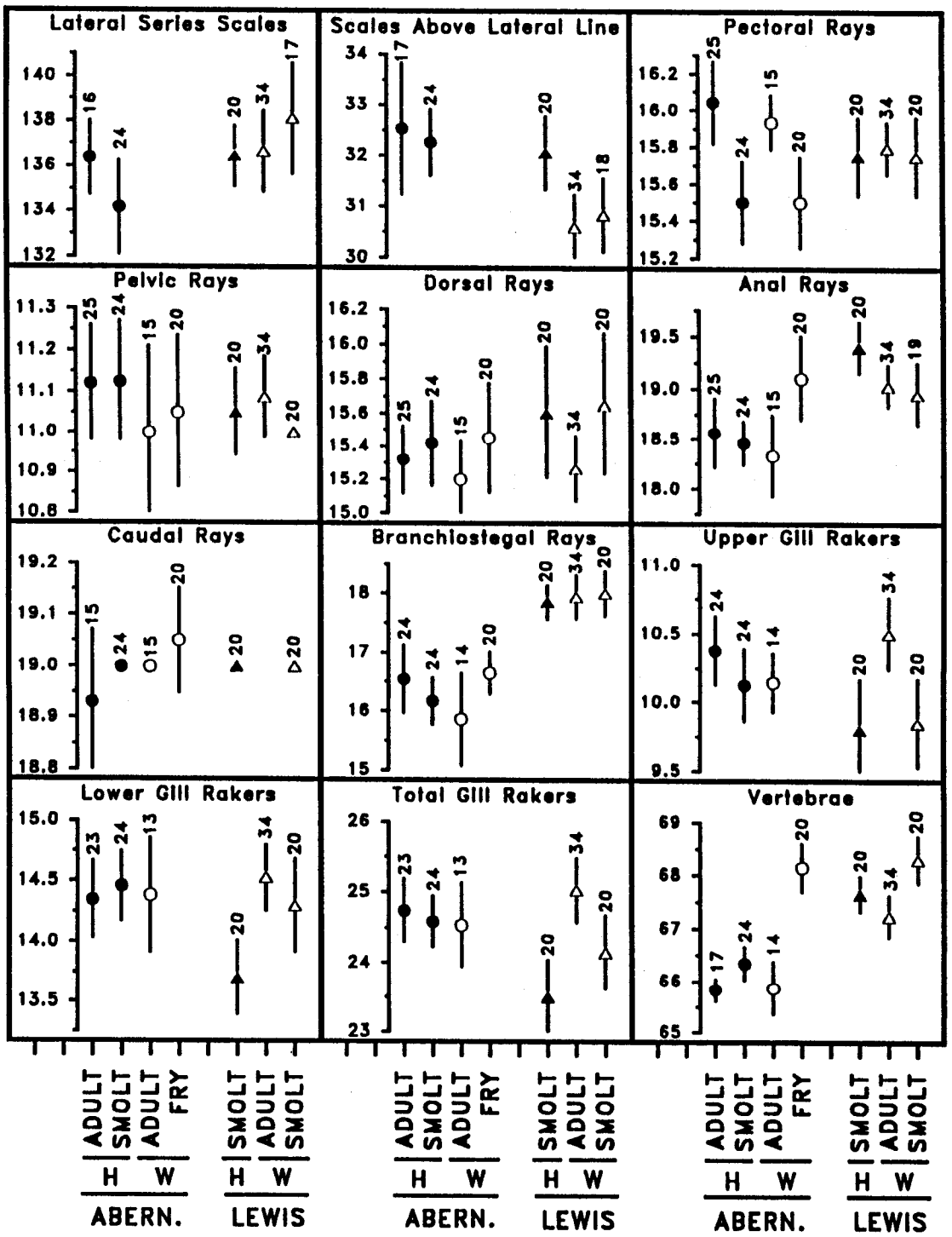

Figure 4. Mean counts, sample sizes and 95% confidence intervals (vertical bars) for meristic characters of adult fall chinook and their juvenile progeny (1984 brood) from Abernathy hatchery (●), Abernathy wild (○), Lewis hatchery (▲) and Lewis wild (△) cohorts.

($P<0.05$) differences were between adults and juveniles and two were between hatchery smolts and wild fry. Hatchery and wild adults did not differ significantly ($P>0.05$) in any meristic traits. Numbers of pectoral fin rays were higher on average in hatchery adults than in wild fry ($P<0.0005$) and were higher in wild adults than in hatchery smolts ($P<0.01$). Wild fry had more anal fin rays than hatchery adults ($P<0.05$) or smolts ($P<0.01$). Likewise, vertebral numbers were much higher ($P<0.00005$) on average in wild fry than in hatchery adults or smolts.

Incubation Temperatures

Comparisons of incubation temperatures for Abernathy hatchery (constant temperature well water) and Abernathy Creek (Figure 5) show that the water temperatures were similar (near 12° C) at the beginning of the incubation period. However, Abernathy Creek cooled rapidly through the fall and winter months to a low of 0.4° C in early February of 1985. This resulted in lower incubation temperatures during the critical periods of meristic development for embryos incubating in Abernathy Creek than for those in Abernathy hatchery. The estimated mean temperatures during those critical periods were lower in Abernathy Creek than in Abernathy hatchery by 1.7° C for vertebrae, 3.8° C for anal fin rays, 4.2° C for pectoral fin rays and 6.0° C for dorsal fin rays. The observed temperature differences were generally within temperature ranges for which Seymour (1959) reported small to moderate influences on meristic traits (Figure 6).

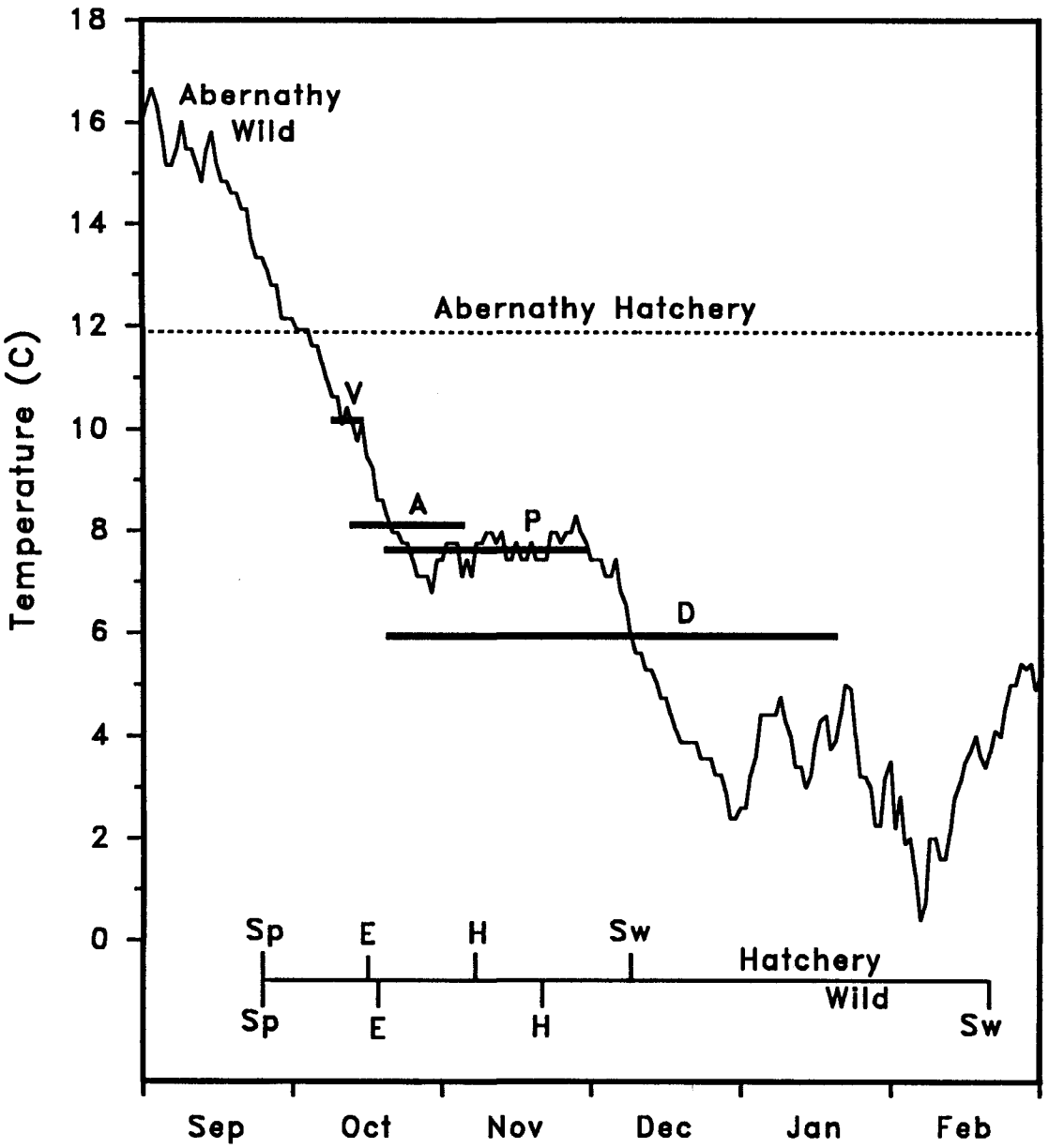

Figure 5. Daily mean water temperatures during the incubation periods (September 1984 through February 1985) of hatchery- and wild-reared fall chinook from Abernathy Creek. Horizontal bars depict the mean temperature and duration of critical periods for determination of vertebrae (V), anal rays (A), pectoral rays (P) and dorsal rays (D) for the wild-reared chinook. The mean dates for time of spawning (Sp) and development of embryos to the eyed (E), hatched (H) and swim-up (Sw) stages are provided as reference points.

