# PACIFIC SALMON COMMISSION 

THE NORTHERN BOUNDARY AND TRANSBOUNDARY RIVER TECHNICAL COMMITTIEES

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STOCK IDENTIFICATION OF SOCKEYE SALMON
USING BIOLOGICAL MARKERS

## Prepared for

The Northern Panel
of
The Pacific Salmon Commission

## TABLE OF CONTENTS

Page
LIST OF TABLES ..... iii
LIST OF FIGURES ..... iv
FOREWORD ..... v
1.0 0bjectives ..... 1
2.0 Description of Biological Markers ..... 1
2.1) Scale patterns ..... 1
2.2) Age composition ..... 2
2.3) Parasite prevalence ..... 4
2.4) Genetic markers ..... 4
3.0 Comparison of Biological Markers ..... 4
3.1) Stock resolution potential ..... 4
3.2) Stability of markers over time ..... 10
3.3) Homogeneity of markers with respect to age and sex ..... 18
3.4) Sampling considerations ..... 18
3.5) Sample collection and analysis costs ..... 22
4.0 Approaches to Estimating Stock Composition ..... 22
4.1) Pooling stocks to simplify analysis ..... 22
4.2) Increasing stock resolution potential ..... 23
5.0 Technical Committee Goals ..... 30
6.0 Literature Cited ..... 31
7.0 Appendix l. Summary of scale samples collected routinely from sockeye spawning escapements in in southern Southeast Alaska and Canada, 1982- 1986. Scale samples have been collected from additional Canadian stocks that are not sampled routinely (see Appendix 3 ) ..... 32
8.0 Appendix 2. Summary of brain parasite (Myxobolus neurobius) samples collected from sockeye spawning escapements in southern Southeast Alaska and Canada, 1982-1986 ..... 35
9.0 Appendix 3. Summary of matched electrophoretic-brain parasite-scale samples available from sockeye spawning escapements in Southeast Alaska and Canada, 1982-1986 ..... 39

## TABLE OF CONTENTS (Continued)

10.0 Appendix 4. Summary of scale samples from commercialand test fishery. sockeye landings in southernSoutheast Alaska and northern British Columbia.This table does not include matched electrophoretic-parasite-scale samples (see Appendix 6)43
11.0 Appendix 5. Summary of brain parasite samples collected from commercial and test fishing sock- eye salmon landings, 1982-1986. This table does not include matched electrophoretic-parasite- scale samples (see Appendix 6) ..... 44
12.0 Appendix 6. Summary of matched electrophoretic- parasite-scale samples from commercial and test fishing sockeye salmon landings, 1982-1986 ..... 45
13.0 Appendix 7. References available or in preparation on sockeye stock identification using biological markers ..... 46
Table Page

1. Year to year variability in biological markers from scales. ..... 11
2. Year to year variability in brain parasiteprevalence and common (100) allele frequenciesfor polymorphic enzymes.............................15
3. Variability with respect to sex of biologicalmarkers from scales19
4. Variability with respect to sex of brainparasite prevalence and common (100) allelefrequencies for polymorphic enzymes................ 20

## LIST OF figures

Figure

1. Sockeye salmon populations in Southeast Alaska and northern British Columbia for which matched brain parasite, electrophoretic, age composition and scale pattern data are available.... 4
2. Similarity dendrogram for 41 sockeye populations based on scale pattern data only.................................
3. Similarity dendrogram for 52 sockeye populations based on freshwater age composition only ..... 7
4. Similarity dendrogram for 52 sockeye populations based on brain parasite (Myxobolus neurobius) prevalence only ..... 8
5. Similarity dendrogram for 52 sockeye populations based on electrophoretic differences in five polymorphic enzymes ..... 9
6. Similarity dendrogram for 52 sockeye populations based on combined data from Figures 4 and 5 ..... 24
7. Similarity dendrogram for 52 sockeye populations based on combined data from Figures 3, 4 \& 5 ..... 25
8. Similarity dendrogram for 41 sockeye populations based on combined data from figures 2, 3, $4 \& 5 \ldots$ ..... 26
9. Simulation results showing the effect of combining biological markers to increase accuracy and precision of stock composition estimates ..... 28
10. Simulation results showing the effect of summing estimates for poorly differentiated stocks to increase the accuracy and precision of stock composition estimates ..... 29

This report was prepared in response to the Northern Panel request generated during the February 17-18, 1986 Commission meetings in Vancouver.

The Panel requested:
"Stock Identification - District 104 and 106
The Northern Panel directs the joint technical committee and national research scientists responsible for the design of the U.S.-Canada cooperative research program to carefully evaluate the research effort directed at sockeye stock separation in the Noyes Island and District 106 fishery in 1986. The objective of the program is to identify stock composition by week by fishery. The Panel realizes that the late stage in the development of the 1986 program and other priorities may not allow a major expansion of effort, but they should determine if program adjustments would better address the stated objectives and could be accomplished within the framework of the existing overall program. Regardless of the outcome of this deliberation the Panel will request a complete review of the sockeye stock separation program for 1987."

The response of the Northern Boundary Technical Committee comprises three parts:

1) An oral presentation to be given at the Pacific Salmon Commission meetings November 16-21, 1986.
2) A written report to accompany the oral presentation.
3) Summaries of sockeye stock identification sampling activities and a bibliography are provided as appendices to the report.

The following report was prepared to evaluate the research directed at sockeye stock identification. It describes and compares the various kinds of biological markers used in differentiating sockeye populations, and explains two different approaches used to estimate sockeye stock composition in the Northern Boundary area.

Stock_Identification of Sockeye Salmon_Using Biological Markers.

## 1.0

OBJECTIVES

Recent advances in statistical methods and computational capabilities have made it feasible to estimate the stock composition of mixed-stock fishery samples by examining biological attributes that differ between stocks. If satisfactory biological "markers" can be found, stock composition could be determined routinely without resorting to expensive and laborious tagging studies. A variety of biological markers have been studied in sockeye salmon populations, and it is generally accepted that the reliability of stock composition estimates using these markers is potentially equal to that of estimates derived from tagging studies. The purpose of this report is first, to describe and compare the various kinds of biological markers which are useful for differentiating sockeye populations, and second, to explain the two different approaches that have been used to estimate sockeye stock composition in the Northern Boundary area (northern British Columbia - Southeast Alaska). This report also summarizes recent sampling activities for sockeye stock identification and includes recommendations for further research.

