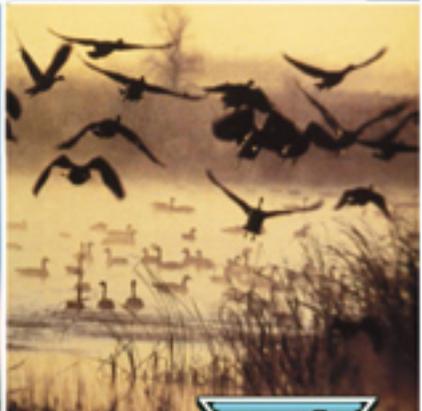
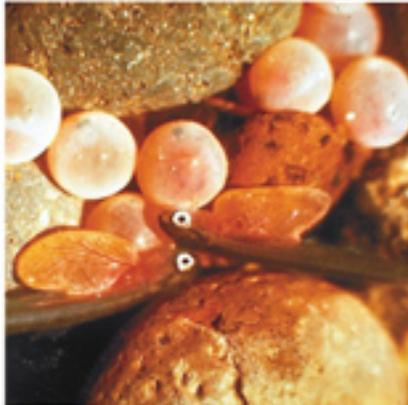


Genetic Analysis of *Oncorhynchus Nerka*

Annual Report 1991

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GENETIC ANALYSIS OF ONCORHYNCHUS NERKA

Annual Progress Report

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**Genetic Analysis of *Oncorhynchus Nerka*
Project Number (90-93)**

Introduction:

The 1990 project to develop DNA assessment techniques for the purpose of determining relationships among populations of *Oncorhynchus nerka* demonstrated differences that had potential for such application. The work was continued in 1991 with specific application of the techniques to develop DNA probes that could be used in separating populations of *O. nerka* associated with the lakes in the upper Salmon River, principally those in Redfish Lake. Research included sockeye-kokanee life history studies that might add supporting evidence for assessing the degree of difference or similarity among populations in the lake systems. This report summarizes the annual activities under the work plan.

Backaround:

The *O. nerka* population in Redfish Lakes consists of the larger (2 to 5 lb) anadromous fish that emigrate as age 1 or 2 year smolts and return from sea at age 4 or 5 years, and smaller resident fish that mature in the lake. The anadromous strain, typically referred to as sockeye, are presently believed to spawn only along a 400 meter section of the northeast shoreline of Redfish Lake (Fig. 1) during the month of October. Their fry emerge from incubation areas in the spring and rear in limnetic environments for one or two years before migrating to sea. A few of the progeny may adopt residence behavior which is a pattern characteristic of anadromous populations in general.

Resident, non-migratory sockeye salmon are commonly referred to as "kokanee". Kokanee are considered to represent the well established long-term resident form isolated from their anadromous progenitors by many generations in contrast to "resident sockeye" identified as more recent descendents. How well kokanee remain isolated from sympatric anadromous sockeye is a matter of their temporal and spatial separation at spawning. The Redfish Lake kokanee population spawns in Fishhook Creek,

