

# Stock Identification of Skeena River Summer Steelhead using Microsatellite DNA Loci

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## Abstract

Variation at 15 microsatellite loci was surveyed in summer run steelhead trout (*Oncorhynchus mykiss*) from 16 populations in the Skeena River drainage. Genetic variation has been examined for Skeena steelhead since 1998; in 2009, a further 900 samples from the Tyee Test Fishery were analyzed from tissues collected in 2007 and 2008. The objective of this investigation was to further resolve/describe Skeena summer steelhead stock composition and abundance. Analysis of simulated mixed-stock samples suggested that variation at the microsatellite loci provided relatively accurate and precise estimates of stock composition for fishery management applications. Microsatellites provided an effective way to determine population structure, and provided reliable estimates of stock composition in mixed-stock fisheries; run timing and stock composition information is provided for Skeena summer steelhead captured at the Skeena estuary in a gillnet test fishery in 2007 and 2008.

## Introduction

Steelhead trout (*Oncorhynchus mykiss*), the anadromous form of rainbow trout, are found in all major coastal river systems in British Columbia. Steelhead trout abundance has become of increasing concern to fisheries managers, because the status of many populations in British Columbia may require increased emphasis on conservation (Slaney et al. 1996). The current management focus is to ensure the continued viability and productivity of existing populations, and after conservation requirements for specific populations have been obtained, to allow for limited exploitation of target populations. Steelhead trout are not abundant enough to support directed commercial fisheries, although some may be incidentally caught in salmon fisheries.

Determination of population structure of exploited species is an essential component in successful management of fisheries. Specifically, this information can be used for applications ranging from the determination of appropriate conservation or management units to estimation of stock composition in mixed-stock fisheries. In British Columbia, surveys of genetic variation have been used to describe population structure in steelhead trout (Parkinson 1984, Taylor 1995, Beacham et al. 1999, Beacham et al. 2000, Heath et al. 2001, Hendry et al. 2002; Beacham et al. 2004). All of these studies, except for Parkinson (1984), have been directed at local populations or regional groups of steelhead trout. Parkinson (1984) surveyed variation at five allozyme loci in primarily fry and parr from 73 presumed steelhead trout and rainbow trout populations in British Columbia. Development of highly polymorphic microsatellite loci has improved the resolution of detectable genetic variation among steelhead trout populations.

Microsatellites are very useful genetic markers to survey genetic variation among salmonid populations. Non-lethal sampling and the abundance of loci make this method very effective for describing population structure in steelhead trout (Nielsen et al. 1994, 1997, Wenburg et al. 1996, Ostberg and Thorgaard 1999, Hendry et al. 2002). Microsatellite loci can also be very useful in estimating stock composition in mixed-stock fisheries that intercept steelhead trout (Beacham et al. 1999, 2000). Microsatellite variation was examined among steelhead trout populations in the Skeena River to resolve population structure and further refine associated stock identification applications. The objective of this study was to estimate stock composition of steelhead trout caught in test fisheries in the lower portion of the Skeena River drainage near Tyee and to assess the reliability of the estimates. Sockeye salmon fisheries in the Skeena River are managed to reduce interceptions of steelhead, and thus information on steelhead timing and abundance is of interest to both Fisheries and Oceans Canada and the Ministry of Environment of British Columbia.

## **Materials and Methods**

### **Collection of DNA samples and laboratory analysis**

Using a chelex resin protocol, DNA was extracted from scales, previously collected frozen samples stored at -20°C, or a punch of operculum or fin tissue preserved in 95% ethanol. We sampled either juveniles or adult fish from 16 steelhead trout populations in the Skeena River. The main method of sampling juveniles and adults were by electro-fishing and angling, respectively, although enumeration fences were used at some locations in some years. Fishery samples of returning adults in the Skeena River were

collected with a gillnet test fishery (Jantz et al. 1990). Populations, year of sampling, and number of fish analyzed are outlined in Table 1. Further sampling details were outlined by Beacham et al. (1999, 2000).

For the survey of baseline populations, PCR products at 15 microsatellite loci: *Ogo4* (Olsen et al. 1998), *Oke4* (Buchholz et al. 1999), *Omm1008*, *Omm1037* (Rexroad et al. 2002), *Omm1276* (Rexroad et al. unpublished), *Omm5140* (Coulibaly et al. 2005), *Ots1*, *Ots2*, *Ots9* (Banks et al. 1999), *Oki10* (Smith et al. 1998), *One111*, *One114* (Olsen et al. 2000), and *Omy325* (O'Connell et al. 1997), *OtsG83b* (Williamson et al. 2002), and *Ssa408* (Cairney et al. 2000) were surveyed. Microsatellites were size fractionated in an ABI 3730 capillary DNA sequencer, and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard. Allele scores derived from GeneMapper were verified by one laboratory personnel.

### **Data analysis**

All annual samples available from a location were combined to estimate population allele frequencies, as was recommended by Waples (1990). The Cavalli-Sforza and Edwards (1967) chord distance was used to estimate genetic distance among populations.

## **Estimation of stock composition**

Analysis of fishery samples was conducted with a Bayesian procedure (BAYES) as outlined by Pella and Masuda (2001). Each locus was assumed to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies and used as model inputs. For BAYES, the initial FORTRAN-based computer program as outlined by Pella and Masuda (2001) required large amounts of computer analytical time when applied to stock identification problems with a baseline as comprehensive as employed in the current study. Given this limitation, a new version of the program was developed by our laboratory as a C-based program which is available from the Molecular Genetics Laboratory website ([http://www-sci.pac.dfo-mpo.gc.ca/mgl/data\\_e.htm](http://www-sci.pac.dfo-mpo.gc.ca/mgl/data_e.htm)). In the analysis, ten 20,000-iteration Monte Carlo Markov chains of estimated stock compositions were produced, with initial starting values for each chain set at 0.90 for a particular population which was different for each chain. Estimated stock compositions were considered to have converged when the shrink factor was  $< 1.2$  for the 10 chains (Pella and Masuda 2001). The last 1,000 iterations from each of the 10 chains were then combined, and for each fish the probability of originating from each population in the baseline was determined. These individual probabilities were summed over all fish in the sample, and divided by the number of fish sampled to provide the point estimate of stock composition. Standard deviations of estimated stock compositions were determined from the last 1,000 iterations from each of the 10 chains incorporated in the analysis.