## 2.0

## DESCRIPTION OF BIOLOGICAL MARKERS

### 2.1 Scale_Patterns

The pattern of circulus formation in sockeye scales records an individual fish's growth history. Most of the scale features used to differentiate stocks are associated with growth in freshwater where different stocks occupy discrete environments that may differ in temperature and in the availability of food. Scale patterns are particularly useful for sockeye salmon because the vast majority of juvenile sockeye inhabit lakes for a year or more, thus most members of a population share a fairly uniform environment. A large number of scale features can be measured from a single scale but usually these can be expressed as two or three uncorrelated markers. The most basic scale features are the number of circuli, and the distance between circuli in each year of freshwater growth.

Scale patterns have proven very useful for in-season management within large river systems (e.g., the Fraser River, Cook and Guthrie 1987) or where a relatively small number of stocks contribute to mixed-stock fisheries (e.g., the Lynn Canal, McPherson 1986). Their utility in analyzing mixed-stock fishery samples with contributions from a large number of stocks ("coastwide mixtures") is less certain owing to probable variation in scale
patterns from year to year and age group to age group which necessitates extensive resampling of individual stocks (see Section 3.2). A total of about 120 stocks have been sampled to
date, but only about 60 are resampled routinely each year (Appendix 1). The Alaska Department of Fish and Game (ADFG) uses scale pattern data to estimate stock composition of sockeye catches in Southeast Alaskan Districts 106, 108 and 111 on a weekly basis (in-season) for principal age classes. Since scales are also collected routinely from many other fishing areas (Southeast Alaskan Districts $102,103,104,105$, and 107 and British Columbia Areas 3,4 and 5 ) as well as all significant escapements to provide age composition data and for post-season stock composition analysis, additional stock identification studies could be conducted without additional collection costs. It is anticipated that scale ageing and digitizing systems which should soon be operational, will significantly reduce the cost and time required for analysis.

### 2.2 Age_Composition

Sockeye salmon populations frequently differ dramatically in both freshwater and marine age composition. Freshwater age typically varies from 0. (i.e., no freshwater annular marks indicating that fry did not spend a winter in freshwater) to 3. (i.e., three winters spent in freshwater). Age l. sockeye are prevalent in most coastal and clearwater interior lake systems whereas 2. sockeye are prevalent in glacial lakes.

Marine age is a useful marker, in principle, since much variation exists among populations. However, its practical value is limited owing to great variability from year to year within a stock and because sockeye of different age groups often migrate through fisheries at different times during a season (McPherson 1986). Even so, age composition can be used for estimating stock composition where samples can be obtained over the entire run, and pooled to provide representative "seasonal" samples of the catch. The age composition in a seasonal mixture sample of fish from a particular stock should then be representative of that stock whereas the age composition of fish from that stock sampled in the fishery in any single week may not be representative.

### 2.3 Parasite Prevalence

Parasite prevalence refers to the proportion of fish in a population that are infected by a particular parasite. Many different parasites are found in sockeye salmon, but for stock identification in the Northern Boundary area, researchers have focused on a protozoan brain parasite (Myxobolus neurobius). Little is known of the lifecycle of this species but the presence of this parasite is thought to be determined by characteristics of the freshwater rearing environment. Again, because sockeye juveniles share the same lake environment, they probably share the same risk of being parasitized. A total of 81 sockeye populations have been examined for the parasite (Appendix 2). The vast majority of Southeast Alaskan stocks appear to be infected heavily whereas relatively few Canadian stocks are infected. For this reason, the proportion of parasitized sockeye in a mixed-stock fishery sample can be interpreted as the maximum contribution from Southeast Alaskan stocks. Brain parasite data have been used to estimate stock composition in weekly sockeye samples from Southeast Alaskan Districts 101, 106 and 111; and Canadian Areas 1, 3, 4, 23, and in the Stikine, Taku and Nass Rivers.

Other parasites have been used to differentiate up to seven stocks in the Fraser River system with excellent precision. However, the prevalence of these parasites is less well studied and their usefulness in the Northern Boundary area has not been demonstrated.

### 2.4 Genetic_Markers

Methods for detecting genetic differences among stocks are gaining widespread popularity in fisheries research. Several different procedures for detecting genetic variation have been developed. The most widely used involves electrophoresis to detect variation in the electrical properties of simple enzymes. The genetic mechanism for this variation is then determined in crossbreeding studies. The sockeye populations (approximately 60) represented in Figure 1 (and Appendix 3) have been screened for differences in the frequency of alternative alleles that code for specific biological characteristics. In sockeye, only about six of these enzymes are polymorphic (i.e., exhibit more than one form), but three of these vary greatly among populations and are very useful for stock identification in the Northern Boundary area. Technology is evolving rapidly in this field, and it seems likely that other useful genetic markers will be discovered in the near future. Already, electrophoretic techniques to assay a fourth polymorphic enzyme have improved to the point where it will soon be added to the stock identification data base.

Other procedures are also being developed to detect genetic differences in mitochondrial tRNA or nuclear DNA itself, rather than in the enzymes that the DNA produces. This should permit

the detection of much more genetic variation among stocks. However, the usefulness or feasibility of these new procedures has not yet been demonstrated for sockeye salmon.
3.0

COMPARISON OF BIOLOGICAL MARKERS

### 3.1 Stock Resolution Potential

Some biological markers permit differentiation of more stocks than others. For example the brain parasite can be used to make minimum estimates of the contribution of $100 \%$ parasitized or $100 \%$ non-parasitized groups but the presence of stocks with intermediate levels of parasitism precludes definitive classification of fish. Similarly, sockeye can be one of four possible fresh water age groups - $0 ., 1 ., 2 .$, or $3 .-$ in varying proportions in each stock making age composition data of limited value in differentiating between stocks. In principle, scale patterns can differentiate more groups because there are measured differences in scale features between stocks. However, in practice there is a limited range of values possible which leads to overlap in the frequency distributions of scale features from stock to stock limiting the number of stocks which can be differentiated. Electrophoretic methods typically detect 2-6 different genotypes for each enzyme in sockeye populations so that each fish sampled must fall into one of only several categories. However, when two different enzymes are considered simultaneously, each with, say 3 genotypes, the potential number of categories increases to 2 or 8. Thus, using markers in combination dramatically increases stock resolution potential. This approach is explored in Section 4.2.

