FLEET MANAGEMENT

Organization and Planning

Planning for managing the fleet operations for the CROOS project began in January of 2006, six months before the project was funded.

The CROOS Team met on a number of occasions to define the roles and responsibilities for a fleet liaison/coordinator and several port liaisons who would report to that person. Tentative contractual arrangements were discussed with the candidates for those positions and were offered conditional to the approval of funding for the project.

The roles of the port liaisons were modified during the course of the project as specific communication and logistical needs were better defined. The liaison functions proved to be best covered on a week-by-week contractual basis by individuals who were chosen to best fit the fleet distribution for that opening period.

Information about the job opportunities for fishermen/vessels for the at-sea research data collection and the port liaison positions were advertised in various locations:

- The OSC June 2006 *Tagline* newsletter that is mailed to all licensed salmon troll permit holders who landed fish in 2005 (565);
- 2006 licensed wholesale first purchasers of troll salmon (73)
- Coastal ports (14)
- Coastal gear/tackle stores (30)
- Sea Grant Extension Agents (4)

From this notification, fishermen responded and were put on a list. Contracts were created for everyone on this list so that if and when they were selected to fish, the paperwork would be complete and they would be ready to go.

Selection of an "optimum compensation level" for the participating fishermen was critical to getting the maximum amount of data while attracting the largest portion of the active fleet to participate. It was determined that each vessel participating would receive $400 per day of charter, plus an additional $100 per day if a crewman was employed. This was intended to encourage fishermen to employ deckhands so that the economic benefits from the project would be spread as far as possible within the industry.

The original plan was to contract with approximately 50 vessels to fish up to 4 openers each (12 days maximum). An opener was defined as the open periods and locations as regulated by the Pacific Fishery Management Council (PFMC) - June 25-28, July 9-11, 16-18, 23-25, August 1-3, September 17-30, and October 17-31 (with each calendar week being an opener in September and October).

Selection of participating fishermen was on a "first come-first served" basis. An original application deadline of June 30 was set, but with the expectation of extending it if available funding allowed more vessels to participate.

Prior to formal project funding approval, four fishermen volunteered to pre-test some of the actual data collection protocols at sea. They fished and collected samples during regular fishery openers, and worked with team members to incorporate their experiences into the initial set of data collection protocols which would be presented to the fishers at a series of training meetings.

Three separate fishermen training sessions were conducted in Newport on June 24, July 8, and September 15. Fishermen traveling from other ports to attend these sessions were paid a mileage allowance.

At these sessions, a brief description of project goals and the science of Genetic Stock Identification were presented. Data collection techniques and protocols were explained. Supplies were distributed, reporting instructions were explained, and the operation of the datalogging equipment was demonstrated. Contracts were explained and signed, and instructions were given for submitting a billing invoice to OSC for each employed fishing period.

Those fishermen selected to use and test electronic dataloggers (four to six fishermen at any one time) were given separate individual training sessions, which involved actual installation and testing of the units on their vessels.

Fleet Sampling Activities and Performance

Contracted data collection began on June 25, immediately after the Grant approval was finalized.

Experimental fish permits were not acquired for this season and therefore all fishing occurred during regulated seasons.

The fishermen were required to record their "fishing track" for the time when their gear was in the water. When each fish was caught and landed, fisherman were required to:

- collect several scale samples and a small clip of a fin for DNA analysis
- enclose both in a piece of blotter paper and enclose in a pre-marked envelope
- measure the length of the fish
- attach a pre-barcoded metal tag to the fish
- record fisherman's name, the date, time, and location of capture in latitude/longitude, the length of the fish, its depth of capture, the presence of any fin clip markings, and other remarks on the envelope
- record pertinent oceanographic data
- record the "track" of the vessel during fishing operations

Although not a formal part of the CROOS project, two or three fishermen in each opener were asked to collect salmon stomach samples on the last day of their trip for NMFS scientists conducting feeding studies.

All oceanographic and track-log recordkeeping was initially accomplished by manual entry into a paper logbook on all vessels not equipped with an electronic datalogger. Data were supposed to be recorded at a minimum of 30 minute intervals by the fishermen. This requirement was cumbersome and not uniformly followed by all fishermen. In addition, difficulties in

standardizing and recording the data seriously affected the time and effort required to analyze the data in the laboratory. By late July, each of the chartered fishermen was provided a handheld GPS unit to automate vessel track logging and to standardize some of the other recordkeeping protocols (the location and time of capture of each fish were also recorded and stored electronically). Using these units eliminated the need to manually record this information on a paper log, which simplified the data recording duties of the fishermen, as well as expediting data entry in the laboratory. Additional training sessions were held to familiarize the fishermen with these units.

In general, the fishermen participants were able to collect samples and record the required data with minimal interruption of their normal fishing operations. Some fishermen were initially concerned that data sampling would negatively impact their production, especially at higher catch rates, but as sampling became more routine, there was little if any impact on fishing production. Several of the original techniques for sampling and recording data were modified based on feedback from the participating fishermen.