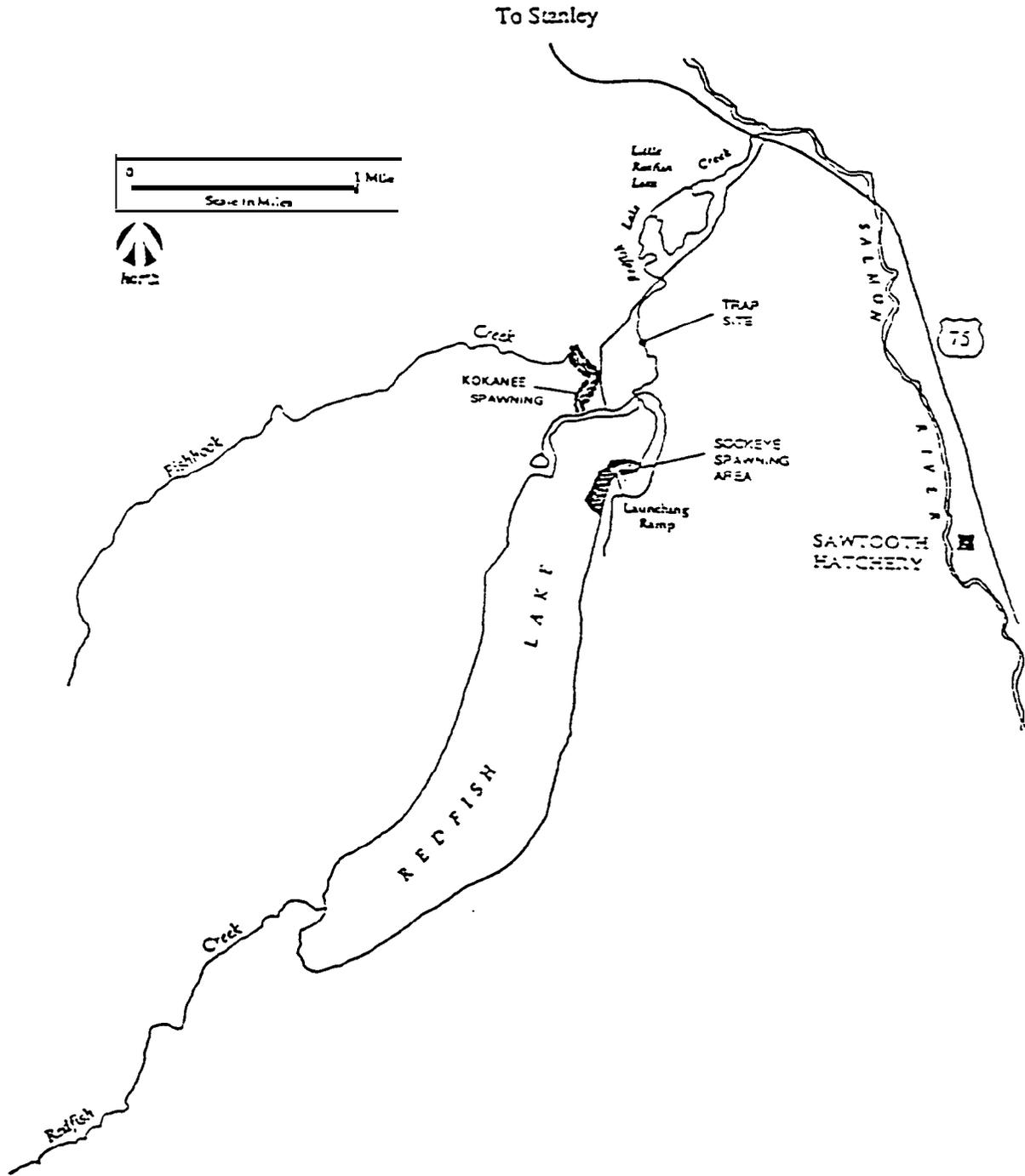


Figure 1. Redfish Lake, showing the sockeye salmon spawning beach in the lake, and the kokanee spawning area in Fishhook Creek

one of the inlet streams of the lake, during August and September. Temporal separation from the beach spawning sockeye is established by the differences in incubation temperatures between the beach and the cooler inlet stream. Although other kokanee populations may exist in Redfish Lake, there has been no evidence of other established spawning sites or observations of spawning at other locations in the lake.

Evermann (1895) reported that large redfish spawned in Redfish Lake in mid-August, 1887-89 and 1893. A mid-August spawning time, however, would identify Fishhook Creek as the spawning site, rather than the beach used by the present anadromous stock. Incubation temperatures appropriate for emergence timing in April and May would occur for August spawners only in Fishhook Creek and the other inlet creek, Redfish Lake Creek. Information at the United States Forest Service Interpretive Center indicates that in the late 1800's a miner constructed an access road along lower Fishhook Creek to harvest sockeye salmon for mining camps in the area, which would indicate that sockeye indeed used Fishhook Creek historically and apparently in large numbers. Sockeye have not been observed in Fishhook Creek recently. Presently, only kokanee are observed to spawn in the stream and at the same August spawning time reported for sockeye by Evermann. It is very likely that the present kokanee population in Fishhook Creek are the best remaining representatives of the original Fishhook Creek sockeye gene pool.