## Results and Discussion

### Population structure

Structure was observed among populations surveyed. The Lakelse River and Kitsumkalum River populations clustered together, as did the Bulkley River and Morice River populations and the Kispiox River and Kitwanga River populations (Figure 5). The upper Skeena mainstem was the most distinct surveyed but it likely that this distinctiveness is a result of sampling error, in this instance, family effects from sampling individuals from the same parentage. Regional similarity was observed among the Bulkley River, Morice River, and Toboggan Creek populations as well as among the Lakelse River, Kitsumkalum River and Zymoetz River populations. In order for a genetic-based method of stock identification to be applied successfully, there must be significant genetic differences among the populations that fishery managers wish to separate. Significant genetic differentiation at the microsatellite loci was observed among steelhead populations surveyed from British Columbia.

### Simulated mixed-fishery samples

Estimates of simulated single population mixtures for Skeena River steelhead (200 steelhead from each population) were accomplished using the Rannala and Mountain correction for small sample size. In the analysis of single-population mixtures, where the expected result was 100% allocation to the target population, all estimates except for two populations were > 90%, the level generally considered acceptable for mixed-stock analysis. For those two populations (Bulkley River, upper Skeena River), the

number of fish surveyed was < 70 steelhead, and thus the lower values for these two populations likely reflect lower sample size, rather than any lack of population differentiation.

Mixtures comprising only a single population illustrate the maximum bias expected in estimated stock compositions for that population. In actual applications, mixed-stock fishery samples will contain a range of populations, and estimated stock compositions from these mixtures may in fact be more reliable than those of single-population mixtures. Mean estimated stock compositions were usually within 2% of the specific population contribution. Simulations may at times provide an optimistic view of model performance when compared with applications to actual mixed fishery samples, particularly if fish from stocks not included in the baseline occur in the mixtures. However, if baseline coverage is improved, bias in the estimated stock compositions as a result of having fish in the mixtures from populations not in the baselines will be reduced.

Accuracy and precision of estimated stock compositions of Skeena River steelhead had previously been investigated by Beacham et al. (2000) during a survey incorporating variation at 8 microsatellite loci analyzed on manual gels. With the new baseline of 15 microsatellite loci analyzed with an automated DNA sequencer, accuracy and precision of the estimated stock compositions was increased relative to the previous analysis.

Estimated percentage stock compositions for the 2007 and 2008 Tyee test fishery are outlined in Table 1 and Figs. 1-4. Under the assumption that the test fishery steelhead are captured in proportion to run abundance throughout the season, and because all

steelhead caught were analyzed, it is possible to estimate the seasonal relative abundance of steelhead. The dominant stock originated from the Bulkley River drainage (Morice, Bulkley rivers and Toboggan Creek), comprising 29% and 40% of the returns in both 2007 and 2008, respectively. Variation was noted for the Bulkley River population, comprising five times less of the overall relative abundance in 2007 than in 2008 (2% and 10%, respectively). In 2007, lower Sustut River steelhead comprised 11% of the returns, but only an estimated 5% in 2008. The Babine River population comprised a similar proportion of the returns in both years (13% and 11%, respectively). Similarly, the Kispiox River population was estimated to comprise 8% and 10% of the returns in 2007 and 2008, respectively

If the test fishery caught steelhead in proportion to population abundance in a constant manner throughout the season, and since all steelhead caught were analyzed, then the estimated stock composition times the weekly sample size should provide an estimate of relative abundance in each week for each population. This would allow determination of timing of specific populations through the lower Skeena River. Timing of different populations is outlined in Figs. 3 and 4. In 2007, peak abundance of steelhead during the August 12-26 period, but in 2008, peak abundance was observed earlier, during the July 29-August 11 period. In 2007, the Kispiox and lower Sustut river populations comprised a significant proportion of the total run (42% and 28%) during the first period of sampling (July 1-July 14). In 2008, the Morice population comprised 49% of the overall abundance during the first period of sampling in contrast to only 2% in 2007 for the same period. The Kispiox population comprising a significant proportion of the late run at 34% and 28% of the overall abundance during the August 26-September 8 and September 9-22 periods. As the test fishery was extended from August 25 to September 22, it was not possible to compare late migration timing populations between these years.

Microsatellite loci provide practical markers for stock identification of steelhead trout only if there is adequate differentiation among populations in the baseline and the level of annual variation of allele frequencies within populations is minimal relative to population differentiation. Practical considerations with respect to baseline sampling and cost require that the differentiation among populations be greater than the variation within populations so that samples may be pooled over several years to obtain representative samples of populations contributing to fishery samples. Evidence to date indicates that the level of annual variation in allele frequencies within populations was 6 times less than differentiation among populations (Beacham et al. 2004), so pooling samples within populations over several years is a viable option.

## **Conclusions**

The objectives of this investigation were met – namely, we were able to further describe/resolve Skeena summer steelhead timing and stock composition issue for 2007 and 2008. Utilizing this information for abundance estimates comprises a critical stock assessment component for Provincial fisheries managers due to the practical challenge of enumerating steelhead in the month of May when discharge and turbidity prevent direct observations and the operation of enumeration weirs. One example is of this application is the Moricetown Canyon mark-recapture population estimate: if the proportion of Skeena summer steelhead migrating through the canyon is 25%, overall watershed abundance can be estimated by multiplying the mark-recapture estimate by a factor of four. It is recommended that sampling and tissue analysis continue to inform managers with regards to seasonal differences and changes in run timing or stock proportions over time.

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## Literature Cited

- Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). J. Hered. 90: 281-288.
- Beacham, T. D., S. Pollard, and K. D. Le. 1999. Population structure and stock identification of steelhead trout in southern British Columbia, Washington, and the Columbia River based on microsatellite DNA variation. Trans. Am. Fish. Soc. 128: 1068-1084.
- Beacham, T. D., S. Pollard and K. D. Le. 2000. Microsatellite DNA population structure and stock identification of steelhead trout (*Oncorhynchus mykiss*) in the Nass and Skeena Rivers in northern British Columbia. Marine Biotechnology 2: 587-600.
- Beacham, T. D., K. D. Le, and J. R. Candy. 2004. Population structure and stock identification of steelhead trout (*Oncorhynchus mykiss*) in British Columbia and the Columbia River based on microsatellite variation. Env. Biol. Fish. In press.