The fleet managers maintained daily records of participation and contact information and shared this information with the liaisons and the lab. Communication between liaisons and charter vessels was sometimes limited due to physical proximity and communications equipment limitations, but was generally not required on a daily basis. Since each of the fishery openings for the 2006 season was limited to less than a week in duration (essentially one fishing trip per opener), it was possible to accurately account for total and individual participation by opener.

When each chartered vessel returned to port to deliver fish, the samples and records were dropped off at pre-arranged locations (a drop box at the Port of Newport office building, by using pre-paid mailers for out-of-Newport vessels, and by hand delivery in some cases), and were then delivered to the lab. Invoices were sent to the OSC.

The logistics involved with these activities proved to be much greater than anyone anticipated. In addition to gathering the samples, reports, and invoices, it was necessary to re-supply the fishermen between trips with tags, envelopes, batteries, etc. and to download the track logs and waypoints from the GPS or datalogger units and to reset these devices between each trip. As the project progressed, many of these problems were addressed and improvements made. In Newport, a convenient drop box, a central supply distribution site, and a check distribution site were all incorporated into the project to accomplish these tasks. By mid August, it was obvious that a single liaison at one central, convenient location would work more efficiently than a handful of fleet coordinators who were also fishing or engaged in other activities.

In late August, the OSC contracted with the Newport Port Outreach Specialist to assist with the logistics of the project. Fishermen were able to take their samples and GPS units to the local Outreach Specialist's office and attend to all responsibilities at one location and time. In addition to downloading and resetting the GPS units, the Outreach Specialist was able to assist with coordinating the disbursement and collection of supplies and equipment, coordinating with and delivery to the lab, and communicating with fleet management and vessels. This greatly reduced the time required by the genetics laboratory to process samples and thus facilitated more rapid genotyping of the fish.

As the project progressed, it became important to track individual "boat-days" of participation consistent with meeting project objectives. These included managing sampling effort throughout the season and across geographic areas, and communicating remaining eligibility to each fisherman.

A spreadsheet model of total fishery participation was updated daily. This model incorporated actual budget expenditures, information from individual fishermen regarding future participation, and estimations of weather and fishing factors. This allowed the management team to produce an estimation of the remaining "boat-days" that the budget would support. In the last weeks of the project, the remaining days of eligibility were calculated and communicated to each fisherman, and additional days of availability were offered to those with remaining eligibility.

Results

87 vessels were on the list with fishermen from the following counties;
Benton, Clackamas, Coos, Curry, Douglas, Garibaldi, Lane, Lincoln, Linn, Marion, Tillamook, and Yamhill
72 vessels participated in at least 1 opener (72 operators, 54 crew members)
707 days were fished
4,278 fish were sampled
$332,100 was distributed to vessels (operators/crew) for participation

Observations and Suggestions

At the beginning of the project, there was some skepticism about the project from within the fishing fleet. This seemed to be primarily focused on two areas. One was the possibility that information gathered from this type of study would be used 'against us' to unfairly limit fishing due to previously undiscovered impacts on critical stocks of salmon, or impacts on small populations which had previously been aggregated into a larger stock composite. The second area of concern was that the project would not fairly distribute the research dollars within the fleet, would favor certain sectors, or would discriminate against very small vessels.

As the project progressed, these concerns diminished and there appeared to be an increase in enthusiasm about the goals and probability of success of the project. (See end of season participant survey results in Appendix 5.) Some of this may have been due to the fact that fishermen were paid for their participation, but the types of questions and positive suggestions that project team members received over the course of the project seemed to indicate a real change in attitude. Comments ranged from 'anything would be better than status quo' to relatively informed discussions about the 'inevitability that better data is going to lead to improved access to healthy stocks of fish'.

The decision that, up until September 1, every Oregon resident permit holder who requested to participate would be offered a contract, helped to gain support for the project. The fact that even those fishermen who were late in contacting the OSC (after the published cutoff date and the July

training session) were still eligible for at least 10 days of work, was perceived by fishermen and the fleet at large as fair.

Comments about sampling and reporting protocols focused primarily on either improving protocols or eliminating unnecessary activities. Early confusion about turning in samples, obtaining supplies, and turning in invoices, were significantly reduced as the project proceeded and more fishermen participated. Information on protocols was passed around by word of mouth and became fairly common knowledge in the fleet.

Future Planning

The fleet management team continues to meet with the overall CROOS Team to refine and plan expansion of the project into the future.

Topics which need to be addressed to support future research include:

1. Planning for fishery sampling outside of normal operating areas. This could include not only preparing for fishing permits and protocols for fishing in areas which are closed to fishing, but also for directing fishing in areas which would not normally be fished by participating vessels (non-volitional fishing). These options would probably require additional training and higher levels of daily compensation.
2. Logistical issues about reporting, sample collection, compensation, etc. Coordinating and fleet management issues may change if fisheries are managed on a continuous basis rather than on the basis of short, weekly openings as it was in 2006.
3. More of the at-sea data recording tasks will need to be done digitally. Disruptions of normal fishing routines will present more problems as catch rates improve, and manual data entry in the laboratory is costly and time consuming.
4. Integrating experience gained from the CROOS project into future, coast wide GSI based research programs. Coordination of fleet management over the entire West Coast will be a particularly important challenge. Contacts have been made with industry representatives from California, and CROOS fleet management representatives have agreed to meet with them to assist in their planning for future GSI projects. Fleet management comments and recommendations were incorporated into the Salmon Advisory Subpanel report to the Pacific Fishery Management Council in November on the Salmon Methodology Review of Future GSI-based management strategies.
5. Consideration of the unique logistical realities which will be encountered at each potential port of landing in the range of the project. Prompt transfer of samples and data to the laboratories, resupplying fishermen, and invoicing and compensation logistics are best accomplished by establishing an office or dropsite in each port staffed by a trained "liaison person". The model ultimately developed in Newport of a drop/distribution site at the Port of Newport Terminal staffed by a trained Port Outreach Specialist was far more efficient than earlier attempts to do these tasks at separate sites or by mail.
6. Communication protocols and daily check-in requirements will need to be clearly defined and explained at the training sessions. The roles of at-sea liaisons will need to be further defined. The use of a shore-based call-in line should be tested.

GENETICS

Introduction

Alternate freshwater drainages where salmon spawn can act as primary delineating forces resulting in isolated populations (stocks) that can have varying levels of genetic uniqueness depending on history and the fidelity of natal homing of the species in question. Among those salmon with high levels of natal philopatry, Chinook salmon display concordantly high levels of genetic structure. These unique genetic signatures can be used to estimate the most likely source population of individual fish (individual assignment) and to estimate the percentage that a particular stock contributes to a total sample of a mixed-stock fishery (mixed-stock analysis, MSA) if an adequate genetic baseline has been developed. The application of molecular genetic data to estimate stock-mixture proportions has made substantial contributions to Chinook management for over two decades (Milner et al. 1983, Teel et al. 1999, Shaklee et al. 1999, Banks 2005). Recent discovery and application of microsatellite molecular markers (Banks et al. 1999, Nelson and Beacham 1999, Williamson et al. 2002, Greig et al. 2003) and advancement of statistical methodologies (e.g. Rannala and Mountain 1997, Banks et al. 2000, Pella and Masuda 2001, Banks et al. 2003, Kalinowksi 2003) have enabled fine-scale detection of genetic differences among populations, increased the accuracy of estimates of mixture proportions, and permitted the assignment of individual fish to their most-likely natal source populations with high levels of confidence (Banks 2000, Beacham et al. 2002, Baudouin et al. 2004, Banks 2005, Banks et al. in prep, Seeb et al. in press).

The distribution of Chinook off the coast of Oregon has traditionally been estimated using coded wire tags (CWT) recovered from a subset of harvested fish sampled dockside by Oregon Department of Fish and Wildlife (ODFW) port-checkers. Coded wire tags are small wires with embedded individual numbers that are inserted into the snouts of some hatchery fish. In-season fisheries management has not been attempted with the current CWT program, probably because data from the small number of tags usually collected in a given fishery are difficult to interpret until all tag returns for the season have been compiled.

The CWT program has recently been under scrutiny because mark-selective fisheries make it more difficult and expensive to sample tags and samples no longer represent unmarked stocks. There are also long-standing concerns over whether the distribution and behavior of hatchery fish is representative of natural stocks, and statistical uncertainties related to sampling, the difficulty of detecting rare stocks, and the need to account for stocks that are unmarked (Hankin et al. 2005). Recommendations by the Expert Panel on CWTs conclude that genetic stock identification techniques could be used to augment the CWT program and assist in modeling stock abundance projections (Hankin et al. 2005).

Recent technological advances in GSI make this a viable option. The Pacific Salmon Commission funded the development of a microsatellite baseline database of Chinook salmon genotypes called GAPS (Genetic Analysis of Pacific Salmonids), that can be used to identify 44 separate reporting groups represented by 160 stocks from California through Alaska (Figure 1, Moran el al. 2005, Seeb et al. in press, Banks et al. in prep). Techniques are now available for rapid scoring, allow near “real time” assessment of the origin of individual fish with unprecedented degrees of accuracy and confidence. Further, because all fish “carry” genetic tags, mixture proportions (contribution rates of fish from many stocks mixed in a single sample, as

would be collected from a mixed-stock ocean fishery) and most-likely source populations of both hatchery and wild Chinook stocks can be estimated.

Ecosystem processes and their affect on the distribution of specific fisheries stocks and their feeding behavior can be investigated by recording the specific location of catch for each fish along with location of fishing effort. Maps of oceanographic conditions can then be matched to these locations. Autonomous underwater vehicles, or gliders, are capable of measuring sea temperature at depth, dissolved oxygen content of water and salinity, and transmitting this information via satellite for "real time" provision of oceanic conditions. Likewise, weather permitting, sea surface temperature and chlorophyll maps are available from satellite imagery and surface currents are available from surface radar. The affordability of onboard handheld Global Positioning Systems (GPS) devices makes the economy of equipping a large fishing fleet to record high resolution special parameters of their fishing efforts feasible. By matching harvest location with genetic stock of origin data we can monitor stock composition by time and area, provide detailed information on the oceanic distribution of different stocks of fish, and explore how oceanic conditions affect fisheries behavior and distribution. Importantly, through long-term datasets we can explore how these factors vary in space and time, seasonally, through decadal oscillations, el Niño events and, potentially, global climate changes.