Similarity among stocks can be defined in terms of a "distance" calculated from the probabilities of drawing a fish with particular scale patterns, (or genotypes) from each population. The similarity dendrograms illustrated in Figures 2-5 show which sockeye populations resemble one another in terms of three scale features (Figure 2), freshwater age (Figure 3), brain parasite prevalence (Figure 4) and five polymorphic enzymes (combined, Figure 5). The distance scale is constant in all of these dendrograms to permit comparison. The vertical lines linking horizontal lines indicate the relative distance separating the linked stock-group; stocks joined by vertical lines on the extreme left hand margin are virtually indistinguishable using the marker in question, whereas those joined together farther to the right are easier to differentiate. Note that the dendrogram based on three scale features (Figure l) shows the greatest stock resolution potential. However, it should be noted that the distance measurement in the dendrogram reflects both the real differences among stocks and differences due to sampling error associated with small samples. Markers which can take on many different values (such as scale circulus counts) can be most

 considered for a\&e 1.3 sockeye wrive-the number of circuli laid down during (1) the first year of freshwater growth, (2) during "spring growth" in fresh water in the second year, and (3) during marine growth in the remainder of the second year. Samples lange in size from 4 to 125 ( 10 samples (15) and were collected botweon 1979 and 1986. Distances between populations abloulated wsine pricedure of Wood et al. (1987b) for finure z-is.


Regions are defined in Figures $2-8$ as: Alsek watershed (01): Lynn Canal Southeast Alaska District 112 (02): Southeast Alaska Districts 101-111 (03): Taku watershed (04); Stikine watershed (05); Nass watershed (06); Skeena watershed (07); British Columbia central and north coast Areas 5-12 (08); Queen Charlotte Islands (09); Vancouver Island (10); lower Fraser watershed (11): upper Fraser watershed, above Hell's Gate (12).

Figure 3. Similarity tendrofram for 52 sorkeye copulations based on freshwater age composition cnly. :imples range in size from 49 to 3010 and were collected between 1979 and 1986.


```
Fi&ur: t similarity dendrofram for 50 sorkeye proulations based
    wrurain parasit, (Myxrbolus nemrnbius) prevalence
    anly "omrlc:: range in size irom 21 to 1039 and were
    collegted between 1982 and 1585.
```

| Stock | Reqlon |
| :--- | :--- |
| Shuswap | $(12)$ |
| Chilco | $(12)$ |
| Stuart | $(12)$ |
| Adams River | $(12)$ |
| Long | $(08)$ |
| Klukshu | $(01)$ |
| Little Tatsamenie $(04)$ |  |
| Fred Wright | $(06)$ |
| Hackett | $(04)$ |
| Bowser | $(06)$ |
| Little Trapper | $(04)$ |
| Stikine Glacial | $(05)$ |
| Great Central | $(10)$ |
| Birkenhead | $(11)$ |
| Weaver Creek | $(11)$ |
| Cultus | $(11)$ |
| Yakoun | $(09)$ |
| Alastair | $(07)$ |
| Chilkat | $(02)$ |
| Chilkoot | $(02)$ |
| Babine | $(07)$ |
| Lakelse | $(07)$ |
| Tahltan | $(05)$ |
| Meziadin | $(06)$ |
| Naha | $(03)$ |
| Mathers | $(09)$ |
| Stikine Mainstem $(05)$ |  |
| Mercer | $(09)$ |
| Damdochax | $(06)$ |
| Hugh Smith | $(03)$ |
| McDonald | $(03)$ |
| Naden | $(09)$ |
| Devon | $(08)$ |
| Henderson | $(10)$ |
| Shipley | $(03)$ |
| Red Bay | $(03)$ |
| Sproat | $(10)$ |
| Klakas | $(03)$ |
| Owikeno | $(08)$ |
| Helm | $(03)$ |
| Luck | $(03)$ |
| Koeye | $(03)$ |
| Salmon Bay | $(03)$ |
| Karta |  |
| Bonilla | $(03)$ |
| Johnson | $(03)$ |
| Thoms | $(03)$ |
| Kegan | $(03)$ |
| Tenas | $(08)$ |
| Warmchuck | $(03)$ |
| Horsefly | $(12)$ |
| Woss |  |
|  |  |

Figure 5. Similarity dendrogram for 52 sockeye populations based on electrophoretic differences in five polymorphic enzymes (Pgm-1, Pgm-2, Ldh-4, Idh-3 and Idh-4). Samples range in size from 26 to 710 and were collected between 1982 and 1985.

Stock Region

affected by sampling errors. Thus, large sample sizes are required to detect real differences among stocks where differences in scale features are not large.

### 3.2 Stability of Markers Over Time

Stability between years in a biological trait within a stock is one of several important criteria for selecting among biological markers. A marker is stable if its probability of occurrence within a population does not change fromyear toyear, so that individual stocks need not be resampled frequently. The stability properties of scale features and freshwater age are summarized for example stocks in Table 1. Because scale patterns reflect growth history, which probably depends on climate, fish density and the availability of food, they can be expected to vary from year to year within populations. This is evident to some extent in Table l. In general, however, variation within stocks seems to be less than variation among stocks. Simulation studies are required to determine the degree to which stock composition estimates are influenced by this kind of variability. Although superior estimates are usually obtained using scale data from the corresponding year (Marshall et al. 1984), it may still prove useful to use historical scale data when current data is unavailable. This is an important topic for further research since the cost effectiveness of scale analysis will be greatly determined by the amount of annual resampling that is required.

Freshwater age composition also varies from year to year in some populations (Table 1) but, again, where variation within populations is less than between populations, it may be possible to get satisfactory stock composition estimates with historical data. Marine age composition often varies dramatically from year to year (Foerster 1968).

The prevalence of the brain parasite appears to be remarkably stable from year to year (Table 2) although the mechanism for this stability is not understood. The best evidence for stability comes from Great Central and Sproat Lakes on Vancouver Island where the parasite prevalence has varied narrowly between 0 8.4\% and 99.4 - $100.0 \%$ respectively over a period of 8 years (Quinnet al. 1987).