- Buchholz, W., S.J. Miller, and W.J. Spearman. 1999. Summary of PCR primers for salmonid genetic studies. US Fish. Wild. Serv. Alaska Fish. Prog. Rep. 99-1.
- Cairney, M., J. B. Taggart, and B. Hoyheim. 2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Mol. Ecol. 9: 2175-2178.
- Cavalli-Sforza, L. L. and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Amer. J. Hum. Genet. 19: 233-257.
- Coulibaly, I., Gharbi, K., Danzmann, R.G., Yao, J. and Rexroad, C.E. III. 2005. Characterization and comparison of microsatellites derived from repeat-enriched libraries and expressed sequence tags Anim. Genet. 36: 309-315 (2005)
- Debevec, E.M., R. B. Gates, M. Masuda, J. Pella, J. Reynolds and L. W. Seeb. 2000. SPAM (Version 3.2): Statistics program for analyzing mixtures. J. Hered. 91: 509-510.
- Goudet, J. 1995. FSTAT A program for IBM PC compatibles to calculate Weir and Cockerham's (1984) estimators of F-statistics (version 1.2). J. Heredity 86: 485-486.
- Heath, D. D., C. Busch, J. Kelly and D. Y. Atagi. 2002. Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). Mol. Ecol. 11: 197-214.
- Hendry, M.A., J. K. Wenburg, K. W. Myers and A. P. Hendry. 2002. Genetic and phenotypic variation through the season provides evidence for multiple populations of wild steelhead trout in the Dean River, British Columbia. Trans. Am. Fish. Soc. 131: 418-434.
- Jantz, L., R. Kadowaki, and B. Spilsted. 1990. Skeena River salmon test fishery, 1987. Can. Data Rep. Fish. Aquat. Sci. 804: iv + 151p.

- Nelson, R. J. and T. D. Beacham. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. *Anim. Genet.* 30: 228-229.
- Nielsen, J. L., C. A. Gan, J. M. Wright, D. B. Morris and W. K. Thomas. 1994. Biogeographic distributions of mitochondrial and nuclear markers for southern steelhead trout. *Mol. Mar. Biol. Biotech.* 3: 281-293.
- Nielsen, J. L., C. Carpanzano, M. C. Fountain and C. A. Gan. 1997. Mitochondrial DNA and nuclear microsatellite diversity in hatchery and wild *Oncorhynchus mykiss* from freshwater habitats in southern California. *Trans. Amer. Fish. Soc.* 126: 397-417.
- Olsen, J. B., P. Bentzen and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. *Mol. Ecol.* 7: 1083-1090.
- Olsen, J. B., S. L. Wilson, E. J. Kretschmer, K. C. Jones and J. E. Seeb. 2000. Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. *Mol. Ecol.* 9: 2185-2187.
- O'Connell, M., R. G. Danzmann, J. M. Cornuet, J. M. Wright and M. M. Ferguson. 1997. Differentiation of rainbow trout populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. *Can. J. Fish. Aquat. Sci.* 54: 1391-1399.
- O'Reilly, P. T., L. C. Hamilton, S. K. McConnell and J. M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Can. J. Fish. Aquat. Sci.* 53: 2292-2298.
- Ostberg, C.O. and G. H. Thorgaard. 1999. Geographic distribution of chromosome and microsatellite DNA polymorphisms in *Oncorhynchus mykiss* native to western Washington. *Copeia* 1999(2): 287-298.

- Parkinson, E. A. 1984. Genetic variation in populations of steelhead trout (*Salmo gairdneri*) in British Columbia. Can. J. Fish. Aquat. Sci. 41: 1412-1420.
- Rexroad, C.E. III, R.L. Coleman, A.L. Gustafson, W.K. Hershberger, and J. Killefer 2002. Development of rainbow trout microsatellite markers from repeat enriched libraries. Mar. Biotechnol. 4: 12-16.
- Slaney, T. L., K. D. Hyatt, T. G. Northcote and R. J. Fielden. 1996. Status of anadromous salmon and trout in British Columbia and Yukon. Fisheries 21: 20-35.
- Smith, C. T., B. F. Koop and R. J. Nelson. 1998. Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Mol. Ecol. 7: 1614-1616.
- Taylor, E.B. 1995. Genetic variation at minisatellite DNA loci among North Pacific populations of steelhead trout and rainbow trout (*Oncorhynchus mykiss*). J. Hered. 86: 354-363.
- Waples, R. S. 1990. Temporal changes of allele frequency in Pacific salmon populations: implications for mixed-stock fishery analysis. Can. J. Fish. Aquat. Sci. 47: 968-976.
- Wenburg, J. K., J. B. Olsen and P. Bentzen. 1996. Multiplexed systems of microsatellites for genetic analysis in coastal cutthroat trout (*Oncorhynchus clarki clarki*) and steelhead trout (*Oncorhynchus mykiss*). Mol. Mar. Biol. Biotech. 5: 273-283.
- Williamson, J. F., K. S. Cordes, and B. P. May. 2002. Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Molecular Ecology Notes 2: 17-19.



Table 1. Estimated percentage stock composition of Skeena summer steelhead past the Tyee Test Fishery site in 2007, 2008. Stock compositions were estimated using 15 microsatellite loci. Standard error of the estimates is in parentheses.