The Pacific Fisheries Management Council (PFMC) sets commercial troll and recreational fishing seasons based on a combination of factors including the projected abundance of fish expected to be encountered in a region and the expected stock mixture compositions by area. Current management practices are aimed at maximizing catch or fishing opportunity while achieving escapement or exploitation rate goals for all stocks in the fishery. As a result, seasons are frequently limited by weak stocks. If fisheries can be designed to avoid these weak stocks in favor of more abundant or healthier stocks, the fishing season can be extended. In 2005 and 2006 concerns over Klamath fall run was the most constraining factor limiting fisheries harvest between Cape Falcon South to the Mexico/US Border (Pacific Fishery Management Council 2006). A fishery resource disaster was declared in 2006 by the Secretary of Commerce and a complete closure of Chinook fishing from Cape Falcon, Oregon to Point Sur, California was only avoided in a collaborative effort by National Oceanic and Atmospheric Administration (NOAA), PFMC, state and tribal representatives to identify a scientific basis to allow a limited fishing season (Gutierrez 2006). Currently, the estimated contribution of Klamath stock to commercial troll and recreational fisheries is based on a complex model that uses, among other parameters, CWT data obtained from years prior. To date, detailed and specific information on timing of return and oceanic distribution of this and other stocks encountered off the Coast of Oregon and California are unknown. Genetic data can be used to identify the stock composition of a mixed stock fishery throughout the season, and to monitor when a particular stock moves in or out of an area being fished. This can be performed rapidly (24-48 hours), allowing for near "real time" monitoring of stocks being impacted by fisheries harvest. Near "real time" availability of such data could enable fisheries managers to apply in-season adjustments to fishing seasons, re-directing fishery efforts towards stocks of harvest intent.

The objective of this study was to test the feasibility of near "real time" genetic analysis coupled with developing fine-scale maps of fish encounter locations. We also developed a prototype system to disseminate this information to fisheries managers and scientists via the world wide web, to be available for "real time" management decisions and ecosystem-based research.

Methods

The feasibility of rapid (24-48 hour) genetic stock identification of individual Chinook salmon harvested by Fishermen during commercial troll fisheries conducted off the Coast of Oregon was tested by the Marine Fisheries Genetics Laboratory at Oregon State University. "Openers" (the time within a calendar week when fishing was permitted) lasted three or four days per week in June - August 2006 and had a retention limit of 75 fish per week (Figure 2; details on openers can be found at http://oregonsalmon.org/2006%20Troll%20Season%20Regs.pdf). Fishermen were instructed to sample up to the first 70 fish retained in openers. September and October openers generally lasted a full week and had a retention limit of 50 fish; every retained fish was required to be sampled by fishermen during these months.

Participants of Project CROOS were issued either a handheld GPS unit (Garmin GPS 60) or an electronic logbook to record track locations while they fished and to record each location where a fish was harvested (brought up on the boat) and sampled. Pre-printed envelopes with barcode labels on the outside and matching metal barcodes inside were provided to fishermen for data collection and tracking barcoded fish. To tag fish, participants removed the barcode from inside the envelope and strung it on a plastic zip tie threaded through a slit cut in the lower jaw of each salmon. Individual fish data were recorded on the matching numbered envelope (unless the participant had an electronic datalogger). Data records included: date and time of harvest, location (waypoint number from GPS), length of fish, depth of capture, whether a hatchery mark was present, if a stomach was saved for content analysis. Check-boxes were provided to indicate that scales and a tissue-sample had been collected. A subset of fishermen was provided temperature-and-depth dataloggers to record oceanographic conditions while fishing. These records will be used to assess whether these devices can detect ocean fronts and to determine how the variation in oceanic conditions influences feeding behavior, spatial distribution, and population specific oceanic distribution and aggregate patterns of Chinook salmon. In the laboratory, data were compiled in an Access database. ArcGIS spatial analysis software was used to analyze fishermen's track logs, fish encounter locations, and to assess spatial and temporal changes in fish encounters off the Oregon Coast.

Each time a participant returned to port after fishing, they were required to check in with the port liaison who, in turn, was responsible for downloading GPS track logs and fish encounter information and transferring this data, along with tissue and scale samples, to the genetics laboratory. The genetics laboratory matched each fish's barcode to the latitude and longitude where that fish was harvested, genotyped a subset of all the fish sampled that week, and used genetic data to estimate the mixture proportions of all stocks present and the individual stock of origin. Critical to rapid sample processing and mapping was the port liaison's ability to collect both fish samples and GPS coordinates from the fishermen, and to provide these data together to the genetics laboratory. After the genetic stock of origin was estimated, the sample envelopes were sent to ODFW for scale aging analysis.