The theory of population genetics predicts that all genetic markers will be stable within isolated randomly mating populations provided there is no survival advantage associated with the alternative genes. Indeed, virtually all sockeye stocks that have been sampled repeatedly exhibit very little variation in gene frequencies (Table 2). It is this property that makes genetic markers attractive for stock identification studies. The data in Tables 1 and 2 span only several years at most. Thus, any gradual trends in population characteristics would not be detected. Even where markers appear to be stable over several

-Continued

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1/ NC1=number of circuli in firat freahwater zone.
NC2enumber of circuli in epring "plus" growth zone.
NC3 $=$ number of circuli in firat ocean zone.
ID1=dietance in mu/100 of firat freshwater zone.
ID2edistance in mim/100 of apring "plus" growth zone.
ID3=diatance in mem/100 of firat ocean zone.
$2 /$ Data not yet analyzed.

Table 2. Year to year variability of brain parasite prevalence and common (100) allele frequencies for polymorphic enzymen of sockeye salmon.



| Stock | Year | Brain Parasite$\qquad$ Sample Prop. Size Infected |  | Frequenc <br> Sample Size | cy ofPGM-1Fraq. | Sampon alSampleSize | lleles for | polymorphic enzymes |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | PGM-2 Freq. |  |  | $\begin{aligned} & \text { Sample } \\ & \text { Size } \end{aligned}$ | LDH-4 Freq. |
| Bowser L. | 1983 | 130 | 0.010 |  | 46 | 0.087 | 65 | 0.923 | 65 | 0.762 |
|  | 1984 | 87 | 0.000 | 86 | 0.145 | 85 | 0.912 | 86 | 0.791 |
| Damdochax | 1983 | 130 | 0.860 | 73 | 0.151 | 100 | 0.860 | 100 | 0.860 |
| L. | 1984 | 100 | 1.000 | 98 | 0.138 | 98 | 0.857 | 99 | 0.818 |
| Long L. | 1983 | 100 | 0.000 | 83 | 0.470 | 84 | 0.845 | 84 | 0.994 |
|  | 1984 | 154 | 0.000 | 100 | 0.540 | 100 | 0.815 | 98 | 0.980 |
| Owikeno | 1982 | 150 | 1.000 | 100. | 0.270 | 99 | 0.833 | 100 | 0.960 |
| L. | 1984 | 100 | 0.990 | 100 | 0.280 | 99 | 0.879 | 100 | 0.980 |

$1 /$ Data soon to be available.
years, it would be prudent to resample populations at regular intervals (perhaps every five years) to be confident of long term stability.

### 3.3 Homogeneity of Markers With Respect To Age and Sex

Another desirable property of biological markers is that their probability of occurrence within a population be independent of age or sex. For example, it has been demonstrated that scale features typically vary from age group to age group within a population (Anas and Murai 1961). This complicates the stock composition analysis and necessitates much larger sample sizes to ensure adequate sampling of all principal age groups. Freshwater and early marine scale features do not appear to be influenced by the sex of the fish (Table 3).

It is well known that marine age composition varies greatly between the sexes, since fish maturing after only one year at sea are almost invariably male ("jacks"). Freshwater age composition does not appear to vary with sex (Table 3).

Brain parasite prevalence and genetic markers are not dependent on sex (Table 4). There is some indication, however, that brain parasite prevalence is slightly lower in sockeye that spend less than one year in fresh water (Wood et al. 1987a) presumably because they are exposed to the parasite source for a shorter time. This is unlikely to be of consequence since age 0 . fish are rare in most populations.

### 3.4 Sampling_Considerations

Scales are collected each year from most significant commercial fisheries and escapements of sockeye salmon to the determine age composition of the population. Scales from commercial catches can be easily collected when the fish are delivered to processing facilities since handing, damage to the fish, and interference with processing routines is minimal. Scale samples can also be collected from spawning escapements without harming fish. However, because environmental conditions change from year to year and the distribution of scale traits is complicated, large sample sizes and independent analyses for all major age classes are required. Trained personnel are required for consistent interpretation of scale features. Duplicate scale impressions can easily be made; these are compact and can be stored for future reference.

Age composition data are collected routinely from most fisheries and spawning populations for other stock assessment objectives. Resorption of scales in escapement and some catch samples can make determination of marine age difficult, but otoliths, fin rays, or length frequency curves can be used to corroborate ages. Freshwater age composition can usually be determined even from badly resorbed scales.


1/Scale features are for age 1.3 mockeye almon only.
NCi number of circuli,in firat freshwater zone.
NC2=number of circuli in epring "plue" growth zone.
NC3 number of circuli in firat ocean zone.
IDI widatance in melioo of firat freshwater zone.
ID2=distance in m/100 of spring "plue" growth zono.
ID3=distance in m/100 of first ocean zone.

|  |  |  |  | Brain | Parasite | -quenc | of co | alle | for | morphi | nzymea |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Stock Year | Sax | Age | $\begin{gathered} \text { Sample } \\ \text { Size } \end{gathered}$ | Proportion Infected | $\begin{aligned} & \text { Sample } \\ & \text { Size } \end{aligned}$ | PGM-1 Freq. | $\begin{aligned} & \text { Sample } \\ & \text { Size } \end{aligned}$ | PGM-2 <br> Freq. | $\begin{aligned} & \text { Sample } \\ & \text { Size } \end{aligned}$ | LDH-4 <br> Freq. |
|  | Iskut R. 1985 | M | 1.3 | 50 | 0.16 | 50 | 0.240 | 50 | 0.750 | 50 | 0.890 |
|  |  |  | All | 96 | 0.20 | 96 | 0.214 | 96 | 0.771 | 96 | 0.885 |
|  |  | F | 1.3 | 132 | 0.17 | 132 | 0.235 | 132 | 0.803 | 133 | 0.891 |
|  |  |  | All | 177 | 0.15 | 177 | 0.209 | 177 | 0.814 | 177 | 0.890 |
| べ | Little 1985 | M | 1.3 | 27 | 0.00 | 27 | 0.185 | 27 | 0.926 | 27 | 0.833 |
|  | Tatsamenie |  | All | 55 | 0.00 | 55 | 0.100 | 55 | 0.927 | 55 | 0.873 |
|  | L. | F | 1.3 | 14 | $0.00{ }^{\circ}$ | 14 | 0.143 | 14 | 0.964 | 14 | 0.821 |
|  |  |  | A11 | 44 | 0.00 | 43 | 0.151 | 43 | 0.884 | 44 | 0.909 |
|  | Owikeno L. 1984 | M | 1.2 | 47 | 0.98 | 47 | 0.245 | 47 | 0.883 | 47 | 0.989 |
|  |  |  | All | 61 | 0.98 | 61 | 0.246 | 60 | 0.900 | 61 | 0.984 |
|  |  | F | 1.2 | 19 | 1.00 | 19 | 0.237 | 19 | 0.868 | 19 | 1.000 |
|  |  |  | All | 39 | 1.00 | 39 | 0.346 | 39 | 0.846 | 39 | 0.974 |
|  | Meziadin L. 1984 | M | 2.2 | 29 | 0.00 | 29 | 0.241 | 29 | 0.948 | 29 | 0.466 |
|  |  |  | All | 48 | 0.00 | 47 | 0.277 | 47 | 0.936 | 47 | 0.426 |
|  |  | F | 2.2 | 36 | 0.00 | 36 | 0.222 | 36 | 0.917 | 36 | 0.389 |
|  |  |  | All | 51 | 0.00 | 52 | 0.192 | 52 | 0.933 | 52 | 0.423 |