| Year            | 2007        |        | 2007        |        | 2007           |       | 2007        |       | 2007        |       | 2008        |        | 2008        |       | 2008        |       | 2008           |       | 2008        |       | 2008          |       | 2008        |        | 2008        |       |
|-----------------|-------------|--------|-------------|--------|----------------|-------|-------------|-------|-------------|-------|-------------|--------|-------------|-------|-------------|-------|----------------|-------|-------------|-------|---------------|-------|-------------|--------|-------------|-------|
| Sampling Site   | Skeena Test |        | Skeena Test |        | Skeena Test    |       | Skeena Test |       | Skeena Test |       | Skeena Test |        | Skeena Test |       | Skeena Test |       | Skeena Test    |       | Skeena Test |       | Skeena Test   |       | Skeena Test |        | Skeena Test |       |
| Weeks           | July 1-14   |        | July 15-28  |        | July 29-Aug 11 |       | Aug 12-25   |       | all         |       | June 17-30  |        | July 1-14   |       | July 15-28  |       | July 29-Aug 11 |       | Aug 12-25   |       | Aug 26-Sept 8 |       | Sept 9-22   |        | all         |       |
| No. Sampled     | 1(0)        |        | 28(0)       |        | 112(1)         |       | 128(1)      |       | 269(2)      |       | 3(0)        |        | 41(0)       |       | 105(1)      |       | 185(0)         |       | 134(1)      |       | 114(2)        |       | 19(0)       |        | 601(4)      |       |
| Stock           | Estimate    | SD     | Estimate    | SD     | Estimate       | SD    | Estimate    | SD    | Estimate    | SD    | Estimate    | SD     | Estimate    | SD    | Estimate    | SD    | Estimate       | SD    | Estimate    | SD    | Estimate      | SD    | Estimate    | SD     | Estimate    | SD    |
| Babine          | 0.3         | (9.7)  | 0.2         | (1.5)  | 18.3           | (4.5) | 13.6        | (3.9) | 13.2        | (2.7) | 0.5         | (8.5)  | 0.4         | (1.5) | 18.3        | (4.7) | 12.7           | (3.4) | 16.0        | (4.0) | 8.6           | (3.6) | 0.0         | (1.1)  | 11.2        | (1.7) |
| Bulkley         | 2.8         | (14.6) | 0.5         | (1.9)  | 0.5            | (1.5) | 3.7         | (3.7) | 2.4         | (2.3) | 6.5         | (15.4) | 2.1         | (4.8) | 11.4        | (5.9) | 11.9           | (4.0) | 4.9         | (3.9) | 1.0           | (2.9) | 0.3         | (2.0)  | 10.4        | (2.8) |
| Kispiox         | 42.3        | (33.1) | 6.3         | (7.8)  | 5.4            | (3.7) | 12.3        | (4.3) | 8.4         | (2.6) | 0.5         | (7.1)  | 0.6         | (1.9) | 3.4         | (3.9) | 9.1            | (3.1) | 7.5         | (5.8) | 34.4          | (6.1) | 28.4        | (11.4) | 10.0        | (2.2) |
| Kitsequela      | 0.0         | (10.2) | 5.2         | (6.2)  | 2.3            | (2.4) | 5.3         | (3.5) | 4.2         | (2.0) | 1.3         | (7.5)  | 0.1         | (0.8) | 3.3         | (3.0) | 4.5            | (2.5) | 6.0         | (2.8) | 12.5          | (4.0) | 0.3         | (1.8)  | 5.4         | (1.3) |
| KitsumKalum     | 0.2         | (10.5) | 2.5         | (4.2)  | 1.1            | (1.7) | 6.6         | (2.8) | 4.7         | (1.7) | 0.0         | (4.7)  | 7.9         | (5.4) | 0.9         | (1.6) | 1.5            | (1.7) | 3.4         | (2.5) | 0.6           | (1.4) | 19.0        | (10.3) | 3.5         | (1.1) |
| Kitwanga        | 3.0         | (12.7) | 1.0         | (3.0)  | 0.1            | (0.4) | 5.5         | (3.2) | 2.4         | (1.8) | 0.0         | (5.3)  | 0.1         | (0.8) | 0.1         | (0.6) | 6.5            | (2.6) | 8.2         | (3.3) | 1.0           | (1.9) | 4.1         | (8.0)  | 3.8         | (1.2) |
| Kluatantan_Klua | 0.3         | (10.7) | 1.9         | (3.5)  | 3.6            | (2.4) | 0.2         | (0.6) | 1.9         | (1.3) | 0.0         | (5.6)  | 2.0         | (3.4) | 1.1         | (1.6) | 0.1            | (0.4) | 0.1         | (0.4) | 2.7           | (2.4) | 2.1         | (4.1)  | 1.2         | (0.8) |
| Kluatantan_Skee | 1.1         | (10.6) | 1.0         | (3.0)  | 0.1            | (0.6) | 0.5         | (1.4) | 0.1         | (0.4) | 0.0         | (5.6)  | 0.5         | (2.1) | 0.3         | (1.0) | 0.3            | (0.9) | 0.5         | (1.2) | 0.8           | (1.5) | 0.3         | (1.8)  | 0.3         | (0.6) |
| L_Sustut        | 28.1        | (28.5) | 2.5         | (4.7)  | 12.2           | (4.3) | 12.5        | (4.6) | 10.9        | (2.8) | 0.1         | (5.9)  | 4.6         | (6.5) | 3.3         | (3.7) | 6.6            | (2.8) | 0.6         | (1.5) | 5.8           | (4.5) | 1.5         | (3.4)  | 5.2         | (1.6) |
| Lakelse         | 0.0         | (9.7)  | 0.0         | (0.9)  | 0.1            | (0.5) | 0.6         | (1.1) | 0.2         | (0.5) | 0.0         | (6.6)  | 1.1         | (3.0) | 0.1         | (0.6) | 0.3            | (0.8) | 0.2         | (0.8) | 0.0           | (0.3) | 9.4         | (7.9)  | 0.8         | (0.6) |
| Morice          | 2.0         | (12.1) | 35.0        | (10.0) | 24.3           | (4.7) | 11.5        | (3.8) | 18.6        | (3.0) | 38.6        | (27.4) | 48.9        | (9.8) | 36.0        | (6.3) | 24.4           | (4.1) | 20.9        | (4.8) | 21.8          | (4.7) | 27.4        | (11.1) | 23.6        | (2.6) |
| Skeena_R_Mosque | 0.6         | (11.5) | 2.2         | (4.2)  | 5.6            | (3.9) | 5.1         | (3.7) | 6.5         | (2.4) | 31.2        | (20.0) | 3.5         | (5.5) | 6.9         | (3.7) | 1.3            | (1.9) | 7.3         | (3.0) | 0.6           | (1.4) | 4.7         | (6.6)  | 4.9         | (1.5) |
| Skeena_Up_Main  | 0.3         | (10.8) | 0.0         | (0.8)  | 0.0            | (0.2) | 0.0         | (0.2) | 0.0         | (0.1) | 0.0         | (5.8)  | 0.0         | (0.6) | 0.0         | (0.2) | 0.1            | (0.4) | 0.0         | (0.2) | 0.0           | (0.2) | 0.0         | (1.0)  | 0.0         | (0.1) |
| U_Sustut        | 0.2         | (10.3) | 18.8        | (7.3)  | 7.6            | (2.9) | 4.3         | (2.1) | 7.1         | (1.8) | 2.0         | (9.8)  | 18.0        | (7.0) | 4.3         | (2.4) | 5.1            | (1.9) | 2.9         | (1.6) | 3.0           | (2.5) | 1.3         | (3.1)  | 4.4         | (1.0) |
| Toboggan        | 18.2        | (25.3) | 10.2        | (8.8)  | 10.0           | (4.1) | 4.2         | (4.0) | 7.8         | (2.6) | 18.6        | (19.9) | 0.3         | (1.4) | 1.0         | (2.2) | 1.0            | (1.8) | 13.5        | (4.3) | 0.6           | (1.8) | 0.9         | (4.0)  | 5.5         | (1.7) |
| Zymoetz         | 0.6         | (9.8)  | 12.7        | (6.7)  | 8.8            | (2.9) | 14.1        | (3.5) | 11.7        | (2.2) | 0.6         | (7.1)  | 9.9         | (6.5) | 9.7         | (3.1) | 14.6           | (2.8) | 7.9         | (2.8) | 6.6           | (2.8) | 0.1         | (1.7)  | 9.8         | (1.4) |