The Marine Fisheries Genetics Laboratory at Oregon State University archived a total of 4,317 fish samples collected by fishermen participating in Project CROOS during the 2006 fishing season (Table 1). This project's initial objective was to identify the stock of origin for 2,000 fish, however it was necessary to process more than 2,000 fish for two reasons: 1) missing genotypic data or 2) low assignment probabilities (< 90%). Fish were required to have data for eight or more of the standardized GAPS 13 loci before they would be included in our genetic

stock assignment dataset. Missing genotypic data results from a sample failing to amplify at a genetic marker. This can occur due to low quality DNA caused by tissue degradation (rotting), inefficient sample digestion in the laboratory, sample contamination, or an inefficient polymerase chain reaction. It is generally difficult to tease out which specific issue contributed to amplification failure. For example the majority of samples provided by fishermen were of excellent quality and taken with obvious care. From the total number of fish processed (3,097), 530 (17.11%) fish did not amplify at 8 or more loci and were subsequently removed from the dataset; the remaining 2,567 fish were used to estimate stock mixture proportions and for individual assignment to baseline populations.

Probability values of stock assignment for the 2,567 fish ranged from 28% - 100%; recent power analyses (Banks et al. in prep) indicate that fish with probability values < 90% are more likely to assign to an incorrect stock of origin. By the end of the season a total of 2,097 fish were assigned probabilities ≥ 90%, while 470 fish with sufficient genotypic data did not meet the 90% criterion (18.3% of the total dataset). Samples not processed in the laboratory (1,221) are in the OSU archive and can be genotyped at a later date.

Laboratory Analysis

Tissue samples were digested and DNA extracted using silica membrane-based kits (Qiagen® DNeasy™ kits) following manufacturer's protocols or by standard chelex methodology. Genomic DNA was arrayed into either 384- or 96- well plates for high throughput genotyping. The polymerase chain reaction (PCR) was used to amplify 13 microsatellite loci standardized by GAPS: *Ogo2, Ogo4* (Olsen et al. 1998)*, Oki100* (unpublished; provided by Canada's Department Fisheries and Oceans)*, OMM1080* (Rexroad et al. 2001)*, Ots201b, Ots208b, Ots211, Ots212, Ots213* (Greig et al. 2003), *Ots3M, Ots9* (Banks et al. 1999)*, OtsG474* (Williamson et al. 2002)*,* and *Ssa408* (Cairney et al. 2000). PCR was performed in 5 ul reactions with 1X Promega buffer or 1x GoTaq® Promega buffer, 1.5 mM $MgCl_2$, 0.1667 - 0.5 uM primer concentration, with annealing temperatures ranging from 50 - 63 degrees C. Locus-specific details for PCR conditions and thermal cycling programs can be obtained by request from R. Bellinger. Forward primers were fluorescently labeled, and PCR products were visualized using an Applied Biosystems® model 3730xl genetic analyzer. GeneMapper software was used to assign standardized GAPS allele calls to allele peaks. Individual fish's unique genotypic profiles were tracked using the unique barcode number, transferred from GeneMapper to Microsoft excel spreadsheets, and archived in the final Microsoft Access "Project CROOS" database.

Genetic Stock Identification

Genetic stock estimates were performed using GAPS baseline v2, which contains 166 Chinook salmon populations from mid-California north to Alaska (Figure 1, Appendix 1). The GAPS baseline uses "reporting regions" for compositional analyses: reporting regions are groups of populations with similar genetic signatures, as previously identified by other allozyme and microsatellite studies, taking into account a combination of geographic features and management applications (Teel et al. 1999, Seeb et al. in press, Banks et al. in prep). Several rivers, such as the Klamath and Rogue, are genetically distinct enough to be considered their own reporting regions. We combined California Central Valley fall and spring runs into one reporting region because of known shortcomings in discriminating fall from spring runs in this drainage and because the relative contribution of these two runs, per se was not of direct importance to the focus of this study.

Genetic-based estimates of stock mixture proportions (mixed stock analysis, MSA) and individual assignment (IA) probabilities were calculated using the computer program Genetic Mixture Analysis (GMA; Kalinowski 2003). GMA uses Bayesian priors to calculate the probability that an individual fish came from a specific population in the baseline. The proportion that a stock contributes to a mixture can also be calculated by summing the number of individual fish assigned to reporting region (IA) and dividing this number by the total. Mixture proportions using IA data were calculated using Microsoft Excel. Compositional results from IA are nearly identical to MSA.

Accuracy of individual assignment rapidly declines with probabilities of less than 90% (Banks et al. in prep), therefore two alternatives to traditional MSA were tested. In the first approach we summed the number of fish with IA ≥ 90% by region, pooled assignments for fish with probabilities of less than 90% into an "uncertain" category, and then recalculated stock mixture proportions (hereafter referred to as the "rigorous dataset"). The second approach calculated mixture proportions only using fish with high IA probabilities (≥ 90%) (hereafter referred to as "conservative dataset").

Pooling individuals with IA < 90% into a separate category and including these in the denominator biases mixture proportions low because some of the fish removed from a reporting region may have been correctly assigned. This amount of bias is dependent on the proportion of fish that were correctly versus incorrectly assigned. The percent contribution of fish with IA < 90% to a reporting region's total allocation was calculated to evaluate the potential range of bias in the rigorous dataset.