Parasite samples (whole heads) can be easily collected in processing facilities but care must be taken to obtain complete and uncontaminated samples. Heads must be removed with a consistent style of cut which some processors do not use. This results in some loss of income, and thus, resistance on the part of the processor. Care must be taken when removing the brain for analysis to avoid contamination. The parasite Myxobolus is encysted and will not deteriorate until the brain rots so that escapement samples can easily be collected from carcasses. If carcasses are not found, it is necessary to kill fish to obtain samples although spawned out or precocious males can often be used. Brains may be removed in the field to save space and weight. Samples should be frozen or refrigerated until analyzed. Large sample sizes are not necessary due to the simple statistical distribution of the trait (i.e., presence or absence). Samples are not usually retained after analysis but they can be preserved by freezing.

Tissues required for electrophoresis are more difficult to obtain from processors because the eye, liver and heart tissues are removed from the fish at slightly different locations during processing. This makes it difficult to cross-reference the samples. Removing muscle tissue reduces the market value of fish and makes samples more expensive to obtain. For this reason, enzymes found only in muscle tissue have not yet been used for sockeye stock composition analysis of commercial fisheries. Samples must be frozen immediately and stored at very low temperatures to preserve enzyme activity. Some loss of enzyme activity has been encountered during long fishery openings when the fish had been dead for up to four days before they were available for sampling. Escapement samples must be obtained from live fish although spawned out or precocious "jack" males may be used. Preservation of tissue samples can be difficult in remote field locations since dry ice or adequate freezer space is usually unavailable locally and the duration of sampling activities is limited by the length of time samples can be kept. These problems can be avoided by preserving samples in liquid nitrogen, although this is a more expensive procedure. Intermediate sample sizes (approximately 100) are required to determine gene frequencies accurately. Enzyme activity cannot be preserved indefinitely but photographic records of electrograms can be stored. Electrophoretic procedures are probably the least subjective of the techniques discussed in this report.

Obtaining the matched scale-parasite and scale-parasite-electrophoretic samples necessary for high resolution stock composition analyses is logistically difficult and requires organization and expertise. It is much easier, although sometimes more expensive, to obtain matched samples from test fishery vessels. However, these samples may be less representative of the actual commercial catch. Sampling activities in processing plants adversely affect processing speed and procedures. The best solution might be to
award sampling contracts to processing plants to provide the required samples under professional direction. Even under the best of conditions care must be taken not to mislabel samples and the procedure requires approximately 50 sampler hours to process 300 fish. Selection of "jack" males on the spawning grounds results in problems for the age-specific scale pattern analyses as fish of these young age classes are not commonly found in commercial catches.

### 3.5 Sample_Collection and Analysis Costs

The cost of collecting a scale averages $\$ 1.87$ (U.S.) while analysis averages $\$ .90$ (U.S.). Collection costs for brain parasites or electrophoretic samples vary widely depending on how and where samples are collected. Generally, once fish specimens are obtained, all types of data can be collected at ittle additional cost. Collection costs in processing plants may be substantially higher for electrophoretic samples, however. Analysis of brain parasites averages $\$ 0.75$ (Canadian) per sample while electrophoretic analysis of a single sample for 5 loci averages $\$ 2.50$ (Canadian).

### 4.0 APPROACHES TO ESTIMATING STOCK COMPOSITION

Each kind of biological marker discussed in Section 3 has proven useful for distinguishing sockeye stocks in particular situations, usually where the number of stocks contributing to a fishery is small. However, for coastwide mixtures in the Northern Boundary area where many stocks are vulnerable to mixed stock fisheries, no single kind of marker can differentiate between all individual stocks. Two approaches have been used to make these stock identification problems tractable. The first approach (described by Marshall et al. 1984), involves pooling samples from major stocks to form a reduced number of "composite stocks", thus simplifying the stock composition analysis by compromising stock resolution. The second approach facilitated by recent advances in statistical methods and computing capabilities, described by Wood et al. (1987b), increases stock resolution potential by using a variety of biological markers simultaneously. To answer detailed management questions raised by the panels, the latter approach offers the best chance of success.

### 4.1 Pooling Stocks to Simplify Analysis

Scale pattern analysis as currently used by ADFG requires that stocks be pooled into groups based on geographical proximity prior to analysis. This method was developed to meet the original objective of the U.S.-Canada Salmon Treaty of providing weekly estimates of the national origin of sockeye salmon taken in interception fisheries. This was convenient because all Alaskan stocks contributing to these fisheries are from relatively small
coastal systems which share a wet, cool maritime climate. In contrast, most Canadian stocks thought to contribute to these fisheries originate from a few large river systems in which the majority of spawning and rearing areas are situated east of the Coast Range mountains where the climate is more continental. These differences in freshwater rearing environment usually result in significant differences in scale patterns. Scale pattern analysis has siece evolved to the point where separate estimates of contributions are routinely provided for the largest Canadian systems. However, scale pattern analysis alone does not provide the capability to separate the many Alaskan or small Canadian stocks contributing to these fisheries. The technique of pooling stocks prior to analysis involves assumptions about the presence or absence and relative abundance of contributing stocks. In addition, it camot detect the presence of stocks not included in the model.