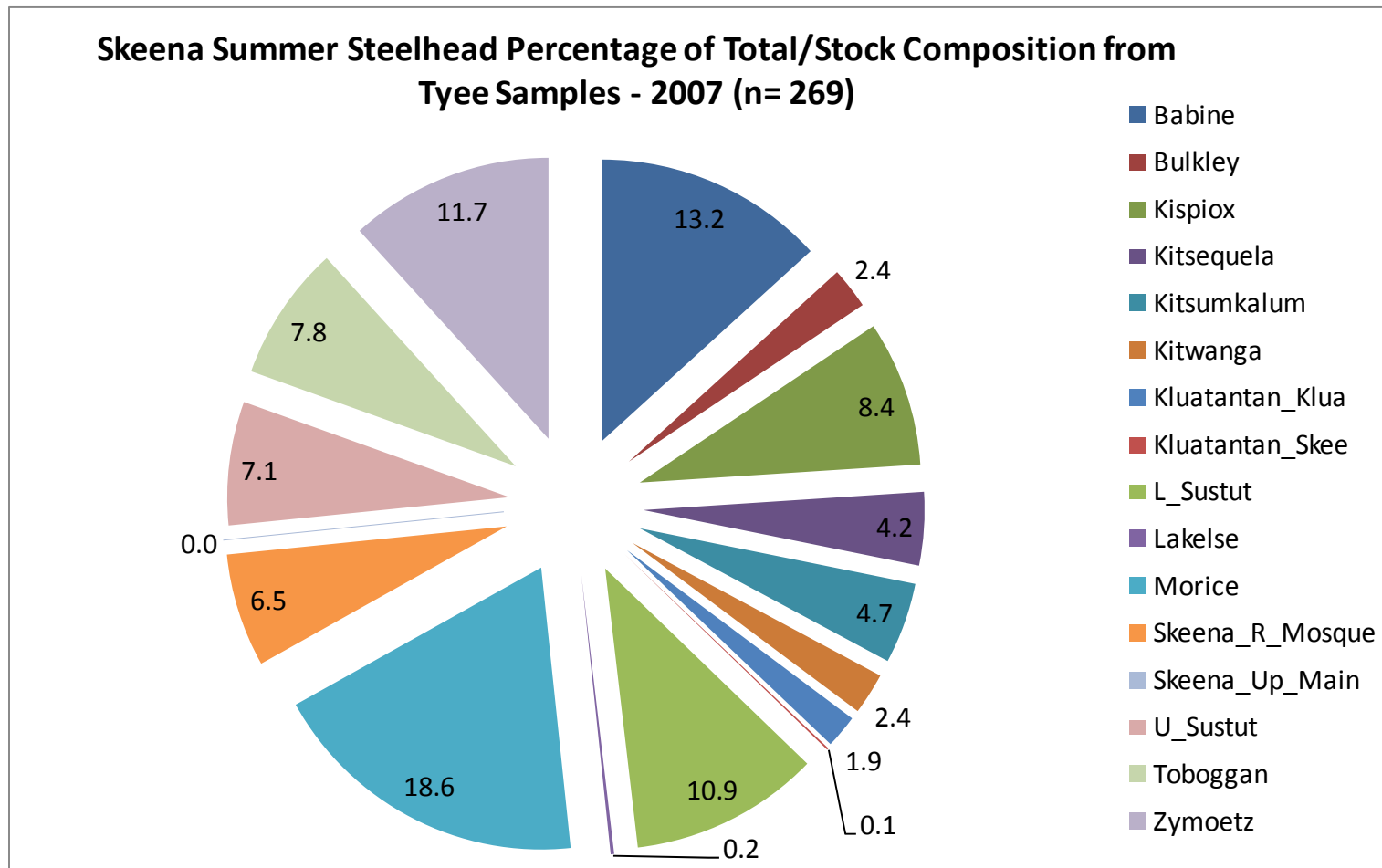


Figure 1 Pie chart illustrating the percentage of total/stock composition from summer steelhead tissue samples obtained from the Tyee Test Fishery, 2007.