Incubation temperatures on the lake shore would place shoal spawning sometime in October. Evermann did not specify any observations of lake shore spawning. However, he may not have been present at Redfish Lake during October to observe lake spawners. Reports from the 1930's-50's indicate all the sockeye were October lake spawners. From 1954 to 1964 a few to several hundred sockeye were counted entering the lake. From 1965 to 1987, weir counts were resumed and adult counts were 11, 29, and 14 respectively. The Redfish Lake Creek trap was not operated from 1989-90, but in 1991, four returning sockeye were counted and held to maturity at the Sawtooth National Fish Hatchery. They too showed the mid-October spawning time which would identify them as lake shore spawners, and representatives of the lake-shore spawning sockeye gene pool.

There has been opportunity for introduced genes in the system from transplants. Reports of spawning activity at various locations in the lake other than the two traditional sites can be explained by such transplants, especially when no imprinting efforts were made to induce homing to the traditional spawning areas. Under such circumstances random distribution would be expected and affinity for particular locations would occur only for release sites.

Redfish Lake was first stocked with 17,500 hatchery kokanee in 1930. The source of the stock is unknown but most likely from Kootenay Lake in British Columbia. Redfish Lake received periodic stockings of kokanee from unknown sources at least until 1945. In 1962, Redfish Lake was stocked with 43,251 late spawning kokanee from North Idaho. Fishhook Creek was stocked with 50,344 early spawning kokanee from an unknown source in 1971. However, the Fishhook Creek stocking is listed as Anderson Ranch Reservoir stock by Hall-Griswold (1990). The Anderson Ranch stock may have originated from Island Park, North Idaho and British Columbia kokanee stocked in Anderson Ranch Reservoir between 1964 and 1967. Redfish Lake received 45,900 kokanee (unknown source) in 1971 and 51,435 early spawning kokanee (unknown source) in 1972. The Idaho Department of Fish and Game (IDFG) stocking records do not show any additional kokanee stocking in Redfish Lake or Fishhook Creek from 1972 to the present.

Laboratory and Field Activities:

The research undertaken in the University of Idaho/Washington State University study is directed at four questions, (1) how similar or dissimilar are the sockeye and kokanee populations in Redfish lake, (2) is the present sockeye or kokanee population represented by more than one deme, (3) are there other populations of *O. nerka* in Redfish Lake, and (4) what are the behavioral stimuli to induce resident sockeye or kokanee to migrate. Tasks in progress have addressed primarily the first three questions.

Task I - DNA Analysis:

The first task was to examine the characteristics of the populations to determine what differences were present other than what was determined by electrophoresis work of the National Marine Fisheries Service. Tissues were collected for DNA analysis in an attempt to identify markers that can be used to separate groups within the system.

With regard to life history information, the adult size forms of *O. nerka* that were intercepted by IDF&G all ripened for spawning in mid-October, indicating that their origin was from a similar spawning time, and corresponding to that recorded for sockeye lake spawners. The survey of Fishhook Creek indicated there were no sockeye sized forms of *O. nerka* present in the stream. These data indicated that in 1992 the forms generally identified as kokanee and sockeye were isolated and created no apparent uncertainty as to their brood origin.

Several genetic differences have been observed in *O. nerka* populations using DNA fingerprinting, which may be useful in elucidating relationships between Redfish Lake sockeye and kokanee, and these populations from other populations of interest. DNA fingerprints of the four returning Redfish Lake sockeye were compared to kokanee populations and Babine sockeye for reference. Redfish sockeye DNA fingerprints were compared in hybridization experiments using two probes; the SNAP probe and a new probe based on the *Drosophila* "Per" sequence. The SNAP probe revealed a band in the 15 kilobase-pair (kbp) range of all four Redfish Lake sockeye that is not present in either the Redfish kokanee or Babine sockeye. Another band at 19 kbp in the Redfish sockeye appears to be present in Redfish and Alturas kokanee but is slightly less intense in these populations. The Per probe showed a doublet of 23 kbp bands and a 14 kbp band appear in all Redfish sockeye and kokanee and Alturas kokanee but is apparently absent in Babine sockeye. Another band at 7 kbp is present in all four populations but shows variation between individuals.