The conservative dataset, limited to fish with high probabilities, would only be more accurate than traditional MSA if confidence limits were equal for all fish considered in the mixture and all fish with lower probabilities were incorrectly assigned. Since it is known that accuracy drops below 90% probability values, a dataset restricted to the most accurate data possible may provide a more reliable estimate of stock mixture proportions if bias factors are known and taken into account, however, bias may confound findings if specific stocks are more likely to be mis-classified than others. Increased accuracy is critical in applications such as considered here because precision of the "weak stock" estimate has overriding importance and this bias factor can be taken into account. Analyses using GAPS baseline v1 and stock mixture proportions calculated using only fish with IA ≥ 90% were used for genetic analysis presented at CROOS meetings and during the 2006 fishing season. After baseline v2 was released, however, we reanalyzed all data using all three treatments of data and present results here. GAPS v2 adds 56 population samples to the 110 population samples in GAPS v1. This should, in principle, reduce the number of mis-classified fish.

The effect of pooling or removing fish with low assignment probabilities was evaluated several different ways. First we verified that estimated stock mixture proportions from MSA were similar to those obtained from IA. The difference between results obtained from the IA conservative and IA rigorous datasets was calculated to determine which stocks were potentially affected the most by pooling or limiting data. A bias factor for the rigorous dataset was estimated by calculating the percent that, if assigned correctly, IA < 90% fish would have contributed to a region's estimated contribution in the mixture.

The region or groups of regions that were assigned the greatest percentage of fish with low probabilities (< 90%) might benefit the greatest from increased baseline coverage or by selection of specific genetic markers to increase genetic assignment power (Banks and Jacobson 2004). We assessed which regions fish were frequently assigned to with low probabilities (IA < 90%) by summing the number of fish with IA < 90% assigned to each region and dividing this number by the total (n = 470).

Power analyses indicate that increasing fishery sample sizes from 100 to 400 fish has a strong affect on the minimum stock component one might be able to estimate in the fishery sample; i.e., the smallest component estimates for fishery sample size of 100 is 0.04, but fishery sample sizes of 200 allow component estimates down to 0.02 (Banks et al, in prep). We estimated stock mixture proportions for weeks with sample sizes of IA ≥ 90% and n > 100. To increase our power and accuracy, we plan to genotype additional fish and attempt to bring the total sample size per week to over 200 fish. This will affect calculations for weeks of July 16-22 and October 22-28 and overall averages for the total dataset.

We evaluated the estimated stock contribution from the Klamath basin on a weekly basis, for weeks with sufficient sample sizes (n > 100 with IA ≥ 90%), and provided a comparison of all treatments of data (IA total, IA rigorous, and IA conservative). Weekly contributions from the Klamath, California Coast, and the Northern California/Southern Oregon Coast were compared on a week-by-week basis using MSA.

Variation in spatial and temporal stock composition and harvest rate per unit effort was calculated in a preliminary test of feasibility for fisheries management and applications. For simplicity purposes, we limited analyses to two weeks, September 17–23 and 24–30. Fishermen's track log records and fish harvest locations were mapped onto the ocean using ArcGIS spatial analysis software. Two areas were hand selected to maximize sample size for assessment of harvest rate per unit effort and to compare relative stock proportions. "Harvest rate" was defined as the location where one fish was landed and "unit effort" was measured as one record in a 5-minute interval recorded by the GPS unit for each fisherman in their track log. Fish harvested by participants not equipped with GPS units were removed from the comparison of harvest rate per unit effort; however, these fish were included in genetic estimates of stock mixture compositions. Three fishermen (different fishermen each week) were not equipped with GPS units and their fish were excluded from the harvest per unit effort calculation to normalize results.

Oregon Department of Fish and Wildlife port samplers at Newport and Garibaldi routinely sample fish for presence of CWTs. Since all fish marked with CWTs are from known populations, these provide a means to validate genetic stock identification methods and scale aging analysis. All fish sampled by ODFW that contained CWTs and were marked with Project CROOS barcodes were recorded and data were provided, with the ODFW snout ID number, to the OSU genetics laboratory. Validation of genetic stock assignments was conducted as a "blind test" as follows. The OSU Genetics Laboratory provided ODFW the genetics results prior to CWT data availability. After the CWT data were available, ODFW personnel matched snout identification numbers and barcodes to determine the true population, and compared these results to those obtained by genetic analyses. To assist in scale analysis conducted by the ODFW scale-aging laboratory, best estimates of river of origin and run-time were provided with the scales when they were given to ODFW.

Results

Feasibility of "real time" analysis and synthesis of findings

By September/October of this project, fish were successfully assigned individual genetic stock estimates and mapped by their harvest location in near "real time" (within 24-48 hours of laboratory receiving the sample). In contrast, during the first few months of the project, genetic analysis was delayed (conducted between 48-96 hours) because personnel in the genetics laboratory were attempting to conduct genetic analysis plus organize project logistics. By the end of the season protocols had been developed, minor laboratory issues had been resolved, and near "real time" analyses were achieved.