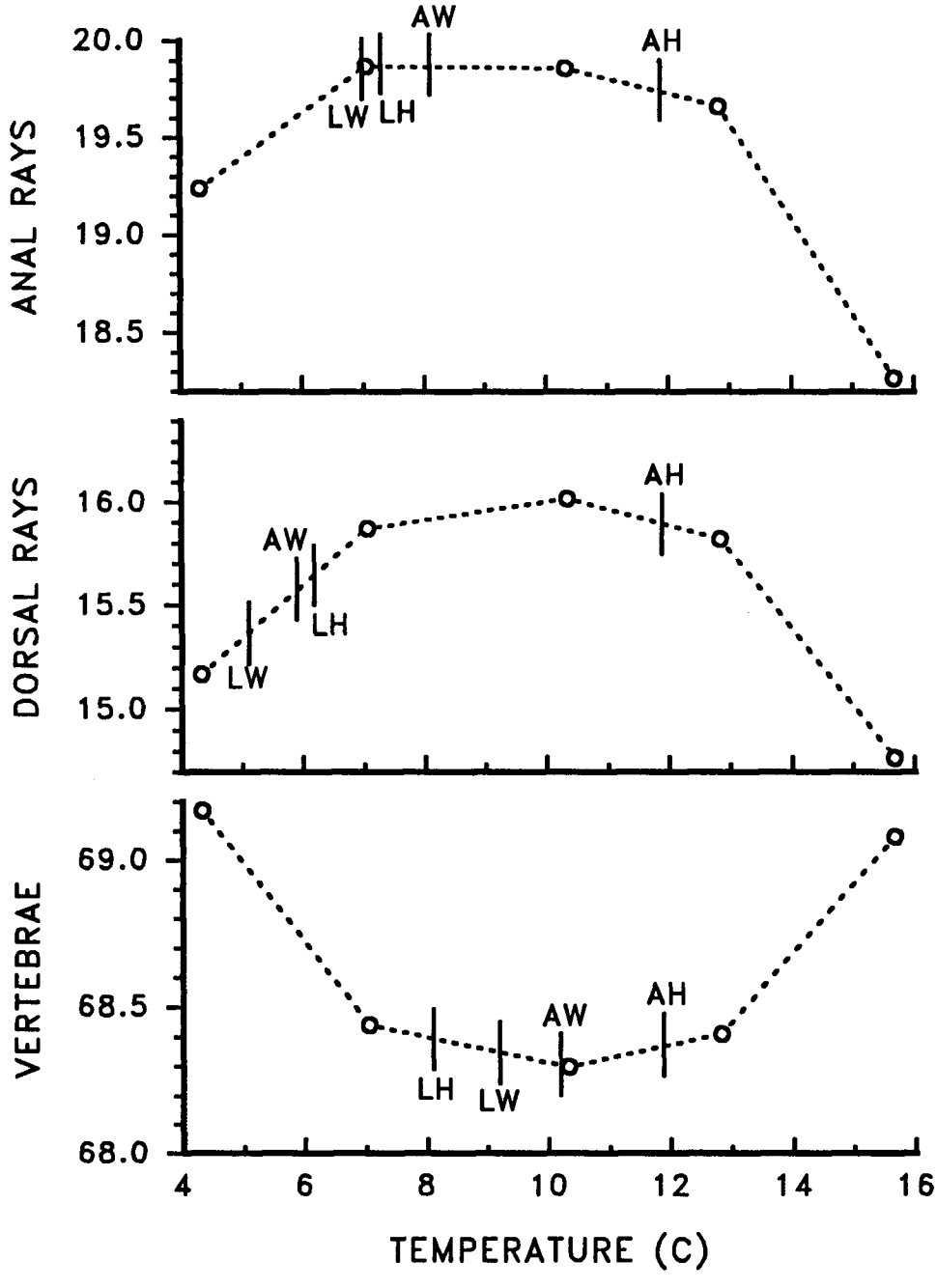

Figure 6. Mean water temperatures during critical periods of determination of three meristic traits for Abernathy (AH) and wild (AW) and Lewis hatchery (LH) and wild (LW) fall chinook of the 1984 brood year. The mean temperatures are plotted along curves depicting the influence of temperature on these three traits, as determined by Seymour (1959).

Lewis Groups

ANOVA Results

Unlike the Abernathy groups, the significant differences in means of six meristic traits among Lewis samples (Table 6) stem largely from differences between hatchery and wild samples (Figure 4). However, this contrast may be an artifact of the lack of data for Lewis hatchery adults, which precluded comparisons of adults and juveniles in the hatchery cohort (as well as comparisons between hatchery and wild adults or smolts). Within the wild cohort, adults had more upper gill rakers ($P<0.005$) and total gill rakers ($P<0.05$) than did their smolt offspring. It should be noted that the difference in total gill rakers is a reflection of the difference in upper gill rakers, since the total count is the sum of the upper and lower counts and the lower gill raker counts did not differ significantly ($P>0.05$). In addition, wild adults had fewer vertebrae ($P<0.0005$) than did their smolt offspring. Hatchery smolts had more scales above the lateral line ($P<0.05$) and more anal fin rays ($P<0.05$) than did wild adults or wild smolts. Numbers of upper gill rakers in hatchery and wild smolts were similarly lower than in wild adults ($P<0.005$). Hatchery smolts had fewer lower gill rakers on average than did either wild adults ($P<0.0005$) or wild smolts ($P<0.05$). As a result, mean numbers of total gill rakers in hatchery smolts were much lower than in wild adults ($P<0.00005$), but were not significantly lower than in wild smolts ($P>0.05$). The mean number of vertebrae in hatchery smolts was slightly less than in wild smolts ($P<0.05$), but not significantly greater than in wild adults ($P>0.05$).

Incubation Temperatures

The water temperatures during early October were substantially higher in the Lewis River (near 16° C) than in Speelyai Creek (about 10° C), which was the water source during the incubation of the hatchery cohort. However, the temperature of the Lewis River declined much more rapidly and steadily through the fall and early winter months. Thus, by January, the Lewis River was cooler than Speelyai Creek, and it remained so until July (Figure 7). In spite of the initial (i.e. autumn) temperature difference, mean temperatures during the critical periods of meristic development were quite similar for Lewis hatchery and wild cohorts. During the critical periods, Speelyai Creek was 1.1° C cooler for vertebrae, 0.3° C warmer for anal fin rays and 1.1° C warmer for pectoral and dorsal fin rays. The main reasons that these values were so similar was because the starting point for incubation (estimated date at which 50% of the adults had spawned) was 22 days later for the wild fish than for the hatchery fish and because the temperature of the Lewis River rapidly approached that of Speelyai Creek during the fall (Figure 7). Again, the observed differences were within temperature ranges for which Seymour (1959) documented rather minor influences of temperature on meristic traits (Figure 6).

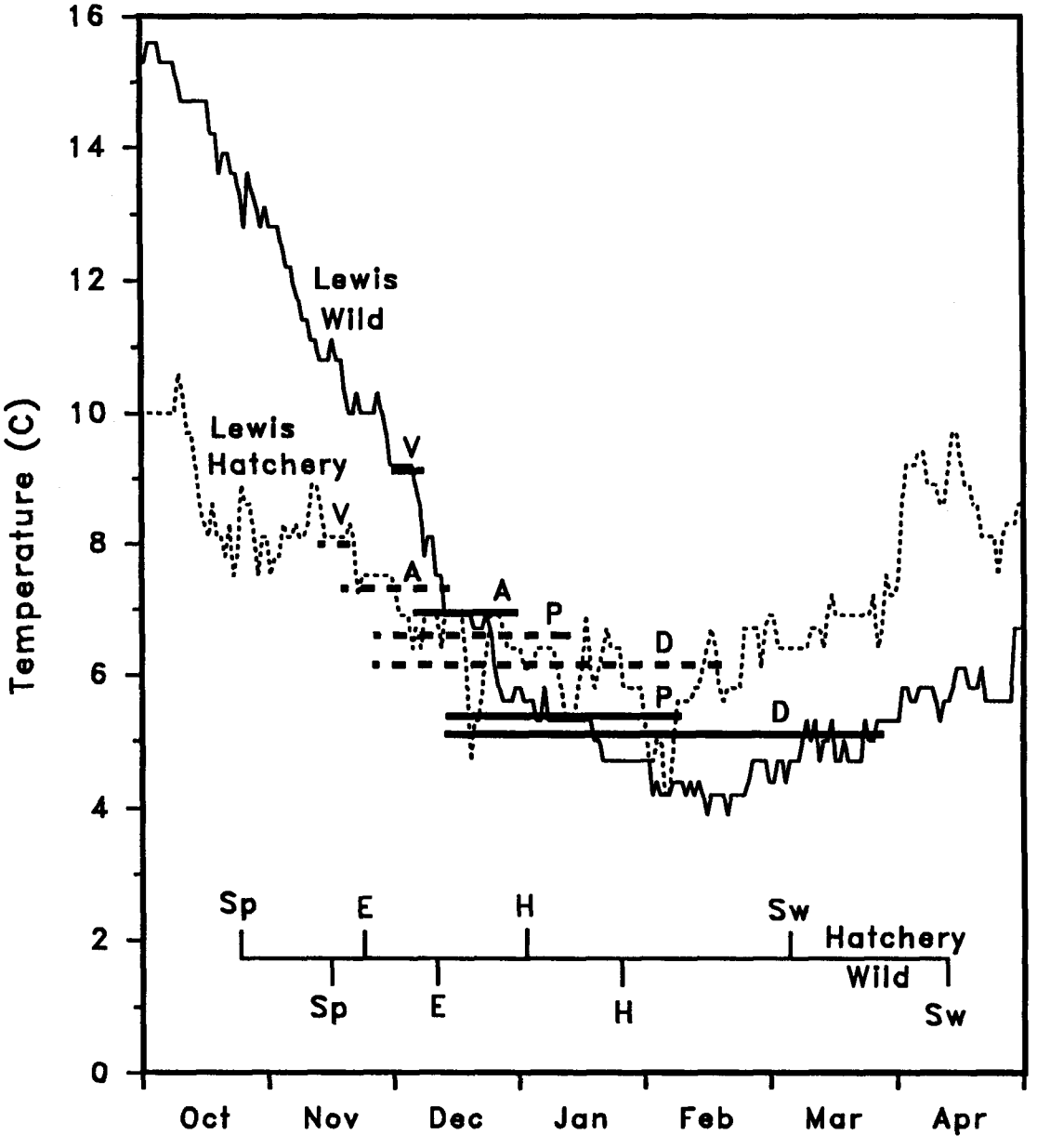

Figure 7. Daily mean water temperatures during the incubation periods (October 1984 through April 1985) of hatchery- and wild-reared fall chinook from the Lewis River. Horizontal bars depict the mean temperature and duration of critical periods for determination of vertebrae (V), anal rays (A), pectoral rays (P) and dorsal rays (D) for the hatchery (broken bars) and wild (solid bars) cohorts. The mean dates for time of spawning (Sp) and development of embryos to the eyed (E), hatched (H) and swim-up (Sw) stages are provided as reference points.