A new technique call "DNA mixing" was used in conjunction with DNA fingerprinting that has made it possible to conduct these comparisons more rapidly. In this techniques the DNA of several individuals of a group or

population are mixed before cutting the DNA with a restriction enzyme. An aliquot of this DNA mix is then cut and used in a hybridization experiment. In theory, the pattern derived from the mixed sample will only show bands that are shared between a majority of individuals. Variable bands (those that appear with low frequency) will be diluted to the extent that they will not appear in the pattern. In a pilot experiment, three individual DNA samples and a DNA mix from each of four populations (Babine sockeye, Redfish sockeye, Redfish kokanee, and Alturas kokanee) in a hybridization experiment was run. For each population, the mixes were prepared by mixing the DNA of eight individuals (except in the Redfish sockeye where only four individuals were mixed). As predicted, the mixed DNA samples showed shared bands while rare bands appearing in individuals did not appear in the mix patterns. The mixed patterns showed the same band differences noted above.

In a second mixing experiment, DNA mixes of the same four populations using four restriction enzymes was run. Several differences were observed among populations. Using two different probes a total of eight band differences were identified between Redfish sockeye and kokanee. Three bands appeared different between Alturas and Redfish kokanee. Babine showed a pattern quite different from the Stanley Basin populations. These data suggest the DNA markers will be identified that can separate origin or relationships. Unfortunately only DNA from the Fishhook Creek kokanee was of adequate quality for the initial DNA hybridization studies. The sockeye and outmigrant samples were sheered to the extent that they did not provide adequate DNA fingerprints. Tests with sockeye and outmigrant samples will continue in order to obtain sufficient resolution.

Table I summarizes the differences observed in DNA fingerprints of *O. nerka* from Redfish and Alturas Lakes. Several differences between Redfish Lake sockeye and kokanee (Fig 2. as an example) may be useful in identifying origin of outmigrants. Differences shown between kokanee from Redfish and Alturas Lakes demonstrate that DNA fingerprinting may reveal fixed differences between closely related populations. Differences observed between these three populations and geographically distant sockeye population from Babine Lake (data not shown) are more extensive and

testify to the closer relationship among the remaining Stanley Basin populations.

A third set of experiments, using mitochondrial DNA, has also been **successful in identifying *O. nerka* stock differences. Mitochondrial DNA** was isolated from coho salmon for use as a hybridization **probe. The probe** was random prime labeled following instructions provided with the Genius kit (Boehringer Mannheim Corp.). Genomic DNA from Babine and **Lake Wenatchee sockeye** and the three kokanee populations of Redfish **Lake, Alturas and Warm Lake** was examined. **Restriction enzyme digests of three enzymes, BGL-I, BGL-II, PST-I ., were examined with the mitochondrial probe. BGL-II is showing an additional restriction enzyme cut site in all kokanee (n = 20) (Fig.31 and a percentage of sockeye (37.5%, n =8) of Babine and (.50%, n = 8) of Lake Wenatchee. Based on analysis to date, a mitochondrial marker appears to be present in all kokanee and a percentage of both Lake Wenatchee and Babine sockeye.**

Task II - Assessment of Subpopulation Differences:

The second task was to determine whether or not more than one deme was represented within the present Redfish Lake sockeye and kokanee populations. Since the number of returning sockeye were so few, it would have been impossible to have made any conclusion about the anadromous population structure. No resident forms of *O. nerka* were observed on the recorded historic sockeye spawning beach in the lake, indicating that if resident sockeye were present in the 1991 brood year, they were in very low numbers. The Fishhook Creek kokanee population was present on its historic spawning grounds over eight weeks. With spawning occurring over such a time period, there is a possibility that more than one deme exists within the kokanee population. To examine for subpopulation differences, incubation rates, and egg sizes were measured from fish sampled during the earlier and later portions of the run. Tissue samples were also taken from the population for DNA analysis of the early and later segments when appropriate markers become available. The kokanee spawner enumeration and egg development work has been completed with the results listed below.