Stock identification and distribution

Population based mixed stock analysis
It has been well documented that population-based methods of stock assignments provide more reliable estimates of stock proportions than individual based methods, primarily because the sub-populations defined within a fishery sample provide more information than is contained within a genotype of a single individual. Results of mixed stock analysis (MSA) (Table 2, Figures 4, 5) indicated that the majority of fish were from California's Central Valley (59.08%). The Rogue River was estimated to contribute the second greatest proportion (7.61%), followed by the Mid Oregon Coast (7.11%) and the Klamath basin (6.58%). The California Coast and Northern California Coast/Southern Oregon Coast regions contributed 2.17% and 1.89%, respectively. The Upper Columbia River summer/fall run was estimated to contribute 3.03% of the total. Twenty other stocks contributed less than 2% each.

Individual assignment and alternate data treatment
The MSA analysis provides the best estimate for overall stock proportions, but does not produce estimates of stock for individual fish. Stock mixture proportions estimated from Individual Assignment (IA) with the conservative dataset (fish with IA $\geq$ 90%) indicated that the majority of fish were from California's Central Valley (70.53%). The Klamath Basin was estimated to contribute the second greatest proportion (6.77%), followed by the Rogue River (5.39%) and the Mid Oregon Coast (4.05%). The California Coast and Northern California Coast/Southern Oregon Coast regions contributed 2.29% and 2.10%, respectively. The Upper Columbia River summer/fall run was estimated to contribute 2.15% of the total. Twenty other stocks contributed less than 2% each. These proportions are similar to the mixture proportions for all stocks except those estimated for the California's Central Valley (CACV) fall and spring run. These two runs were grouped into a single reporting region so the criterion for accepting a CACV fish was essentially widened and more fish assigned to this group with high probability, inflating their stock proportion. Rigorous comparison of differences between the two analytical techniques will require confidence limits for each estimate.

The greatest proportion of fish with low probabilities (IA < 90%, n = 470) was assigned to the Mid Oregon Coast (20.00%), followed by the Rogue River (18.72%) and the Central Valley (10.21%; Table 3). The lower Columbia fall run and upper summer/fall run contributed 6.17% – 6.81% respectively, followed by the Klamath (5.11%) and the mid Oregon Coast (4.89%). Northern California/Southern Oregon Coast and California Coast were estimated to contribute

3.62% and 1.91%, respectively. The remaining 17 stocks contributed less than 5% to the IA < 90% dataset.

Distribution patterns over time
We collected sufficient sample sizes (n > 100) for weekly estimates of stock composition in seven weeks (Figure 3). The number of samples collected per weekly opener ranged from 0 – 1173 and sampling efforts were highly dependent on weather conditions. Sample sizes were sometimes limited by low catch rates. Weekly stock proportions from IA and MSA averaged over all weeks were similar: Central Valley fall and spring contributed the greatest percent (MSA weekly average 61.01 %; range 43.91% - 71.49%, Table 4). The Klamath ranged from 3.82% to 11.32% (weeks of 9 July 2006 and 17 September 2006, respectively) with an average over all weeks of 6.47%. The Rogue River spiked at 19.13% during October, up from 1.70% in the first week of August (average 7.26%). Stocks from the California Coast reporting region averaged 2.20% (range 0.67% - 5.38%), and the Northern California/Southern Oregon Coast contributed an estimated average of 2.25% (range 0.60% - 5.75%). Weekly trends for the Klamath, California Coast, and Northern California/Southern Oregon stocks were largely comparable (Figure 7). One of Project CROOS's objectives was to test our ability to provide genetic stock estimates for management and protection of fish from the Klamath basin. Of all treatments of data considered, the conservative dataset estimated the greatest contribution from the Klamath basin on a weekly basis (Figure 7) but the method that would be most useful for management remains to be determined.

Global position system technology was successfully implemented to record fishermen's track logs and harvest locations throughout the season (Figure 8). Oceanographic data were collected with temperature-at-depth dataloggers during the last portion of the season (September and October), and by the OSU COAS AUV glider. These results will be discussed separately in the Oceanographic section.

Preliminary analysis of distribution patterns over space
Catch per unit effort (CPUE) differed between Sections 1 and 2 during the week of 17–23 September (Figure 9). A total of 3,918 and 1,362 units of effort were exerted in Sections 1 and 2, yielding a harvest of 956 and 19 fish (0.239 and 0.041 fish/unit effort, respectively). The following week, fishermen exerted 1,541 and 6,781 units of effort to harvest 63 and 351 fish from Sections 1 and 2 (0.014 and 0.052 fish/unit effort, respectively), so it appears that CPUE in Section 1 dropped. Considerable work remains to be done before we can analyze the CPUE and distribution data with confidence.

Stock mixture composition (MSA) in Sections 1 and 2 followed previously identified trends, dominated by contributions from the California Central Valley (Figure 10; n = 468, 19 fish in Sections 1 and 2, respectively for week 1; n = 35 and 192, Sections 1 and 2, respectively for week 2). It is premature for us to attempt to compare contribution rates for other stocks.