DISCUSSION

The results of this study clearly indicate that biochemical and meristic traits changed among parental adult and various juvenile stages of their offspring within the hatchery and wild cohorts. Furthermore, natural selection appears to have been the causal mechanism for a majority of these changes, although differential dispersal cannot strictly be ruled out for the wild cohorts. The effects of random genetic drift were probably minor in most cases, as will be discussed later.

Causal Mechanisms for Specific Cases

Biochemical Traits

Within Hatchery Cohorts

The increased frequency of *sMDH-3,4*121* in Abernathy hatchery juveniles from that observed in their parents could reflect either differential reproductive success of adults or differential survival of juveniles prior to the fry stage (recall that *sMDH-3,4** allele frequencies did not change between fry and parr or smolt stages). The magnitude of change observed would require a fairly large difference in the partial fitnesses of *sMDH-3,4** genotypes for the adult-to-smolt period (W_{11} = 0.341, vs. W_{12} = 1.000).

Selection increased the frequency of *PGK-2*90* between the fry and smolt stages of the Lewis hatchery cohort, reflecting substantial differential survival of fish according to their *PGK-2** genotypes between those stages: W_{11} = 0.200, W_{12} = 0.365, W_{22} = 1.000. In addition, selection may have been responsible for an increase in the

frequency of *PEPA-1*90* between the adult and smolt stages of that cohort. However, the moderate significance level of the difference and the extremely small sample size for adults (n = 15) prohibit a firm conclusion in this case.

Within Wild Cohorts

I was unable to distinguish between the effects of differential survival and differential migration in causing differences among stages within the Lewis wild cohort at three loci: *PEPA-1**, *sIDHP-3,4** and *sSOD-1**. Partial fitness values are given nonetheless, for purpose of comparison. The increase in frequency of *PEPA-1*90* between adult and smolt stages would correspond to a partial fitness of W_{11} = 0.477, relative to W_{12} = 1.000. It is unclear whether the change occurred throughout the juvenile stages, since fry did not differ significantly from either adults or smolts. In addition, variant alleles of *sIDHP-3,4** increased in frequency from the adult to smolt stage by a magnitude that would correspond to a partial fitness of W_{11} = 0.427 relative to W_{12} = 1.000. Although the increase in frequency of *sSOD-1*-152* from the adult to smolt stage was only marginally significant, a more substantial difference existed in their *sSOD-1** genotype frequencies (P=0.005). The observed magnitude of change in genotype frequencies would correspond to partial fitness values of W_{11} = 0.504 and W_{12} = 0.823, relative to W_{22} = 1.000.

Between Hatchery and Wild Cohorts

The genetic profiles of the hatchery and wild cohorts from the Lewis River differed at two loci: *ADH** and *sSOD-1**. In both cases,

determination of the causal mechanism for differences between the hatchery and wild cohorts is complicated by the inability to conclusively determine whether allele frequencies differed between the hatchery and wild adults. Nevertheless, these differences are thought to reflect the differential effects of natural selection on hatchery- vs. wild-reared juveniles with respect to *ADH** and *sSOD-1** genotypes.

Considering the following information, it is doubtful that the Lewis hatchery and wild adults differed in allele frequencies by more than would be expected due to random genetic drift within a single generation (to be discussed later). A vast majority of the hatchery broodstock have typically been of Lewis River wild origin (e.g. 100% in 1982, 81% in 1983 and 100% in 1985, based on coded wire tag recoveries; WDF unpublished data). Such a level of migration would easily counteract the effects of genetic drift between generations, as well as any moderate selective pressures. Differential contribution of non-Lewis origin (stray) stocks to Lewis hatchery and wild spawning groups (though suspected to occur) is also unlikely to have caused differentiation of Lewis hatchery and wild cohorts. Stock composition estimates based on recoveries of coded wire tags and stock-specific fin clips for the 1982, 1983 and 1985 spawners (WDF, unpublished data) indicate that strays (lower Columbia River "Tule" stocks) comprised an average of 17.8% of the fish trapped for hatchery broodstock versus 9.5% of the wild spawning population. Although all of the tagged adults spawned at Speelyai Hatchery in 1984 were of Lewis origin, untagged strays could still have been

present among the 120 spawners because fewer than 10% of the fish from most stocks were tagged. The origin of Lewis hatchery broodstock based on the data described above (and excluding stocks contributing <1%) averaged about 86% Lewis (over 90% of which was wild), 8% Cowlitz Hatchery, 5% Kalama Falls Hatchery and 1% Washougal Hatchery. Composite allele frequencies for this composition (using data of Schreck et al. 1986) were negligibly different from those of the Lewis River wild stock. This is as might be expected given the predominance of the Lewis River wild component in the composite stock, and given the relatively minor differences in the allele frequencies of the Tule stocks and the Lewis stock.

In contrast to the above examples of possible divergent selective pressures in the hatchery and wild environments, selective pressures appear to have been parallel regarding the *PEPA-1** locus in Lewis hatchery and wild cohorts. The increased frequency of *PEPA-1*90* from the adult to smolt stages in both cohorts suggests the effects of selection were similar whether in the hatchery or the wild environment. *PEPA-1** data from Abernathy samples showed no evidence of selection in either environment. However, the results were inconclusive for Abernathy wild samples due to the small sample size (n = 26) for adults.

Hardy-Weinberg Expectations

Natural selection seems the most probable cause for three of the four cases in which the observed genotype frequencies differed significantly from Hardy-Weinberg (H-W) expectations. Hatchery spawning procedures greatly reduce the potential for non-random

mating to have influenced genotype frequencies of Abernathy hatchery adults at *MPI** or of Lewis hatchery (1985) adults at *PGK-2**. Assortative mating of Lewis wild adults with respect to *sSOD-1** also seems unlikely, although it cannot be ruled out. The relatively large number of spawners for these three cohorts (600, 400 and 8000, respectively) make the influence of chance assortative mating unlikely. The influence of a Wahlund effect (reduced heterozygosity) caused by overlapping generations would be expected to be minor (Waples 1990). Likewise, heterozygote deficiency resulting from stock admixtures is expected to be very slight (at least for the Lewis cases) due to the limited contribution and similar genetic composition of strays, as previously discussed.

Although an unusually high proportion of variant alleles were expressed as variant homozygotes at the *PGK-2** locus for Abernathy wild fry, the cause of this departure is uncertain. Because the small number of fry collected could be the progeny of relatively few parents, genotype ratios could have been affected by chance assortative mating with respect to *PGK-2** genotypes. Alternatively, increased mortality or earlier outmigration of heterozygotes, and to a lesser extent common homozygotes, could have been responsible.

It may have been a matter of chance that three of four significant departures from H-W expectations involved adult samples. However, one might expect departures resulting from selection to be more frequent in adults, which have been subjected to selective pressures over a longer period of time and have incurred higher cumulative mortality than juveniles.

That departures in each case were in the form of heterozygote deficiencies would indicate that $W_{12}^2 < W_{11} \times W_{22}$. Furthermore, it is likely that $W_{22} >> W_{11}$, since a small fitness differential among homozygotes would not likely be detected for sample sizes less than 200 (Lewontin and Cockerham 1959).

For the most part, no departures from H-W expectations were detected for cases in which allele frequencies differed among samples within a cohort. This may be counter to what is intuitively expected, but should not lead one to conclude that the conditions of H-W equilibrium (e.g. no selection) have been met. The ability of H-W tests to detect the effects of different forms of selection varies greatly, depending on the relationships of the genotypic partial fitnesses (Lewontin and Cockerham 1959). As an extreme example, no departure from H-W expectations is caused if $W_{11} \times W_{22} = W_{12}^2$, even though selection may be intense (e.g. $W_{11} = 1.00$, $W_{22} = 0.01$ and $W_{12} = 0.10$). Furthermore, extremely large sample sizes are required to detect selection for cases in which the relationship among the genotypic partial fitness values approaches that described above. Nevertheless, inability to detect departures from H-W expectations for some forms of selection does not detract from the possible significance of those departures that are detected.

Meristic Traits

The most likely causal mechanism for the observed variation in meristic traits is believed to have been natural selection (and/or, in the case of wild cohorts, differential outmigration of juveniles). Although consideration of all possible alternative mechanisms was

beyond the scope of this study, the influences of some key alternatives were assessed and concluded to be minor. The rationale for dismissing the potential influences of contribution by Tule strays (to the Lewis hatchery cohort), variation in incubation temperatures, and bias from variation in counting prodedures are discussed at the end of this section.

The greater mean numbers of scales above the lateral line in Lewis hatchery smolts (32.05) than in either adults (30.59) or smolts (30.83) of the Lewis wild cohort (Figure 4), may have been caused by differential selective pressures. It seems unlikely that the hatchery smolts inherited the greater scale numbers from their parents, considering the previous discussion regarding the potential for differentiation of hatchery and wild stocks in the Lewis.