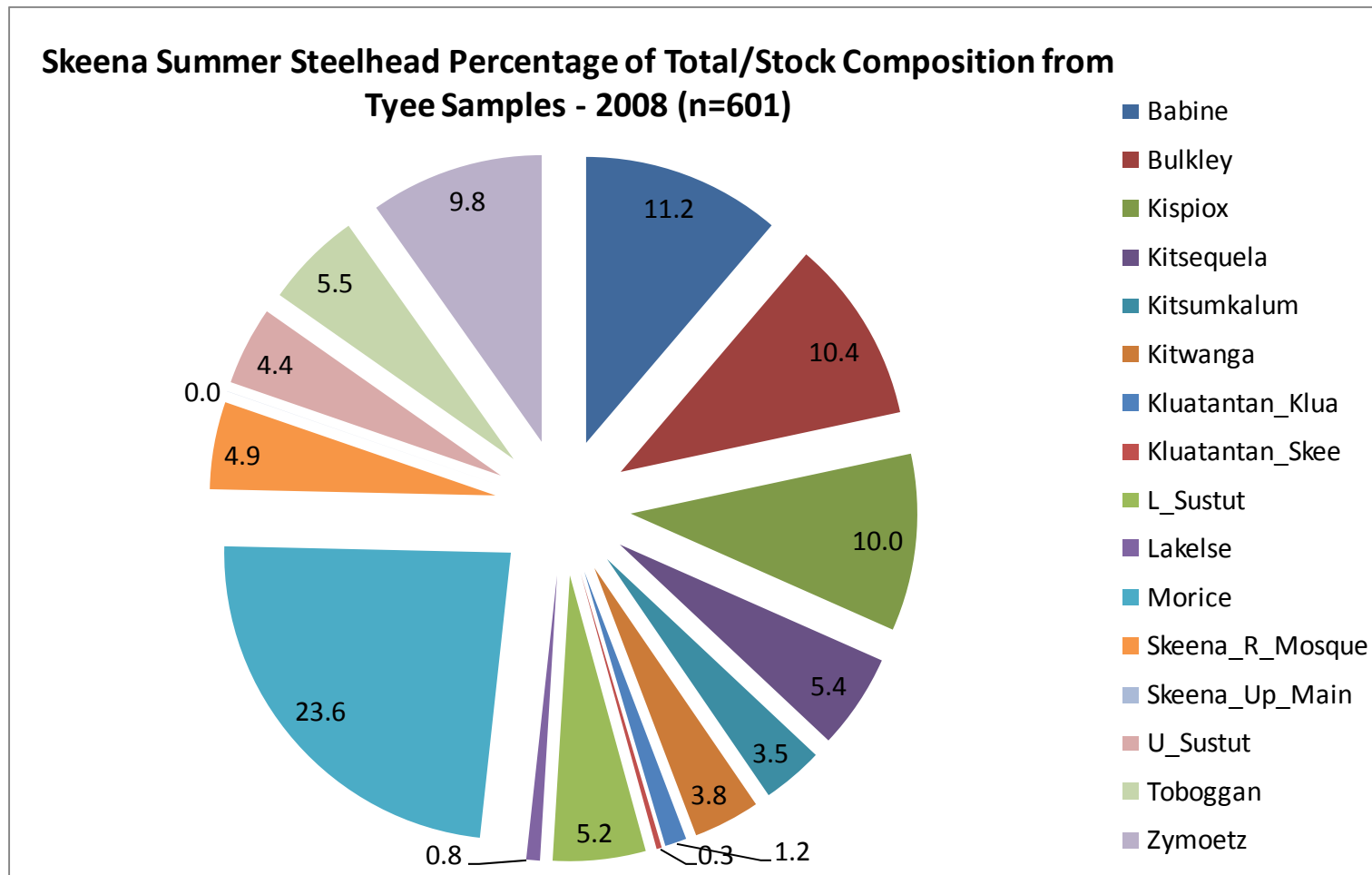


Figure 2. Pie chart illustrating the percentage of total/stock composition from summer steelhead tissue samples obtained from the Tyee Test Fishery, 2008.

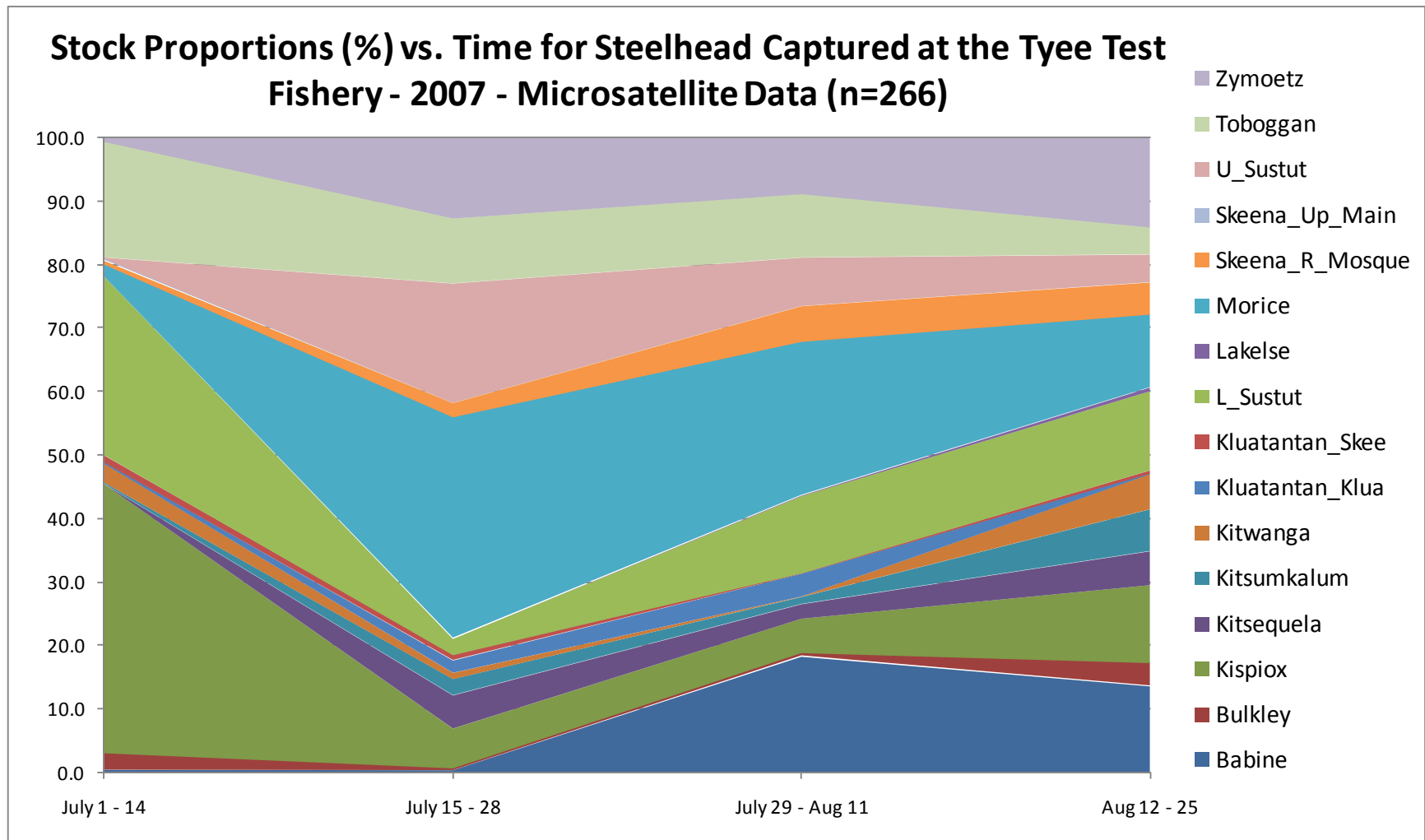


Figure 3. Graph illustrating the percentage of total/stock composition over time for summer steelhead captured at the Tye Test Fishery, 2007.