### 4.2 Increasing Stock_Resolution_Potential

Stock resolution can be enhanced by using several kinds of biological markers simultaneously. The similarity dendrograms in Figures $6-8$ illustrate this improvement. The dendrogram in Figure 6 is produced by combining the brain parasite with five electrophoretic markers; adding freshwater age (Figure 7) and freshwater age and three scale features (Figure 8) provide still greater stock resolution potential. (The distance scale is identical in Figures 6-8 but twice that in Figures 2-5.) These dendrograms are calculated from data collected over several years, and the scale features pertain only to the most common age group (age 1.3). Thus, the reliability of the dendrogram depends very much on the stability of these markers over time.

Although stock resolution can always be improved by combining markers, some stocks will usually remain difficult to differentiate. For exampe, in Figure 8, Hackett River and mainstem Stikine River socteye cluster together far to the left and hence, will be very difficult to detect individually. While their individual conitributions to a mixed-stock fishery cannot be identified, their collective contribution can be estimated more precisely. Sieilarly, the collective contribution by Hackett River, mainstes Stikine River, Stikine glacial lakes and Long Lake can be estmated still more precisely. In fact, any required level precision on stock composition estimates can be achieved by suming estimates of contributions by individual stocks which difficult to differentiate. This improvement in precision comes at the cost of decreased stock resolution--the numbers below the five vertical lines in Figures 6-8 indicate the actual number stock-groupings resolved when estimates are summed at the corresponding distance value.

Similarity demegrams provide perspective on the relative capabilities of different markers or combinations of markers, and predict which stocks will be difficult to differentiate. Simulations are required, however, to determine the reliability of

Figure 6. Similarity dendrogram for 52 sockeye populations based on combined data from Figures 4 and 5. This data set. is abbreviated "PG" to denote the combination of parasite and genetic markers.



Figure 8. Similarity dendrngram for 41 sncuey popuiations based on combined data irom Figures $2,3,4$ and 5 . This data set is abbreviated "PGAS" to denote the combination of parasite, genetic, freshwater ant ape ind ranle pattern markers.

Stock
Region


Number of Stock-Groupings Resolved
estimates of stock composition at any particular level of stockgrouping. To illustrate this point, two hypothetical mixture samples were generated each containing 500 fish. The first contained fish from Tahltan Lake only, and the second contained fish from McDonald Lake only. Each mixture was drawn randomly from the respective stocks. In addition, standards from each of the individual stocks were drawn randomly from the original samples to simulate sampling error. Stock composition of the mixture samples was estimated three times using a maximum inkelihood mixture model (Fournier et al. 1984) with the brain parasite and five electrophoretic markers (PG), the brain parasite, electrophoretic and freshwater age markers (PGA), and finally, the brain parasite, electrophoretic, freshwater age and three scale markers (PGAS). This procedure was repeated 100 times for each of the two mixture types to determine the variance of the estimates due to random sampling. The results are summarized in

Figure 9 and 10 for each combination of markers, and at seven different levels of grouping to improve precision. It is clear that contributions from McDonald Lake are badly underestimated using the brain parasite and electrophoretic markers (mean estimated proportion $=0.17$ or $17 \%$; the correct value is $100 \%$ ), but that error is reduced dramatically when additional markers are used (mean estimated proportion $=0.87$ or $87 \%$ using all markers) (Figure 9). Similarly, estimates improve in both accuracy and precision as they are summed within larger stock groupings, until error is virtually eliminated (Figure 10). Including freshwater age as a marker allows accurate detection of Tahltan Lake sockeye which otherwise could not be distinguished reliably from Meziadin Lake sockeye. This improvement could be very helpful in assessing stock composition of sockeye catches in Alaskan Districts 104 and 106.
The simulation results in Figures 9 and 10 demonstrate that, in principle, techniques are currently available to estimate stock composition in complex mixed-stock fisheries with considerable precision. However, it should be noted that these results depend on the assumption that all individual stocks have been sampled randomly, and that these samples are representative of their respective contribution to the mixture sample. Because, samples were obtained in different years, it has been assumed implicitly that the markers are stable. Since this is not strictly true (see Section 3.2), results from real mixed-stock fishery problems are likely to be inferior to those presented here, unless all stocks are resampled annually. It is also important to assess the reliability of stock composition estimates based on historical data in further simulation studies.


Fieure 9 . Simulation results showing the eifect of combining biological markers to increase accuracy and precision of stock composition estimates without compromising the number of stocks to be differentiated. PG denotes the use of parasite and genetic markers: PGA, parasite, genetic and freshwater age markers; and PGAS, parasite genetic, freshwater age and scale pattern markers. The correct solution is 100\% McDonald Lake in Figure 9A and 10.0\% Tahltan lake in Figure 9B.


Eigure 10. Simulation results showing the effect of summing estimates for poorly differentiated stocks to increase the accuracy and precision of stock composition estimates for a reduced number of stock-groupings. Only parasite and genetic markers were used. The correct solution is $100 \%$ McDonald Lake in Figure 10A and 100\% Tahltan Lake in Figure 10B.

Recent research activities by the Northern Boundary and Transboundary River Technical Committees have led to the development of sophisticated stock identification techniques for sockeye salmon. It is now possible, in principle, to detect contributions to mixed-stock fisheries by up to 40 or 50 individual sockeye stocks. In practice, the need for annual resampling must be evaluated to determine if the benefits derived from reliable estimates justify the sampling costs. Therefore, the Technical Committee recommends the following research objectives:

1. Resample several sockeye populations to assess long-term stability of biological markers used for stock identification.
2. Undertake simulation studies to assess the influence of annual variability in biological markers on the reliability of stock composition estimates based on historical data.
3. Continue to evaluate and to search for additional stable parasite and genetic markers.
4. Complete collection of baseline samples.
5. Investigate the potential for using biological markers for stock identification of other Pacific salmon species.