Likewise, the higher mean numbers of pectoral rays in Abernathy hatchery (16.04) and wild (15.93) adults than in Abernathy hatchery smolts or wild fry (both 15.50, Figure 4), would seem to have been caused by natural selection. However, differential outmigration (with respect to pectoral ray phenotypes) cannot be ruled out for the wild fry. In any case, the direction and intensity of the mechanism was similar in the hatchery and stream environments: decreased survival (or stream residency) of juveniles having greater numbers of pectoral rays. Although the way in which the selection was manifested is unknown, it must be variable among brood years or life stages for the scale counts of adults and their progeny to differ by this amount. If the selection pressure were constant among life stages and brood years, then it would seem that a stable, "optimal"

value would already have been reached in the population. This would be especially true for the hatchery population, which has been cultured for more than 20 years (about five generations) at the current facility.

Greater mean numbers of anal fin rays in Abernathy wild fry (19.10) than in Abernathy wild adults (18.33), hatchery adults (18.56) or hatchery smolts (18.46) may reflect higher mortality or earlier outmigration of fry with fewer anal rays in Abernathy Creek. Selection also appears to have influenced anal fin rays in Lewis hatchery smolts. However, the higher mean number of anal fin rays in Lewis hatchery smolts (19.40) than in Lewis wild adults (19.03) or smolts (18.95) appears to reflect an increase in the number of rudimentary rays in the hatchery smolts. The data of Schreck et al. (1986) from the same specimens show the average number of primary anal fin rays to be equal (16.05) among hatchery and wild smolts. Thus, the observed difference appears to be the result of selection for greater number of rudimentary anal fin rays in Lewis hatchery smolts.

The factors affecting numbers of upper and lower gill rakers appear to be somewhat independent as evidenced by the different results for upper and lower gill rakers among Lewis hatchery and wild samples (Figure 4). Mean numbers of upper rakers were higher in wild adults (10.50) than in either wild (9.85) or hatchery (9.80) smolts, whereas mean numbers of lower rakers were higher in wild adults (14.53) and smolts (14.30) than in hatchery smolts (13.70). In general, there was a poor correlation between the number of upper and

lower gill rakers in individual fish. A positive correlation existed in Lewis wild adults (r^2=0.214, P=0.006), but not in Lewis wild (r^2=0.008, P=0.712) or hatchery (r^2=0.085, P=0.211) smolts, nor in any Abernathy samples (r^2<0.124, P>0.232). Leary et al. (1985b) found the heritability of upper rakers (h^2=0.67, SE=0.11) to be higher than that of lower rakers (h^2=0.37, SE=0.21) in rainbow trout. This may be part of the reason for the lack of correlation in numbers of upper and lower rakers seen here. Differences in the function of upper and lower gill rakers, and subsequent differences in the selective pressures acting on them, might also contribute to low correlation of upper and lower rakers.

Although not evident from these data alone, it doubtful that differences in the number of gill rakers in Lewis wild adults and hatchery smolts reflect a difference between the hatchery and wild parental adults (for reasons previously stated regarding biochemical data). Assuming the number of gill rakers in hatchery and wild adults were similar, the lower number of rakers in the hatchery smolts may reflect selection for fewer rakers in juveniles. If this is the case, selection would appear to have acted similarly on hatchery and wild juveniles in regards to upper, but not lower, gill rakers.

The difference between numbers of upper gill rakers in wild adults and wild smolts probably reflects differential mortality (or differential outmigration) during the juvenile stage. If so, the selective pressures would have to vary between adult and juvenile stages, or among different brood years, to account for the

persistence of variation for selection to act on. The existence of opposing selective pressures during adult and juvenile stages is plausible considering the functional role of gill rakers in feeding and the magnitude of difference expected in the feeding habits of adults and juveniles in their respective marine and freshwater environments.

Two relationships regarding vertebral numbers were evident from chinook in both stream systems: (1) adults had fewer vertebrae than juveniles within each hatchery and wild cohort, and (2) hatchery juveniles had fewer vertebrae than wild juveniles in each stream (Figure 4). I conclude that this reflects selection for greater number of vertebrae in juveniles (from that of their parents) and that the selection appears to have been more intense for wild than for hatchery juveniles.

In summary, there was evidence of selection acting on meristic traits within a cohort in at least 6 cases, involving 3 cohorts (Abernathy hatchery and wild and Lewis wild) and 3 traits (pectoral rays, gill rakers and vertebrae). Selection caused a decrease in the mean number of pectoral rays in juveniles (as compared to their parents) of both the Abernathy hatchery and wild cohorts. Likewise, selection decreased the mean number of upper gill rakers in juveniles of the Lewis wild cohort. Selection also increased the mean number of vertebrae in juveniles (relative to that of their parents) of Abernathy hatchery and wild cohorts and of the Lewis wild (and perhaps also hatchery) cohort.

In addition, there were at least 6 cases, involving 4 traits, of differing direction or intensity of selection between hatchery and wild cohorts of a given stream. Selection increased the mean number of anal rays in Abernathy wild fry (relative to that of their parents), but not in Abernathy hatchery smolts. Conversely, selection increased the mean number of anal fin rays and scales above the lateral line, and decreased the mean number of gill rakers, in Lewis hatchery smolts, but not in Lewis wild smolts. Finally, the intensity of selection for higher mean number of vertebrae in juveniles (relative to that of their parents) was greater in wild than in hatchery juveniles of Abernathy and Lewis populations.

The influences of three other potential sources of variation in meristic traits were considered negligible. The first of these, the influence of Tule strays on Lewis cohorts, was previously discussed regarding biochemical traits. Its influence on meristic traits is expected to be minor for the same reasons discussed earlier: small reproductive contribution by the Tule stocks and only slight differences in meristic traits of Tule vs. Lewis River stocks.

The effects of incubation temperature (a potential source of non-genetic variation) on meristic trait was unlikely to have influenced the results of this study. Two types of temperature differences were considered: between years (as a source of variation between adults and their progeny) and between hatchery and wild environments (as a source of variation between hatchery and wild cohorts). The temperature of well water used for incubation at Abernathy hatchery was not variable between years, so incubation

temperature should have no bearing on differences between Abernathy hatchery adults and juveniles. Annual variation in Abernathy Creek temperatures was not of concern regarding meristic variation between wild fry and their parents, because the majority of adults spawning in Abernathy Creek were likely of hatchery origin. Thus, the temperature differences between Abernathy hatchery and wild environments (discussed later) are the ones of concern regarding comparisons of meristic traits between Abernathy wild adults and fry. The difference between the incubation temperatures of Lewis wild adults and their progeny is presumed to be relatively small for two reasons. First, annual differences in incubation temperatures in the Lewis River are moderated by the thermal stabilizing effect of three large reservoirs upstream of the spawning area. Secondly, the adults that returned in 1984 originated from several brood years: 12% 1982 (age 2), 15% 1981 (age 3), 44% 1980 (age 4), 28% 1979 (age 5) and 1% 1978 (age 6) (n=465; WDF, unpublished data). This would dampen the effects of brood year specific differences in incubation temperatures. Finally, differences between incubation temperatures of hatchery and wild environments (Figures 5 and 6) were not responsible for observed differences in meristic traits of hatchery and wild fish. In all cases, the expected effect (based on Seymour 1959 and Kwain 1975) of existing temperature differences was either much smaller than, or in the opposite direction of, the observed difference in meristic traits.

It should be noted that the potential influence of environmental variables other than temperature cannot be ruled out, as assessment

of such variables was beyond the scope of this study. Difficulty in distinguishing between environmental versus genetic variation is an inherent problem when studying traits (such as meristic characters) that are heritable but not of a purely genetic basis.

Errors in enumeration of meristic characters are not believed to have contributed to observed differences in meristic traits. However, because of the size difference between adults and juveniles and differences in counting procedures, some discussion of the rationale for this conclusion is warranted for three traits: pectoral fin rays, gill rakers and vertebrae. Counting bias with regard to pectoral rays (Abernathy hatchery cohort) is unlikely because pectoral rays were not difficult to count in adults or juveniles and because differences in adult and juvenile pectoral ray counts were not seen in the Lewis wild cohort. The same argument can be made for gill rakers, for which differences were seen in Lewis cohorts but not in Abernathy cohorts. Finally, counting bias is not believed to account for differences in vertebral numbers of adults and juveniles, despite the size differences and separate methodologies. Enumeration of the vertebrae was relatively straightforward and unambiguous, whether making counts directly from the exposed vertebral column of adults, from radiographs of smolts or from cleared and stained fry. Radiography and clear-and-stain methods yielded identical counts for all nine fry examined using both techniques.

Role of Random Genetic Drift

In the conventional sense, random genetic drift refers to stochastic changes in gene frequencies between generations. Such changes between generations are beyond the scope of this study. However, random genetic changes within a generation have relevance to this study in that they could have contributed to observed differences between adults and their juvenile progeny, between juvenile stages of a given cohort, or between hatchery and wild samples of various stages. Such changes may be brought about in two phases: a reproductive phase in which changes result from the chance failure of mature adults to contribute equally to the production of zygotes, and a zygotic phase in which changes result from the chance failure of zygotes to survive to the juvenile stage of concern.

In the hatchery cohorts, the greatest potential for random genetic change stems from variation in contribution of gametes by males. This is because the adults sampled are known to have spawned, and because variation in the contribution of gametes from females is driven primarily by fecundity differences (which can be expected to be relatively minor). Furthermore, mortality from zygote to smolt stages was too low (about 10-30% for these cohorts) to effect stochastic changes of a magnitude that would influence the results of this study. Nevertheless, because pooled-milt spawning procedures (which were used in both hatcheries) are known to increase the variation in genetic contribution by males (Withler 1988), this factor alone could lead to random changes if the number of spawners were small to begin with. Thus, measurable stochastic changes may

have occurred in the Lewis hatchery cohort (120 spawners), but probably not in the Abernathy hatchery cohort (600 spawners).

In the wild cohorts, there is potential for random genetic change in both the reproductive and zygotic phases. Although little is known about the magnitude of such changes in naturally spawning salmonids, it is unlikely to have been of significance for the Lewis wild cohort because of the large number of spawners (8000). However, it could have been a factor for the Abernathy wild cohort, in which the success of attempted natural spawning appears to have been quite low.