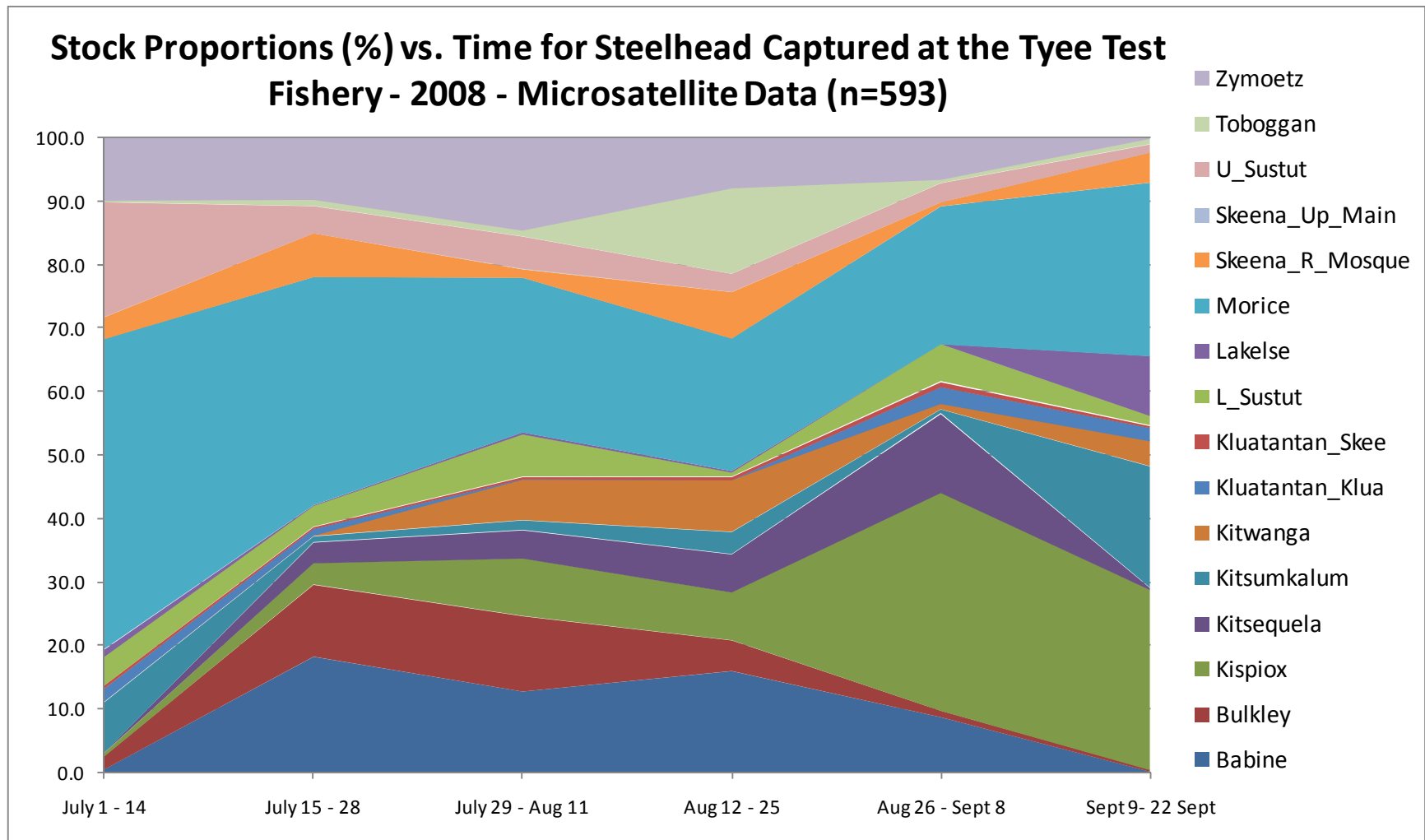


Figure 4. Graph illustrating the percentage of total/stock composition over time for summer steelhead captured at the Tye Test Fishery, 2008.

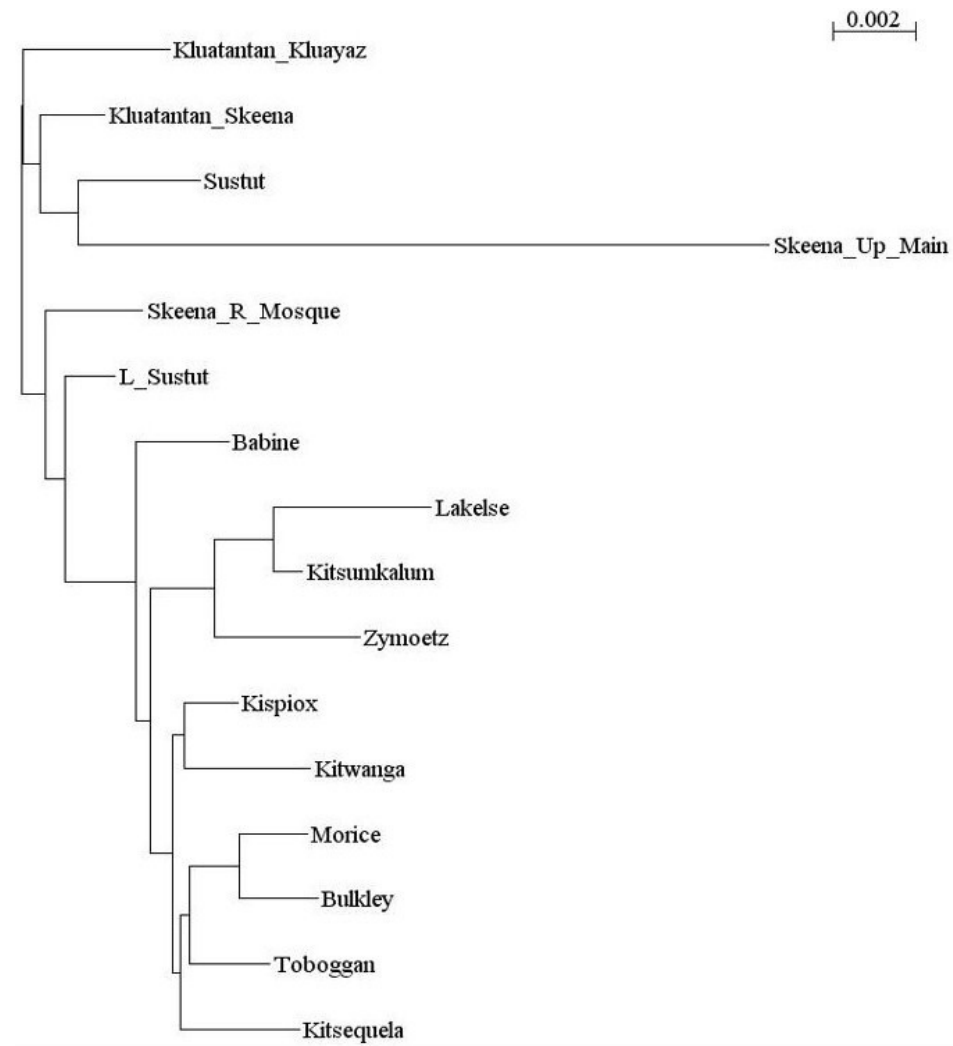


Figure 5. Dendrogram outlining the relationships between the 16 steelhead trout population in the Skeena River drainage based on chord distances. The scale at the top is outlining a Cavalli-Sforza and Edwards chord distance.