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| Appendix | 1. Summary of scale amplea collected routinely from mockeye almon apawing eacapamenta in mouthern Southeast Alaska and Canada, 1982-1986. Thia table does not include matched electrophoretic-parasite-scale amplee (aee Appendix 3). |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | Year |  |  |
| Statel | Statistical |  |  |  |  |  |  |
| Province | Area | Syatem | 1982 | 1983 | 1984 | 1985 | 1986 |
|  |  |  | , |  |  |  |  |
| Alaska | 101-11 | Filmore L. | 143 | 146 |  |  |  |
|  |  | Hugh Smith L. | 3,009 | 1,107 | 1,591 | 1.170 | 2.172 |
|  | 101-45 | Leask L. | 257 | 58 |  | 35 | 370 |
|  | 101-80 | McDonald L. | 629 | 1.366 | 927 | 537 | 610 |
|  | 101-90 | Neha L. | 184 | 1,648 | 500 | 405 | 1.001 |
|  |  | Helm L. | 276 | 274 | 29 | 367 | 350 |
|  | 102-20 | Paul L. | 106 |  | 123 |  |  |
|  | 102-30 | Johnson L. | 337 | 563 | 240 | 358 | 14 |
|  |  | Kegan L. | 1,776 | 1,699 | 321 | 444 | 240 |
|  | 102-60 | Karta L. | 1.429 | 921 | 224 | 1851 | 595 |
|  | 103-15 | Klakas L. | 356 | 691 | 180 | 318 |  |
|  | 103-25 | Hette L. | 745 | 114 | 199 | 436 | 500 |
|  | 103-E0 | Klawock L. | 725 | 100 | 191 |  | 700 |
|  | 103-80 | Warm Chuck L. | 332 | 1,755 | 572 | 686 | 414 |
|  | 103-90 | Sarkar L. | 538 | 140 | 316 | 457 | 416 |
|  | 105-31 | Kuahneahin L. | 30 | 29 | 50 | 100 | 104 |
|  | 105-42 | Sutter L. | 177 |  | 69 |  | 65 |
|  | 105-43 | Shipley L. | 92 |  | 105 |  |  |
|  | 106-10 | Trumpeter L. | 120 | 452 | 75 | 480 | 276 |
|  |  | Luck L. | 365 | 445 | 335 | 265 | 488 |
|  | 106-30 | Sweetwater L. | 346 |  |  |  | 276 |

Appendix 1. Summary of ecale amples collected routinely from sockeye almon apowning escapements in southern Southeast Alama and Canada, 1982-1986. This table doea not include matched electrophoretic-paranite-acale amplea (continued) (aee Appendix 3).

| State/ StatiaticalProvince Area |  | Year |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | 1982 |  |  | 1985 | 1986 |
|  |  | System | 1982 | 1983 | 1984 | 1985 | 1986 |
|  | 106-41 | Salmon Bay L. | 1,302 | 527 | 592 | 1,342 | 1528 |
|  |  | Red Bay L. | 316 | 367 | 271 | 520 | 608 |
|  | 106-42 | Kah Sheets L. | 25 | 194 | 161 |  | 26 |
|  | 106-44 | Peteraburg L. | 31 | 242 | 474 | 237 | 551 |
|  | 107-30 | Thome L. | 508 | 419 | 607 | 337 | 530 |
|  | 109-20 | Falle L. | 532 | 727 | 688 | 879 |  |
|  | 109-52 | Kutlaku L. | 345 |  | 492 | 413 | 525 |
|  | 109-62 | Alecks L. | 396 |  |  |  | 520 |
|  | 111-32 | Creacent | 611 | 1648 | 1140 | 1303 | 1047 |
|  | 111-50 | Speel | 312 | 793 | 765 | 396 | 1203 |
|  | 01 | Yakoun L. |  | 100 |  |  |  |
|  | 02 | Mercer L. |  | 300 |  | 125 |  |
| B.C. | 03 | Bowser L. | 20 | 65 |  | 164 |  |
|  |  | Damdochax L. | 85 | 173 | 50 | 100 |  |
|  |  | Fred Wright L | 23 | 295 | 50 | 100 |  |
|  |  | Meziadin L. | 1075 | 1200 | 1200 | 1200 |  |
|  | 10 | Long L. | 65 |  |  | 135 |  |
|  | 12 | Nimpkish L. |  |  | 44 | 80 |  |

## Appendix 1. Summary of scale amplee collected routinely from mockeye almon apaning eacapements in eouthern Southeast Alama and Canede, 1982-1986. Thie teble does not include matched electrophoretic-paresite-scele samples (continued)(see Appendix 3)



| Province | Area | System | 1982 | 1983 | 1984 | 1985 | 1986 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| B.C. | 23 | G. Central L. | 800 | 800 | 800 | 400 | 400 |
|  |  | Henderson L. | 200 | 200 | 200 | 5 | 100 |
|  |  | Sproat L. | 1000 | 1000 | 1000 | 450 | 400 |
|  | 29 | Adame R. | 350 | 253 | 120 | 368 |  |
|  |  | Birkenhead R. | 720 | 585 | 328 | 596 | $1 /$ |
|  |  | Chilko L. | 595 | 209 | 498 | 588 | 1/ |
|  |  | Cultus L. | 130 | 120 | 262 | 84 | $1 /$ |
|  |  | Hormefly L. | 240 | 134 | 202 | 473 | $1 /$ |
|  |  | Sushwap R. | 360 | 221 | 4 | 62 | $1 /$ |
|  |  | Stellako R. | 136 | 258 | 247 | 273 | $1 /$ |
|  |  | Weaver Ck. | 480 | 710 | 168 | 570 | 11 |
|  |  | Stuart L. | 458 | 450 | 1900 | 1064 | 11 |
| Traneboundary Syetemes |  |  |  |  |  |  |  |
| Stikine |  | Stikine R. (mainatem) |  | 100 |  | 490 | 66 |
|  |  | Chriatina L. |  |  | 68 | 130 | 168 |
|  |  | Chutine R. |  |  | 54 | 90 | 109 |
|  |  | Imkut $R$. |  | 24 | 125 | 159 |  |
|  |  | Skud |  |  | 40 | 68 |  |
|  |  | Tahltan L. | 1460 | 2125 | 2197 | 2641 | 760 |
| Taku |  | Hackett R. |  |  |  | 278 | 250 |
|  |  | Kuthai L. | 219 |  | 242 |  |  |
|  |  | L. Tateamenie |  | 255 | 183 | 1000 | 750 |
|  |  | L. Trapper L. | 611 | 1939 | 1300 | 1358 | 850 |
| Aleek |  | Klukehu R. | 523 | 1565 | 400 | 1700 | 1006 |