Thus, differences observed in comparisons involving Lewis hatchery and Abernathy wild samples may have been influenced by random genetic changes. Such comparisons include those between Lewis hatchery adults and their progeny at *PEPA-1** and between Lewis hatchery and wild cohorts at *ADH** and *sSOD-1**. They also include comparisons between Abernathy hatchery and wild cohorts at *sAH-1**, *sIDHP-3,4**, *sMDH-3,4** and *PEPB-1**. Of these, only the differences between Lewis hatchery and wild cohorts (*ADH** and *sSOD-1**) are of much consequence to the main findings of this study. Furthermore, it is unlikely that drift alone could account for the magnitude of heterogeneity observed (P=0.0001) between the hatchery and wild cohorts at *sSOD-1**.

Although polygenically controlled meristic traits might be expected to be influenced less by random processes (through the buffering effect of multiple genes), some influence may still be possible. If so, differences in the mean numbers of scales above the

lateral line, anal fin rays, gill rakers and vertebrae between Lewis hatchery and wild cohorts may have been due in part to random genetic change. The same could be said of mean numbers of anal and pectoral fin rays and vertebrae regarding comparisons involving Abernathy wild adult or fry.

Overall, random genetic drift (though possibly a contributing factor in the cases noted above) was not thought to have played a major role in causing the more prominent genetic differences observed in this study.

Summary and Implications of Findings

The findings with respect to the study objectives are summarized as follows:

(1) Changes in biochemical and meristic traits within hatchery and wild cohorts were documented.

(2) Changes in some biochemical and meristic traits were probably caused by natural selection, even in populations that have been in the same environment for many generations (i.e. Abernathy hatchery and Lewis wild populations).

(3) The direction and/or degree of change in some biochemical and meristic traits differed between hatchery and wild cohorts of a given stream (Table 7), suggesting differences in the selective pressures of the hatchery and wild environments in those cases.

Many of the biochemical traits that varied temporally (within cohorts) in this study were also shown to vary temporally (between years) in other studies. Parkinson (1984) found significant between-year variation in steelhead populations of British Columbia at three

Table 7. Comparative summary of the genetic profiles of biochemical and meristic characters of hatchery (H) and wild (W) cohorts from Abernathy and Lewis populations. For each cohort, "NC" indicates no change, "+" indicates an increase and "-" indicates a decrease in the frequency or the mean value of the listed character from parent to progeny (or from earlier to later juveniles stages) of progeny, and a blank indicates no profile was obtained (i.e. data available for fewer than two life stages). Asterisks indicate differences, and equal signs similarities, between the profiles of hatchery and wild cohorts within populations, and among corresponding hatchery and wild cohorts between populations. Symbols in parentheses indicate the results may have been influenced by small sample size.

Biochemical and meristic characters	Within Abernathy			Between Abernathy and Lewis	Within Lewis		
	H		W		H		W
*sIDH-3,4*74,127*	NC		NC_s	(*)	NC_s	(*)	+
*sMDH-3,4*121*	+	(*)	NC_s	(*)	NC_s		NC
*PEPA-1*90*	NC		NC_s	*	$+_s$	=	+
*PGK-2*90*	NC			*	+	*	NC
sSOD-1-152*	NC				NC_s	*	+
Pectoral fin rays	-	=	-	*			NC
Anal fin rays	NC	*	+	*			NC
Upper gill rakers	NC						-
Vertebrae	+	=	+	=			+

$_s$ small sample size limited conclusiveness of the genetic profile

biochemical loci: *sIDH-3,4**, *sMDH-3,4** and *sSOD-1**, each of which varied temporally within at least one cohort of chinook in my study. Parkinson (1984) also found significant variation between life stages within a given cohort at those three loci. To a variable degree, the loci that differed between samples within cohorts in my study were also found to differ between year classes of Columbia River stocks of chinook and steelhead (Schreck et al. 1986). For example, 7 of 15 cases tested by Schreck et al. (1986) differed at *sIDH-3,4** and 5 of 10 at *PGK-2**, but only 1 of 10 at *sMDH-3,4** and 1 of 13 at *sSOD-1**. Further, *MPI** and *PEPB-1** did not differ among samples in my study, but differed between years in 4 of 11 and 2 of 6 cases tested, respectively, by Schreck et al. (1986). Leary et al. (1989) provide evidence of another source of temporal variation at biochemical loci in salmonid stocks. Those authors found significant variation among spawning dates within a given spawning season for two strains of rainbow trout at a total of 10 loci. Those loci included *sIDH-3,4**, *sMDH-3,4**, *PEPA-1** and *sSOD-1**, which were temporally variable (among life stages) in my study as well.

The meristic traits that varied temporally within cohorts in my study were generally the same ones found to vary between years in other studies. Pectoral fin rays, scales above the lateral line, and upper gill rakers varied between years in 3 cases, vertebrae in 2 cases, and dorsal fin rays and branchiostegals in 1 case, of the 6 cases tested by Schreck et al. (1986). Of those characters, all but the last two varied within cohorts in my study. Blouw et al. (1988) documented extensive and persistent between-year variation over a 10

year period in mean numbers of vertebrae, dorsal and anal pterygiophores, pectoral fin rays and gill rakers in Atlantic salmon smolts.

Evidence of temporal changes in biochemical and meristic characters within hatchery and wild cohorts of fall chinook has important implications regarding the use of those traits to characterize stocks or to serve as genetic tags. It is clear from the above discussion that the assumption of temporal stability of biochemical and meristic traits within a cohort or between successive cohorts should be applied with caution. Sampling strategies of studies involving these characters should be designed to account for the possibility of temporal and/or spatial heterogeneity. For example, samples of adults may not accurately represent the genetic characteristics displayed in their progeny and vice versa. The relative importance of intra- and inter-population variation in biochemical and meristic characters depends on the particular application of the data. Temporal variation within populations may be of minor consequence relative to inter-population variation for studies of diverse populations. For example, the significant between-year variation in biochemical and meristic traits of chinook and steelhead reported by Schreck et al. (1986) did not obscure the relationships of the major stocks of those species in the Columbia River drainage. Such might not be the case for studies attempting to examine population structure on a finer scale.

Both Schreck et al. (1986) and Blouw et al. (1988) found poor correlation of meristic variation with incubation temperatures,

suggesting little environmental influence from that source (as was also the case in my study). If the variation in the meristic traits of these three studies is largely due to selective pressures, these traits may be good candidates for further study of selection acting on meristic variation. Although relatively scarce in the literature, evidence of the effects of natural selection on meristic traits of fishes has been documented by other authors. Evidence for selection acting on vertebrae number in peamouth (Mylocheilus caurinus) fry was reported by Swain (1988). Swain and Lindsay (1984) reported similar evidence of selection acting on the number of vertebrae in the young of the stickleback Gasterosteus aculeatus. Both studies showed the favored vertebrae number to shift upwards as the length of the fry increased, until fry reached a threshold size, beyond which mortality was independent of vertebrae number. The authors postulate that the selection may be related to differing optimal vertebrae number for fry of different lengths due to the influence of vertebrae number on body flexibility as it affects the swimming performance, and hence predator avoidance, of the fry. The relative complexity of the proposed selective mechanism serves to illustrate the inherent difficulty in demonstrating the effects of natural selection in wild populations.

The implication of natural selection as a source of variation in biochemical traits in my study further emphasizes the desirability expressed by several authors (e.g. Allendorf and Utter 1979; Seeb et al. 1986; Chandler and Bjornn 1989) to test the assumption of selective neutrality in studies that use biochemical loci as genetic

tags. An important feature in the design of selective neutrality studies is consideration of the minimum selection coefficient that would be detectable for a given sample size (Gharrett et al. 1984). Hartl and Clark (1989) suggest that studies should be designed to detect selection differentials as small as 10%. However, that would require a sample size of more than 1000 individuals (Gharrett et al. 1984), which is impractical for most studies of selection in natural environments. Thus, practical consideration may often prohibit detection of selection coefficients of small to moderate magnitudes. It is therefore important that workers report the minimum detectable selection coefficient for the sample size of their study, particularly when no selection differential was detected. I further recommend that selective neutrality studies use the same genetic markers, stocks and environmental conditions as would apply in the intended application of the genetic marker. In determining the level of effort one is willing to expend on a selective neutrality study, one should carefully consider the cost (to the resource, management program or experimental conclusions) of using a genetic marker that has a biologically significant but undetected effect on survival or other performance traits of interest.

A review of several studies that employed, or proposed for use, specific biochemical genetic markers in fish reveals a considerable range in the effort expended to test the assumption of neutrality of the marker. For example, three studies assumed selective neutrality of allozyme markers used to determine paternal contribution (to eyed

egg or fry stages) of salmonids under hatchery (Gharrett and Shirley 1985; Withler 1988) and simulated natural (Hutchings and Myers 1988) spawning conditions. Schweigert et al. (1977) assumed neutrality of an allozyme marker in their study of the contribution of walleye (Stizostedion vitreum vitreum) to production in a lake. Seeb et al. (1986) likewise assumed selective neutrality of the genetic markers they used to assess the contribution of chum salmon (O. keta) released as fry to production at smolt and returning adult stages. The design of two other studies made it possible to confirm the neautrality of genetic marks directly from the results of their marked study groups, without the need for a separate selective neutrality study (Reisenbichler and McIntyre 1977; Fields et al. 1987). Taggart and Ferguson (1984) detected no selective effects of a genetic tag proposed for use in monitoring the success and genetic impacts of hatchery stocking programs, but conducted their study under hatchery conditions. Chandler and Bjornn (1989) detected no survival effects of a genetic mark four months after steelhead fry were stocked in natural streams. However, given their sample sizes they would not have been expected to detect selection differentials smaller than 45%. Chilcote et al. (1986) found that their genetic marker reduced the survival of steelhead stocked as fry in natural streams and that the selection coefficient differed for fish recovered as subyearlings and as post-yearlings. However, the selective effect of the genetic mark during the smolt-to-adult stage was not evaluated, forcing an untested assumption about the effects of the mark on adult return rates of marked (hatchery-parented) and

unmarked (wild-parented) steelhead (Leider et al. 1990). In all fairness to the workers of the studies reviewed here, it is much easier to find flaws in studies addressing selective neutrality of allozymes than it is to design and successfully conduct a flawless selective neutrality study. Still, these examples serve to illustrate the need for more rigorous testing of the assumption of selective neutrality of biochemical genetic marks in future works.