1. The kokanee population was present in Fishhook Creek from August 2nd to September 28th, with a population size estimate (cumulative fish days/stream life) of 7200 fish (4235 max live count).
2. Fish spawning was observed on August 9th, first death on August 17th, with peak mortality occurring between September 4th and 9th.
3. Dates of artificial spawning for rate of development and timing studies were August 22nd and September 5th. A period of 14 days separated egg lots.
4. The eggs were incubated at the IDF&G Eagle Laboratory, Eagle, ID, in near constant temperatures (12.5°C to 13.8°C) in separate upwelling incubators.
5. The September 5th egg lots had a faster rate of development than the August 22nd egg lots, showing less yolk at comparable time intervals following fertilization, and reached yolk absorption 12 days after the earlier lots, or a two day reduction (14% compensation) in the 14 days separating spawning dates.
6. These data suggest that separate demes exist within the Fishhook Creek kokanee population, and that development rate differences serve to compress emergence timing centrally for the population.

Task III - Lake Survey for Spawning Sites

Task three was to survey the lake for differences in DNA patterns and egg developmental rates. The lake was surveyed at intervals from August to November for spawning representative of *O. nerka*, and for temperature differences on the surface of the lake substrate that would be appropriate for spawning, given the timing/temperature relationship necessary to correspond to spring emergence timing. No *O. nerka* forms were observed in the lake, and no temperature differences were detected that would suggest spawning areas other than the recorded historic sockeye spawning beach. A slight temperature difference has been shown along the historic

sockeye spawning beach, and spots off the lodge along the eastern shore. The temperature difference suggests only a slight infiltration of surface or ground water, but that appears to have provided sufficient irrigation of the incubation area to assure embryo survival with reduced metabolic needs of the embryo at the low temperatures characteristic of that elevation.

In conclusion, differences in rates of development between the early and late spawning segments of the kokanee population using Fishhook Creek, suggest that at least two demes exist in the population. Further, the kokanee in Fishhook Creek demonstrate sufficient temporal separation from the present beach spawning sockeye population to effect isolation between the present kokanee and anadromous stocks. The fact that such a difference in spawning time exists between the sockeye and kokanee populations is evidence that stocks in Redfish Lake have evolved unique differences at least with regard to spawning times associated with the different incubation habitats they use. However, it cannot be concluded these populations are reproductively isolated to the extent that no gene transfer exists between them.

The fact that significant numbers of kokanee were transplanted in Redfish Lake raises the issue that neither of the *O. nerka* populations may be totally representative of their founding populations. Fishhook Creek transplants would have homed to Fishhook Creek, and may have mixed with endemic stock. Beach spawning kokanee from transplants may have spawned with sockeye, or may have even been the progenitors of the beach spawning stock. The conclusion that present spawning populations of sockeye and kokanee are not members of the same stock, however, is justified by the behavioral information, and the DNA patterns are appearing to support that conclusion. Moreover, DNA work to date has indicated that Redfish Lake sockeye and kokanee, and Alturus Lake kokanee are sufficiently different from other populations of *O. nerka* examined that contribution genetic material from transplants made in the past has not been substantial.

Literature Cited

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Hall-Griswold, J. 1990. Sockeye of Stanley Basin, a summary. IDF&G report.

Table 1. Summary of band differences observed between population of *O. nerka*. Observed differences between Redfish and Alturas Lake kokanee (RK-AK) and between Redfish Lake kokanee and sockeye (RK-RS) are shown. Each number represents the molecular weight of the variable band. Bold numbers represent the clearest and most useful differences. ND means no difference was observed between the populations. Double dashes represent enzyme and probe experiments that were not performed..

<u>ENZYME</u>	<u>PROBE</u>					
	<u>SNAP</u>		<u>MI3</u>		<u>PER</u>	
	<u>RK-AK</u>	<u>RK-RS</u>	<u>RK-AK</u>	<u>RK-RS</u>	<u>RK-AK</u>	<u>RK-RS</u>
Taq I	ND	23 5.5	ND	ND	ND	7
RsaI	20	20	ND	12	ND	17
Bgl II	ND	5	--	--	ND	8
Hinf I	20	20 7	ND	ND	ND	ND
Msp I	ND	19	ND	ND	ND	ND
BamH-II	5	ND	--	--	--	--