[^0]Appendix 2. Summary of brain parasite samples collected from sockeye
salmon spawning escapements in southern Southeast Alaska
and Canada. $1982-1986$.

|  |  |  | Year |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| State/ Statistical |  |  |  |  |  |  |  |
| Province | Area | Systerm | 1982 | 1983 | 1984 | 1985 | 1986 |
| Alaska | 101-11 | Hugh Smith L. | 30 | 75 |  |  |  |
|  | 101-45 | Leask L. |  |  |  | . | 41 |
|  | 101-80 | McDonald L. | 30 | 100 |  |  | 50 |
|  | 101-90 | Naha L. |  | 57 | 50 |  | 50 |
|  |  | Helm L. |  | 50 |  |  | 51 |
|  | 102-30 | Johnson L. |  | 50 |  |  |  |
|  |  | Kegen L. | 30 | 50 |  |  | 50 |
|  | 102-60 | Karta.L. | 30 | 50 |  |  | 10 |
|  | 103-15 | Klakas L. |  | 50 |  |  |  |
|  | 103-80 | Warm Chuck L. |  | 50 |  |  | 32 |
|  | 103-90 | Sarkar L. |  |  |  |  | 44 |
|  | 105-42 | Sutter L. |  |  |  |  | 36 |
|  | 105-43 | Shipley |  | 49 |  |  |  |
|  | 106-10 | Luck L. |  | 50 |  |  | 21 |
|  | 106-41 | Salmon Bay L. | 30 | 45 |  |  | 51 |
|  |  | Red Bay L. |  | 70 |  |  | 52 |
|  | 106-44 | Petersburg L. |  |  |  |  | 50 |
|  | 107-30 | Thome L. |  | 50 |  |  | 50 |
|  | 111-33 | Speel L. |  | 50 |  |  | 100 |
|  | 111-35 | Crescent L. |  | 50 |  |  | 50 |

```
Appendix 2. Summary of brain parasite samples collected from sockeye salmon spawning escapements in southern Southeast Alaska and Canada, 1982-1986 (continued).
```

State/ Statistical
Srovince Area
Pr-nstern

| B.C. 01 | Naden R. | 50 | 26 |
| :---: | :---: | :---: | :---: |
|  | Yakoun L. | 50 |  |
|  |  | Mercer L. | 50 |
|  | Mathers L. | 50 | 100 |
|  |  |  |  |


| Bowaer L. |  | 64 | 87 | 183 |
| :---: | :---: | :---: | :---: | :---: |
| Damdochax L. |  | 100 | 100 | 100 |
| Fred Wright L. |  | 100 | 100 | 190 |
| Meziadin L. | 100 | 100 | 100 | 294 |
|  |  | 100 | 100 |  |

Babine L.
(early) 100158
Fulton R. $100 \quad 100300$

Four Mile R. $\quad 71$
$\begin{array}{llll}\text { Pinkut R. } 100 & 151 & 200\end{array}$
Lakelse L. 48100
05 Banks L. 100

Bonilla L. 100
Devon L. 100
Lowe L. ..... 93
Mikado L. ..... 100
06 Canoona R. ..... 100

Appendix 2. Sumary of brain parasite samples collected from sockeye salmon spawning escapements in southern Southeast Alaska and Canada, 1982-1986 (continued).


B.C.

07

Kitlope L.
50

Tankeeah L. 100
08 Kimsquit L. 100
Koeye L. 100
Tenas L. 100
09 Owikeno L. 100100
10 . Long L. 84154
12 Wose L. 100
23 Cheewhat L. 100
G. Central L. 143

Henderson L. 154
Sproat L. 183
29 Adamer R. 100100
Birkenhead R. 72150
Chilko L. 100
Cultus L. 100
Horsefly R. 100150
Shuswap R. 100150
Takla L. 100100
Stellako R. 100150

```
- continued -
```



Appendix 3. Sumary of matched electrophoretic-parasite-scale samples collected from spawning escapements in southern Southeast Alaska and Canada. 1982-1986.

| Statel Province | Statistical Area | System | Year |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | 1982 | 1983 | 1984 | 1985 | 1986 |
| Alaska | 101-11 | Hugh Smith L. | 50 | 96 |  |  |  |
|  | 101-45 | Leask L. |  |  |  |  | 41 |
|  | 101-80 | McDonald L. | 50 | 100 |  |  | 50 |
|  | 101-90 | Naha L. |  | 100 | 50 |  | 50 |
|  |  | Helm L. |  | 50 |  |  | 51 |
|  | 102-30 | Johnson L. |  | 80 |  |  |  |
|  |  | Kegan L. | 100 | 100 |  |  | 50 |
|  | 102-60 | Kerta L. | 50 | 100 |  |  | 10 |
|  | 103-15 | Klakes L. |  | 100 |  |  |  |
|  | 103-80 | Warm Chuck L. |  | 100 |  |  |  |
|  | 103-90 | Sarkar L. |  |  |  |  | 44 |
|  | 105-42 | Sutter L. |  |  |  |  | 36 |
|  | 105-43 | Shipley |  | 50 |  |  |  |
|  | 106-10 | Luck L. |  | 50 |  |  | 21 |
|  | 105-41 | Salmon Bay L. | 69 | 75 |  |  | 51 |
|  |  | Red Bay L. |  | 70 |  |  | 52 |
|  | 106-44 | Petersburg L. |  |  |  |  | 50 |
|  | 107-30 | Thome L. |  | 75 |  |  | 50 |
|  | 111 | Speel L. |  | 50 |  |  | 100 |
|  | 111 | Crescent L. |  | 50 |  |  | 50 |






1/ Sampling goal for all eignificant fioharies in Southeat Alake is 700 acales and esmociated length and aex dsta for each week a District is open to fishling. In instances where few fieh were caught weokly ample aizes may be leas then 700.

2/ 1986 figure are preliminary.
3/ Includes 6,095 gemplem from the clarence Streit portion and 5,987 from the Sumar Streit portion of the diatrict.

4/ Includes 4,826 samples from the Clerence Strait portion and 6,095 from the Sumer Strait portion of the district.

3/ Approxinate figures.



1/ Matched parasite data not collected.

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