The biological and management implications of the observed divergence in genetic traits between hatchery and wild cohorts are not clear. Although this study provides direct evidence that such changes can occur within a single generation, other questions remain unanswered. For example, the extent to which genetic changes within a cohort might vary between year classes or generations is not known. The divergence observed could have been similar, absent, or reversed in other years depending on the temporal stability of selective pressures in the hatchery, stream and marine environments. This has bearing on another issue: whether the observed divergence of traits reflects general differences in hatchery and stream environments, or whether it reflects population-specific responses to site-specific environmental conditions. If the divergence between hatchery and wild cohorts were due to differences in hatchery and stream environments in general, one might expect some similarity between the comparisons of genetic profiles of hatchery and wild cohorts from Abernathy and Lewis populations. However, this was generally not the case, as evidenced by the comparisons of genetic profiles in Table 7. In most cases, the relationship between the genetic profiles of the

hatchery and wild cohorts differed between Abernathy and Lewis populations (denoted by asterisks in the center column of Table 7). Although it could be argued that the incongruity of results from the two populations could be due to differences in their respective genetic backgrounds, that issue cannot be resolved from these data.

Furthermore, it is unclear whether, or to what extent, the divergence of the hatchery and wild cohorts should be viewed as being genetically detrimental to the hatchery population. The biological significance of the effects of the observed divergence in specific traits on short and long term fitness of the populations is unknown and beyond the scope of this study. In the absence of specific evidence to the contrary, it is reasonable to assume that selection for characteristics that maintain or improve fitness (in terms of continued reproductive success of a population) in a natural environment is best achieved by the action of the selective pressures of that particular environment. This view is supported by the failure of attempts to artificially breed anadromous fish for increased ocean survival (McIntyre et al. 1988). Considering this, genetic changes that lead to divergence from the wild state might generally be expected to lessen adaptation for survival in the wild, to the extent that such changes have some bearing on fitness. However, the management implications of divergence of this type will depend largely on the particular situation of each population. In the Abernathy case, available evidence suggests that naturally spawning and hatchery spawned adults are both derived primarily from hatchery production and are, as such, two sub-units of the same

population. Therefore, maintenance of the genetic integrity of the naturally spawning segment of the Abernathy population would not likely be an issue of management or stock conservation concern. The situation is quite the reverse for the Lewis population. There, the parents of hatchery and wild cohorts were sub-units of a wild population that accounts for a vast majority (e.g. 84%, 1980-1984) of the naturally produced fall chinook of the lower Columbia River (Howell et al. 1985). In this case, maintenance of the genetic integrity of the wild population is most certainly of concern, both from a production management and from a stock conservation perspective.

The results of this study have demonstrated divergent genetic changes in hatchery and wild reared cohorts of two populations of fall chinook within a single generation. Whether such changes are due to general and persistent differences between hatchery and stream environments, and thus reflect natural selection in the hatchery for "optimal hatchery-type" fish as hypothesized by Helle (1981), remains to be seen. Furthering our understandings of the short-term and long-term effects that various artificial production techniques have on the genetic make-up of hatchery stocks, and of the genetic effects of interactions between hatchery and wild stocks, are vital to successful integration of management for natural and artificial production of salmonids.

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APPENDICES

Appendix 1. Enzyme name, Enzyme Commision (E.C.) number and mobility of alleles (% mobility of common allele) identified in hatchery and wild chinook salmon from Abernathy Creek and Lewis River. Peptide substrates used to resolve the peptidases are listed in brackets.

Enzyme name (E.C. number)	Locus abbreviation	Allele mobilities 1	2	3
Aspartate aminotransferase (2.6.1.1)	*sAAT-1,2**	100		
	*-3**	100	90	
	*-4**	100		
Adenosine deaminase (3.5.4.4)	*ADA-1**	100	83	
	*-2**	100	105	
Alcohol dehydrogenase (1.1.1.1)	*ADH**	-100	-52	
Aconitate hydratase (4.2.1.3)	*sAH-1**	100	86	116
Creatine kinase (2.7.3.2)	*CK-1**	100		
	*-2**	100		
Glucose-6-phosphate isomerase (5.3.1.9)	*GPI-1**	100		
	*-2**	100	60	
	*-3**	100	93	
Glutathione reductase (1.6.4.2)	*GR**	100	85	
Isocitrate dehydrogenase (NADP+) (1.1.1.42)	*sIDHP-2**	100		
	*-3,4**	100	127	74
L-lactate dehydrogenase (1.1.1.27)	*LDH-4**	100		
	*-5**	100	90	
Malate dehydrogenase (1.1.1.37)	*sMDH-1,2**	100		
	*-3,4**	100	121	70
Mannose-6-phosphate isomerase (5.3.1.8)	*MPI**	100	109	95
Dipeptidase (3.4.-.-) [glycyl-L-leucine]	*PEPA-1**	100	90	
Tripeptide aminopeptidase (3.4.-.-) [DL-leucylglyclyglycine]	*PEPB-1**	100	130	
Peptidase-C (3.4.-.-) [glycyl-L-leucine]	*PEPC**	100		
Proline dipeptidase (3.4.13.9) [L-phenylalanyl-L-proline]	*PEPD-2**	100	107	
Peptidase-LT (3.4.-.-) [L-leucyl-L-tyrosine]	*PEP-LT**	100		
Phosphogluconate dehydrogenase (1.1.1.44)	*PGDH**	100		
Phosphoglycerate kinase (2.7.2.3)	*PGK-2**	100	90	
Phosphoglucomutase (5.4.2.2)	*PGM-1**	-100	-70	
	*-2**	-100	-70	
Superoxide dismutase (1.15.1.1)	*sSOD-1**	-100	-260	
Triose-phosphate isomerase (5.3.1.1)	*TPI-4**	100	105	

Appendix 2. Test statistic values and degrees of freedom (df) for pairwise log likelihood ratio tests of independence of allele frequencies at nine loci among different life stages of hatchery (H) and wild (W) cohorts of Abernathy fall chinook. Asterisks denote significance: P<0.05 (*), P<0.01 (**) and P<0.001 (***).

Locus (df)	Stage	Hatchery			Wild	
		Fry	Parr	Smolt	Adult	Fry
ADH (1)	H Adult	-	-	-	-	-
	H Fry		-	8.79**	-	-
	H Parr			-	-	-
	H Smolt				-	-
	W Adult					-
sAH-1 (2)	H Adult	-	-	-	-	-
	H Fry		-	-	-	-
	H Parr			0.10	-	4.54*
	H Smolt				-	5.13*
	W Adult					-
sIDHP-3,4 (2)	H Adult	-	4.89	1.62	0.00	7.64*
	H Fry		-	-	-	-
	H Parr			1.86	1.59	0.47
	H Smolt				0.40	4.62
	W Adult					2.51
sMDH-3,4 (1)	H Adult	11.34***	4.91*	6.45*	4.19*	0.73
	H Fry		0.68	1.04	0.02	5.93*
	H Parr			0.00	0.35	1.92
	H Smolt				0.40	2.55
	W Adult					2.25
MPI (2)	H Adult	-	2.52	4.67	1.23	-
	H Fry		-	-	-	-
	H Parr			0.79	0.48	-
	H Smolt				0.09	-
	W Adult					-
PEPA-1 (1)	H Adult	0.00	0.42	1.02	0.64	1.73
	H Fry		0.45	1.25	0.72	2.07
	H Parr			0.06	1.32	0.37
	H Smolt				1.77	0.21
	W Adult					2.28
PEPB-1 (1)	H Adult	0.44	0.42	0.57	2.88	0.26
	H Fry		0.01	0.01	4.56*	1.66
	H Parr			0.00	4.33*	1.39
	H Smolt				4.78*	1.93
	W Adult					2.01
PGK-2 (1)	H Adult	-	-	-	-	-
	H Fry		2.00	2.98	-	0.17
	H Parr			0.23	-	2.58
	H Smolt				-	3.55
	W Adult					-
sSOD-1 (1)	H Adult	-	-	0.08	0.22	-
	H Fry		-	-	-	-
	H Parr			-	-	-
	H Smolt				0.12	-
	W Adult					-

Appendix 3. The number of genotypes observed (obs) and those expected under Hardy-Weinberg equilibrium (exp) for fall chinook of Abernathy hatchery (AH), Abernathy wild (AW), Lewis hatchery (LH) and Lewis wild (LW) cohorts. Asterisks denote significance of the log likelihood ratio test statistic (G) at the appropriate degrees of freedom (df): *, P<0.05; **, P<0.01; and ***, P<0.001. LH adult[85] are parents of the 1985 brood (others are from 1984 brood, see text).

Locus / Sample		Genotype 1/1	1/2	2/2	1/3	2/3	3/3	G (df)
ADH								
AH fry	obs:	76	7	1				1.62
	exp:	75.2	8.5	0.2				(1)
AH smolt	obs:	148	45	4				0.07
	exp:	147.6	45.9	3.6				(1)
LH smolt	obs:	115	13	3				5.59*
	exp:	112.7	17.6	0.7				(1)
LW fry	obs:	79	2	0				0.03
	exp:	79.0	2.0	0.0				(1)
LW smolt	obs:	181	13	0				0.45
	exp:	181.2	12.6	0.2				(1)
sAH-1								
AH parr	obs:	73	6	0				0.24
	exp:	73.1	5.8	0.1				(1)
AH smolt	obs:	166	16	0				0.74
	exp:	166.4	15.3	0.4				(1)
AW fry	obs:	61	15	0				1.65
	exp:	61.7	13.5	0.7				(1)
LH adult	obs:	6	2	1				1.02
	exp:	5.4	3.1	0.4				(1)
LH smolt	obs:	81	43	5	1	1	0	0.92
	exp:	80.1	42.5	5.6	1.6	0.4	0.0	(3)
LH adult[85]	obs:	66	30	5	1	0	0	0.85
	exp:	65.1	32.0	3.9	0.8	0.2	0.0	(3)

Appendix 3. Continued.