## Appendix I. Project budget form.

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Name of Project: Skeena River Steelhead Genetics

| ELIGIBLE COSTS  |           |                   |                  |                  |  | TOTAL<br>BUDGET   | OTHER<br>FUNDING | PSC<br>N. FUND<br>GRANT<br>AMOUNT |
|---|-----------|-------------------|------------------|------------------|--|-------------------|------------------|-----------------------------------|
| <b>Labour</b>   |           |                   |                  |                  |  |                   |                  |                                   |
| <b>Wages &amp; Salaries</b>   |           |                   |                  |                  |  |                   |                  |                                   |
| Position  | # of crew | # of work<br>days | hrs per day      | rate per<br>hour | Total (In-<br>kind & cash<br>+ PSC<br>Amount ) | In-Kind &<br>Cash | PSC Amount       |                                   |
| DFO Tyee Test Fishery staff   | 2         | 90                | 1                | 25               | 4,500  | 4,500             |                  |                                   |
| MOE Fisheries Biologists  | 4         | 10                | 7                | 35               | 9,800  | 9,800             |                  |                                   |
| PBS Genetics Lab staff  | 2         | 40                | 7.5              | 30               | 18,000   |                   | 18,000           |                                   |
|   |           |                   |                  |                  |  |                   |                  |                                   |
| Person Days (# of crew x work days)                                 |           | 300               | <b>sub total</b> |                  | 32,300   | 14,300            | 18,000           |                                   |
| <b>Labour - Employer Costs ( percent of wages subtotal amount )</b> |           |                   |                  |                  |  |                   |                  |                                   |
| rate  |           | 20%               | <b>sub total</b> |                  | 6,460  | 6,460             |                  |                                   |
| <b>Subcontractors &amp; Consultants</b>                             |           |                   |                  |                  |  |                   |                  |                                   |
|   | # of crew | # of work<br>days | hrs per day      | rate per<br>hour |  |                   |                  |                                   |
| Insurance if applicable   | rate      | 0%                | <b>sub total</b> |                  |  |                   |                  |                                   |
| <b>Volunteer Labour</b>   |           |                   |                  |                  |  |                   |                  |                                   |
|   | # of crew | # of work<br>days | hrs per day      |                  |  |                   |                  |                                   |
| Skilled TSES/Angling guides   | 8         | 4                 | 10               |                  | 6,400  | 6,400             |                  |                                   |
| Un-skilled  |           |                   |                  |                  |  |                   |                  |                                   |
| Insurance if applicable   | rate      | 0%                | <b>sub total</b> |                  | 6,400  | 6,400             |                  |                                   |
| <b>Total Labour Costs</b>   |           |                   |                  |                  |  | 45,160            | 27,160           | 18,000                            |

## Page 2 of 3

**Site / Project Costs****Detail (use additional page for details if needed )**

|  |   |               |
|--|---|---------------|
| Travel (do not include to & from work) | Heli charters to collect tissue samples           | 35,000        |
| Small Tools & Equipment                |   |               |
| Site Supplies & Materials              | PBS genetics lab - markers/chemicals/lab supplies | 4,000         |
| Equipment Rental                       |   |               |
| Work & Safety Gear                     |   |               |
| Repairs & Maintenance                  |   |               |
| Permits                                |   |               |
| Technical Monitoring                   |   |               |
| Other site costs                       |   |               |
| <b>Total Site / Project Costs</b>      |   | <b>39,000</b> |

|        |       |
|--------|-------|
| 35,000 |       |
|        |       |
|        | 4,000 |
|        |       |
|        |       |
|        |       |
|        |       |
|        |       |
| 35,000 | 4,000 |

**ELIGIBLE COSTS****BUDGET****OTHER  
FUNDING****CONTRIBUTION  
FUNDING**

| <b>Training (e.g Swiftwater, bear aware, electrofishing, etc).</b> |           |           |  | <b>Total (PSC<br/>+ In-kind +<br/>cash)</b> | <b>In-Kind &amp;<br/>Cash</b> | <b>PSC Amount</b> |
|--|-----------|-----------|--|---|-------------------------------|-------------------|
| Name of course   | # of crew | # of days |  |   |                               |                   |
|  |           |           |  |   |                               |                   |
| <b>Total Training Costs</b>  |           |           |  |   |                               |                   |

**Overhead / Indirect Costs (not to exceed 20% of PSC Amount)**

|  |              |  |
|--|--------------|--|
| Office space; including utilities, etc.              |              |  |
| Insurance  |              |  |
| Office supplies                                      |              |  |
| Telephone & long Distance                            |              |  |
| Photocopies & printing                               |              |  |
| Other overhead costs                                 |              |  |
| 5% for MOE in-kind project administration (\$53 160) | 2,658        |  |
|  |              |  |
|  |              |  |
| <b>Total Overhead Costs</b>                          | <b>2,658</b> |  |

|       |  |
|-------|--|
|       |  |
|       |  |
|       |  |
|       |  |
|       |  |
|       |  |
| 2,658 |  |
|       |  |
|       |  |
| 2,658 |  |



**Capital Costs / Assets                      Detail (use additional page for details if needed )**

Assets are things of value that have an initial cost of \$250 CAN or more and which can be readily misappropriated for personal use or gain or which are not, or will not be, fully consumed during the term of the project.

|                            |  |        |        |        |
|----------------------------|--|--------|--------|--------|
| <b>Total Capital Costs</b> |  |        |        |        |
| <b>Project Total Costs</b> |  | 86,818 | 64,818 | 22,000 |

**Budget Summary**  
(PSC + in-kind + cash)

|                                   |        |
|-----------------------------------|--------|
| <b>Total Labour Costs</b>         | 45,160 |
| <b>Total Site / Project Costs</b> | 39,000 |
| <b>Total Training Costs</b>       | -      |
| <b>Total Overhead Costs</b>       | 2,658  |
| <b>Total Capital Costs</b>        | -      |
| <b>Project Total</b>              | 86,818 |