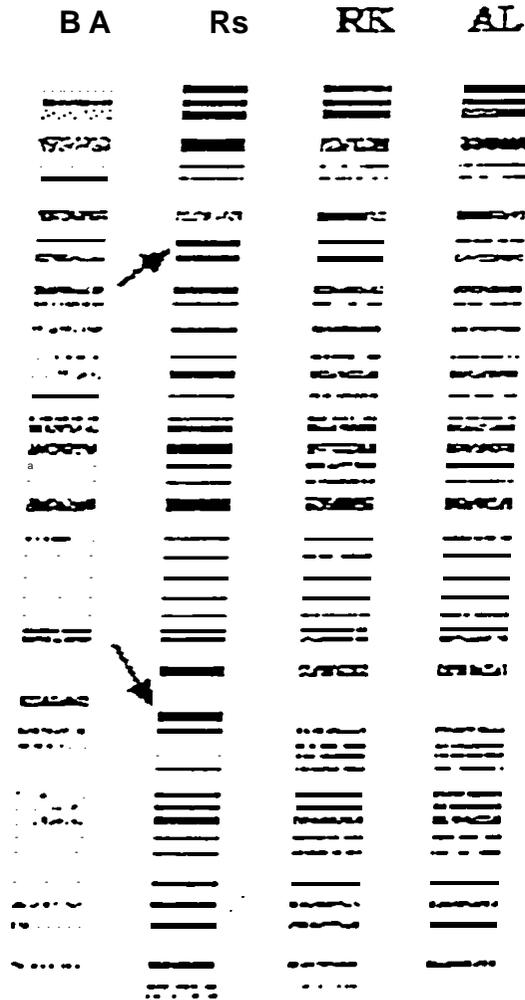


Figure 2. DNA fingerprints of DNA mixes from Babine Lake sockeye (BA), Redfish Lake sockeye (RS), Redfish Lake kokanee (RK), and Alturas Lake kokanee (AL). DNA samples were cut with restriction enzyme Taq I and probed with SNAP. Arrows indicate bands differentiating Redfish Lake sockeye from kokanee.

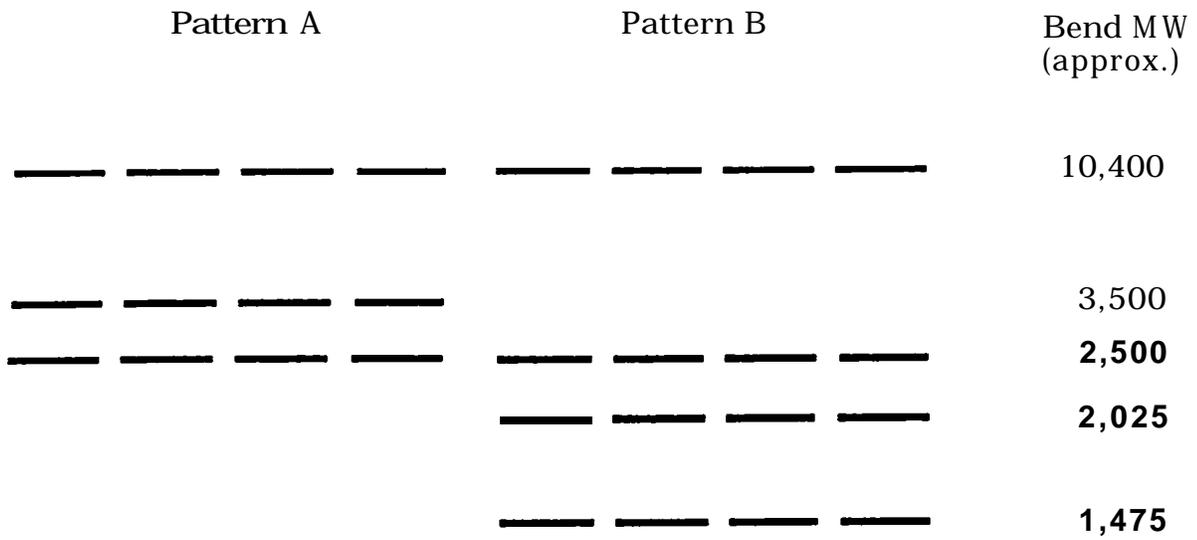


Figure 3. Bands observed on southern blot of genomic DNA cut with restriction enzyme BGL-II and probed with mitochondrial DNA. Pattern A has been observed only in anadromous sockeye. Pattern B has been seen in both anadromous sockeye and resident kokanee.