Locus / Sample		Genotype 1/1	1/2	2/2	1/3	2/3	3/3	G (df)
sAH-1 (continued)								
LW adult	obs:	100	36	6	1	1	0	2.40
	exp:	97.5	40.3	4.2	1.6	0.3	0.0	(3)
LW smolt	obs:	115	64	6	7	2	0	0.79
	exp:	117.5	59.9	7.6	7.0	1.8	0.1	(3)
MPI								
AH adult	obs:	45	37	29	1	0	0	12.55**
	exp:	36.6	54.3	20.1	0.6	0.4	0.0	(3)
AH parr	obs:	23	54	14	3	1	0	4.38
	exp:	27.9	45.0	18.1	2.2	1.7	0.0	(3)
AH smolt	obs:	65	80	34	7	3	0	1.91
	exp:	62.3	86.7	30.2	5.7	4.0	0.1	(3)
AW adult	obs:	7	10	2	0	1	0	2.31
	exp:	7.2	9.0	2.8	0.6	0.4	0.0	(3)
LH smolt	obs:	26	69	32	4	0	0	6.94
	exp:	29.8	63.4	33.8	1.9	2.0	0.0	(3)
LH adult[85]	obs:	24	27	33	1	3	0	11.19*
	exp:	16.4	41.5	26.2	1.7	2.2	0.0	(3)
LW adult	obs:	20	23	28	1	1	0	8.58*
	exp:	14.0	35.1	21.9	0.9	1.1	0.0	(3)
LW smolt	obs:	58	81	45	5	5	0	2.76
	exp:	52.6	91.6	39.9	5.2	4.5	0.1	(3)
PEPA-1								
AH adult	obs:	111	8	1				1.84
	exp:	110.2	9.6	0.2				(1)
AH fry	obs:	183	17	0				0.76
	exp:	183.4	16.3	0.4				(1)
AH parr	obs:	91	7	2				5.45*
	exp:	89.3	10.4	0.3				(1)

Appendix 3. Continued.

Locus / Sample		Genotype 1/1	1/2	2/2	1/3	2/3	3/3	G (df)
PEPA-1 (continued)								
AH smolt	obs:	177	22	1				0.11
	exp:	176.7	22.6	0.7				(1)
AW adult	obs:	25	1	0				0.02
	exp:	25.0	1.0	0.0				(1)
AW fry	obs:	108	13	2				2.63
	exp:	106.6	15.8	0.6				(1)
LH fry	obs:	84	13	0				0.93
	exp:	84.4	12.1	0.4				(1)
LH smolt	obs:	106	23	2				0.30
	exp:	105.4	24.2	1.4				(1)
LH adult[85]	obs:	77	22	0				2.76
	exp:	78.2	19.6	1.2				(1)
LW adult	obs:	147	19	0				0.18
	exp:	147.5	17.9	0.5				(1)
LW fry	obs:	162	33	2				0.05
	exp:	161.7	33.5	1.7				(1)
LW smolt	obs:	153	39	2				0.08
	exp:	153.4	38.2	2.4				(1)
PEPB-1								
AH adult	obs:	68	29	5				0.63
	exp:	66.7	31.5	3.7				(1)
AH fry	obs:	119	70	7				0.75
	exp:	121.0	66.0	9.0				(1)
AH parr	obs:	61	33	5				0.04
	exp:	60.7	33.7	4.7				(1)
AH smolt	obs:	121	71	8				0.38
	exp:	122.5	68.1	9.5				(1)

Appendix 3. Continued.

Locus / Sample		Genotype 1/1	1/2	2/2	1/3	2/3	3/3	G (df)
PEPB-1 (continued)								
AW adult	obs:	22	3	1				1.94
	exp:	21.2	4.5	0.2				(1)
AW fry	obs:	82	33	4				0.09
	exp:	81.5	33.9	3.5				(1)
LH fry	obs:	84	11	3				5.42*
	exp:	81.7	15.5	0.7				(1)
LH smolt	obs:	108	21	2				0.58
	exp:	107.2	22.6	1.2				(1)
LH adult[85]	obs:	88	15	1				0.14
	exp:	87.7	15.6	0.7				(1)
LW adult	obs:	147	19	1				0.18
	exp:	146.6	19.7	0.7				(1)
LW fry	obs:	166	25	1				0.00
	exp:	165.9	25.1	0.9				(1)
LW smolt	obs:	164	29	1				0.06
	exp:	164.2	28.5	1.2				(1)
PGK-2								
AH fry	obs:	71	26	2				0.05
	exp:	71.3	25.5	2.3				(1)
AH parr	obs:	55	9	2				2.56
	exp	53.6	11.7	0.6				(1)
AH smolt	obs:	37	7	0				0.61
	exp:	37.3	6.4	0.3				(1)
AW fry	obs:	40	8	5				9.12**
	exp:	36.5	14.9	1.5				(1)

Appendix 3. Continued.

Locus / Sample		Genotype 1/1	1/2	2/2	1/3	2/3	3/3	G (df)
PGK-2 (continued)								
LH fry	obs:	59	34	2				1.50
	exp:	60.8	30.4	3.8				(1)
LH smolt	obs:	59	62	10				1.36
	exp:	61.8	56.3	12.8				(1)
LH adult[85]	obs:	60	21	11				10.91***
	exp:	54.0	33.0	5.0				(1)
LW fry	obs:	26	23	5				0.00
	exp:	26.0	22.9	5.0				(1)
LW smolt	obs:	100	78	16				0.02
	exp:	99.6	78.8	15.6				(1)
sSOD-1								
AH adult	obs:	21	64	30				1.66
	exp:	24.4	57.1	33.4				(1)
AH smolt	obs:	40	108	51				1.57
	exp:	44.4	99.2	55.4				(1)
AW adult	obs:	6	9	6				0.43
	exp:	5.3	10.5	5.3				(1)
LH adult	obs:	5	5	5				1.70
	exp:	3.8	7.5	3.8				(1)
LH fry	obs:	24	27	22				4.98*
	exp:	19.3	36.5	17.3				(1)
LH smolt	obs:	31	64	36				0.06
	exp:	30.3	65.4	35.3				(1)
LW adult	obs:	65	45	26				10.45**
	exp:	56.3	62.4	17.3				(1)
LW fry	obs:	65	71	27				1.00
	exp:	62.0	77.1	24.0				(1)
LW smolt	obs:	61	96	37				0.01
	exp:	61.2	95.5	37.2				(1)

Appendix 4. Test statistic and degrees of freedom (df) for log likelihood tests of allele frequencies at nine loci among different life stages of hatchery (H) and wild (W) cohorts of Lewis River fall chinook. Asterisks denote significance: P<0.05 (*), P<0.01 (**) and P<0.001 (***). Adult^{85} = 1985 spawners (others = 1984, see text).

Locus (df)	Stage	Hatchery			Wild		
		Adult	Fry	Smolt	Adult	Fry	Smolt
ADH (1)	H Adult^{85}	-	-	-	-	-	-
	H Adult		-	-	-	-	-
	H Fry			-	-	-	-
	H Smolt				-	9.08**	4.88*
	W Adult					-	-
	W Fry						2.14
sAH-1 (2)	H Adult^{85}	0.11	-	0.20	0.56	-	3.24
	H Adult		-	0.17	0.31	-	0.69
	H Fry			-	-	-	-
	H Smolt				1.11	-	2.47
	W Adult					-	4.18
	W Fry						-
sIDHP-3,4 (2)	H Adult^{85}	0.43	-	0.68	7.03*	-	3.12
	H Adult		-	0.71	3.23	-	1.80
	H Fry			-	-	-	-
	H Smolt				10.39**	-	1.67
	W Adult					-	9.52**
	W Fry						-
sMDH-3,4 (1)	H Adult^{85}	0.00	8.02**	7.69**	4.73*	-	1.47
	H Adult		1.12	0.95	0.55	-	0.14
	H Fry			0.11	1.20	-	4.26*
	H Smolt				0.70	-	3.72
	W Adult					-	1.40
	W Fry						-
MPI (2)	H Adult^{85}	-	-	1.04	0.35	-	4.01
	H Adult		-	-	-	-	-
	H Fry			-	-	-	-
	H Smolt				0.58	-	2.34
	W Adult					-	4.04
	W Fry						-
PEPA-1 (1)	H Adult^{85}	6.19	2.34	0.08	4.81*	0.43	0.00
	H Adult		3.52	5.80*	3.05	5.35*	6.42*
	H Fry			1.83	0.20	1.24	2.99
	H Smolt				4.22*	0.15	0.10
	W Adult					3.45	6.68**
	W Fry						0.61
PEPB-1 (1)	H Adult^{85}	-	0.03	0.27	0.68	0.25	0.01
	H Adult		-	-	-	-	-
	H Fry			0.10	1.02	0.48	0.08
	H Smolt				2.14	1.29	0.47
	W Adult					0.16	0.78
	W Fry						0.25
PGK-2 (1)	H Adult^{85}	-	0.62	3.40	-	1.79	1.60
	H Adult		-	-	-	-	-
	H Fry			7.33**	-	4.11*	4.79*
	H Smolt				-	0.02	0.65
	W Adult					-	-
	W Fry						0.20
sSOD-1 (1)	H Adult^{85}	-	-	-	-	-	-
	H Adult		0.02	0.04	2.27	1.50	0.42
	H Fry			0.40	6.58*	4.35*	0.99
	H Smolt				14.34***	10.81**	4.10*
	W Adult					0.46	4.42*
	W Fry						2.19

Appenxix 5. Enzyme allele frequencies of Abernathy hatchery and wild fall chinook from the 1984 cohort (parental adults and their juvenile progeny). Only allelic sample sizes (in parentheses) are given for loci that were monomorphic in these samples.

Enzyme locus	Allele mobility	Hatchery cohort				Wild cohort	
		Adult	Fry	Parr	Smolt	Adult	Fry
*sAAT-1,2**	100	(360)	(0)	(372)	(748)	(0)	(456)
*sAAT-3**	100	(0)	(0)	(324)	(258)	(0)	(224)
*ADA-1**	100	(0)	(0)	(0)	(214)	(0)	(0)
*ADA-2**	100	(0)	(0)	(150)	(258)	(0)	(94)
*ADH**	-100		0.946		0.865		
	-52		0.054		0.135		
		(0)	(168)	(0)	(394)	(0)	(0)
*sAH-1**	100			0.962	0.956		0.901
	86			0.038	0.044		0.099
	116			0	0		0
		(0)	(0)	(158)	(364)	(0)	(152)
*CK-1**	100	(180)	(0)	(0)	(384)	(0)	(0)
*CK-2**	100	(180)	(0)	(0)	(398)	(0)	(0)
*GPI-1**	100	(240)	(0)	(198)	(398)	(52)	(244)
*GPI-2**	100	(240)	(0)	(198)	(398)	(52)	(244)
*GPI-3**	100	(240)	(0)	(198)	(398)	(52)	(244)
*GR**	100			0.774	0.730		0.664
	85			0.226	0.270		0.336
		(0)	(0)	(146)	(326)	(0)	(238)
*sIDHP-3,4**	100	1.000		0.986	0.995	1.000	0.980
	127	0		0.006	0.002	0	0.010
	74	0		0.008	0.003	0	0.010
		(292)	(0)	(364)	(612)	(88)	(412)
*LDH-4**	100	(240)	(0)	(198)	(394)	(52)	(242)
*LDH-5**	100	1.000		1.000	1.000		0.996
	90	0		0	0		0.004
		(176)	(0)	(190)	(378)	(0)	(242)

Appendix 5. Continued.

Enzyme locus	Allele mobility	Hatchery cohort				Wild cohort	
		Adult	Fry	Parr	Smolt	Adult	Fry
*sMDH-1,2**	100	(480)	(0)	(396)	(800)	(104)	(488)
*sMDH-3,4**	100	0.987	0.955	0.965	0.965	0.952	0.980
	121	0.013	0.045	0.035	0.035	0.048	0.020
		(480)	(800)	(400)	(800)	(104)	(460)
*MPI**	100	0.571		0.542	0.574	0.600	
	109	0.424		0.437	0.400	0.375	
	95	0.005		0.021	0.026	0.025	
		(224)	(0)	(190)	(378)	(40)	(0)
*PEPA-1**	100	0.958	0.958	0.945	0.940	0.981	0.931
	90	0.042	0.042	0.055	0.060	0.019	0.069
		(240)	(400)	(200)	(400)	(52)	(246)
*PEPB-1**	100	0.809	0.786	0.783	0.782	0.904	0.828
	130	0.191	0.214	0.217	0.218	0.096	0.172
		(204)	(392)	(198)	(400)	(52)	(238)
*PEPC**	100	(204)	(0)	(140)	(398)	(32)	(66)
*PEPD-2**	100	(0)	(0)	(0)	(400)	(24)	(0)
*PGDH**	100	(160)	(0)	(194)	(360)	(42)	(228)
*PGK-2**	100		0.848	0.902	0.920		0.830
	90		0.152	0.098	0.080		0.170
		(0)	(198)	(132)	(88)	(0)	(106)
*PGM-1**	-100	1.000		1.000	0.992	1.000	1.000
	-70	0		0	0.008	0	0
		(220)	(0)	(184)	(400)	(52)	(222)
*PGM-2**	-100	0.995		1.000	1.000	1.000	1.000
	-70	0.005		0	0	0	0
		(220)	(0)	(192)	(400)	(52)	(244)
*sSOD-1**	-100	0.461			0.472	0.500	
	-260	0.539			0.528	0.500	
		(230)	(0)	(0)	(398)	(42)	(0)
*TPI-4**	100			0.955			0.964
	105			0.045			0.036
		(0)	(0)	(200)	(0)	(0)	(248)

Appendix 6. Enzyme allele frequencies of Lewis hatchery and wild fall chinook from the 1984 cohort (parental adults and their juvenile progeny) and parental adults of the 1985 hatchery cohort ($Adult^{85}$). Only allelic sample sizes (in parentheses) are given for loci that were monomorphic in these samples.

		Hatchery cohort				Wild cohort		
Locus	Allele	Adult	Fry	Smolt	$Adult^{85}$	Adult	Fry	Smolt
*sAAT-1,2**	100	(56)	(0)	(520)	(384)	(664)	(0)	(776)
*sAAT-3**	100			1.000	1.000			0.997
	90			0	0			0.003
		(0)	(0)	(260)	(110)	(0)	(0)	(386)
*sAAT-4**	100	(0)	(0)	(60)	(0)	(0)	(0)	(274)
*ADA-1**	100			0.992	1.000			
	83			0.008	0			
		(0)	(0)	(260)	(172)	(0)	(0)	(0)
*ADA-2**	100			0.988	1.000	1.000		
	105			0.012	0	0		
		(0)	(0)	(252)	(146)	(154)	(0)	(0)
*ADH**	-100			0.927			0.988	0.966
	-52			0.073			0.012	0.034
		(0)	(0)	(262)	(0)	(0)	(162)	(388)
*sAH-1**	100	0.778		0.786	0.799	0.823		0.776
	86	0.222		0.206	0.196	0.170		0.201
	116	0		0.008	0.005	0.007		0.023
		(18)	(0)	(262)	(204)	(288)	(0)	(388)
*CK-1**	100	(0)	(0)	(180)	(60)	(0)	(0)	(188)
*CK-2**	100	(0)	(0)	(218)	(100)	(0)	(0)	(380)
*GPI-1**	100	(28)	(0)	(262)	(206)	(262)	(0)	(388)
*GPI-2**	100	1.000		1.000	1.000	1.000		0.928
	60	0		0	0	0		0.072
		(28)	(0)	(262)	(206)	(262)	(0)	(388)
*GPI-3**	100	1.000		1.000	1.000	1.000		0.997
	93	0		0	0	0		0.003
		(28)	(0)	(262)	(206)	(340)	(0)	(388)

Appendix 6. Continued.

Locus	Allele	Hatchery cohort				Wild cohort		
		Adult	Fry	Smolt	Adult[85]	Adult	Fry	Smolt
*GR**	100		0.824	0.738				0.885
	85		0.176	0.262				0.115
		(0)	(74)	(42)	(0)	(0)	(0)	(394)
*sIDHP-2**	100	(8)	(0)	(252)	(176)	(230)	(0)	(388)
*sIDHP-3,4**	100	0.942		0.943	0.952	0.976		0.944
	127	0.019		0.034	0.025	0.020		0.042
	74	0.039		0.023	0.023	0.004		0.014
		(52)	(0)	(524)	(400)	(656)	(0)	(764)
*sMDH-1,2**	100	(60)	(0)	(262)	(408)	(732)	(0)	(776)
*sMDH-3,4**	100	0.977	0.997	0.996	0.976	0.992		0.985
	121	0.023	0.003	0.004	0.024	0.008		0.014
	70	0	0	0	0	0		0.001
		(44)	(400)	(524)	(412)	(656)	(0)	(776)
*MPI**	100			0.477	0.432	0.438		0.521
	109			0.508	0.545	0.548		0.453
	95			0.015	0.023	0.014		0.026
		(0)	(0)	(262)	(176)	(146)	(0)	(388)
*PEPA-1**	100	1.000	0.933	0.897	0.889	0.943	0.906	0.889
	90	0	0.087	0.103	0.111	0.057	0.094	0.111
		(30)	(194)	(262)	(198)	(332)	(394)	(388)
*PEPB-1**	100		0.913	0.905	0.918	0.937	0.930	0.920
	130		0.087	0.095	0.082	0.063	0.070	0.080
		(0)	(196)	(262)	(208)	(334)	(384)	(388)
*PEPC**	100	1.000		1.000	1.000	0.973		1.000
	105	0		0	0	0.027		0
		(12)	(0)	(262)	(172)	(294)	(0)	(386)
*PEPD-2**	100	1.000		1.000	1.000	1.000		0.997
	107	0		0	0	0		0.003
		(12)	(0)	(260)	(206)	(288)	(0)	(386)
*PEP-LT**	100	(0)	(0)	(258)	(170)	(0)	(0)	(0)
*PGDH**	100	(24)	(0)	(262)	(210)	(188)	(0)	(388)

Appendix 6. Continued.

		Hatchery cohort				Wild cohort		
Locus	Allele	Adult	Fry	Smolt	Adult[85]	Adult	Fry	Smolt
*PGK-2**	100		0.800	0.687	0.766		0.694	0.716
	90		0.200	0.313	0.234		0.306	0.284
		(0)	(190)	(262)	(184)	(0)	(108)	(388)
*PGM-1**	-100	1.000		0.988	0.957	1.000		0.972
	-70	0		0.012	0.043	0		0.028
		(22)	(0)	(260)	(210)	(260)	(0)	(388)
*PGM-2**	-100	1.000		1.000	1.000	1.000		0.997
	-70	0		0	0	0		0.003
		(30)	(0)	(258)	(210)	(356)	(0)	(388)
*sSOD-1**	-100	0.500	0.514	0.481		0.643	0.617	0.562
	-260	0.500	0.486	0.519		0.357	0.383	0.438
		(30)	(146)	(262)	(0)	(272)	(326)	(388)
*TPI-4**	100			0.988				0.962
	105			0.012				0.038
		(0)	(0)	(244)	(0)	(0)	(0)	(208)