Blind testing against CWT results
Port samplers removed a total of 56 snouts from fish thought to contain CWTs; of these, five were false-positives and did not contain a CWT. From 51 fish with CWTs, 31 were assigned with >90% stringency and each of these were correctly assigned to the CWT stock of origin using genetics. Eight fish did not meet the criteria of ≥ 90% IA, thus we did not consider these

for the empirical validation of genetic assignment, however data are reported for comparison purposes (Table 5). One fish was initially reported as having a genetic identity that did not match the true source population, however, the scale-derived age of the fish also did not match CWT data and further investigation revealed this tag number was read incorrectly. The majority (n = 5) fish with probabilities < 90% incorrectly assigned to their true source population, while three correctly assigned. Eleven fish failed to amplify at ≥ 8 loci and will be reanalyzed. Of the 31 CWT fish that could be assigned with ≥ 90%, all 31 fish (100%) were assigned correctly.

During this first CROOS season we culled a substantial portion (17%) of fish from the genotype dataset due to missing data. The first few months of the project we used Chelex to extract DNA because it is less expensive and faster than using prepared extraction kits. However, kits such as Qiagen, using silica-based columns, produce cleaner DNA, which has a better amplification efficiency. By the end of the project we had switched to Qiagen DNA kits and reduced the number of genotypes requiring removal from the dataset (3-10%).

Discussion

Project CROOS's approach of associating genetic stock identification of individual fish with at-sea data harvest locations and oceanic conditions provides high levels of resolution for stock behavior studies of Pacific salmonids. Combining genetic stock of origin data with other analytical techniques such as otolith microchemistry may enable us to elucidate vexing questions such as where fish go after they enter the ocean and whether they remain as aggregated stocks or mix freely in the ocean. This project demonstrates the feasibility of using molecular genetic technology and stock assignment techniques for stock identification of fish harvested off the Coast of Oregon. We provide proof in principle for generating near "real time" stock origin and distribution estimates for in-season management of fisheries. Internet technology, spatial analysis software (ArcGIS) and Arc IMS interface are the key to successfully distributing this information to fishermen, managers, scientists and consumers.

Genetic data holds great promise for fisheries management; however, statistical and analytical biases need to be evaluated to ensure the best data for fisheries management is provided. Stocks which are a low percentage of a mixed-stock fishery sample are problematic because power declines for smaller contributions (Reynolds and Templin 2004). Three other factors which also contribute to accuracy and bias of genetic estimates of mixture proportions are: 1) marker power, 2) genetic similarity of stocks, and 3) baseline coverage. Marker power for the GAPS baseline has been evaluated by Seeb et al. (in press) and Banks et al. (in prep); results indicate that MSA estimates are accurate within 1–5% of the true value more than 90% of the time. Genetic similarity of stocks can reduce the accuracy of mixture proportions and individual assignments because individuals from similar populations tend to mis-assign. The genetic relationship of stocks in the GAPS baseline has been evaluated to group closely related populations into reporting regions. In some cases, for management purposes, it is desirable to maintain separate reporting regions despite similarity of genetic signatures. Consequently, bias is introduced because measures of baseline accuracy decrease.

Genetic similarity of stocks can cause biased results because of potential mis-assignment. For example, if two rivers included in the baseline, "River A" and "River B" were known to be

genetically similar and 10% of all River "B" fish consistently, but incorrectly, assign to River "A", a 10% bias correction factor could be applied. This might occur because stock from River A was moved to River B, thus clouding the genetic difference between the two populations. Chinook salmon stock transfers between basins have occurred for establishment and maintenance of hatchery breeding programs. Current Oregon legislation mandates that 35% of all hatchery brood stock must originate from the wild stock. However, the long established hatchery brood stock population may contain genetic heritage from any of several out-of-basin stock transfers. For example, the Rogue River, a southward migrating stock, has been transferred to the Coos and lower Columbia Rivers as an attempt to establish southward instead of northward migrating stocks. While populations from these three rivers are genetically distinct, fish with intermediate Coos or Columbia /Rogue genetic signatures may assign to both basins with low probabilities.

The third factor affecting accuracy and bias of genetic stock estimation can be attributed to inadequate baseline coverage. All individuals included in a mixed stock fishery sample must assign to a population in the baseline, regardless of whether its source population is represented in the baseline. Statistical methods to address this shortcoming are being developed by Pella and Masuda (2006, program *HWLER*), however this computer analysis method currently requires an excessive amount of computer time and is not publicly available. Since every fish must assign back to a baseline population, a fish from a population not in the baseline will assign to the stock that it is the most similar to. This can inflate the estimated contribution from such most similar baseline stocks.

To explore the relative contribution of fish that might not originate from a stock represented in the baseline we created the category "uncertain" for all fish with probabilities less than 90%. These fish might represent a different stock, or they might be fish at the perimeter of the "average" genotypic profile of a river. Given that the true stock of origin is only known for fish with CWTs, we assessed the contribution of fish with low probabilities by reporting region. The Mid Oregon Coast and Rogue River comprised the greatest component among this group of assignments with low probability. Either fish from genetically similar stocks are mis-assigning to these regions, or fish assigning to these regions are from other perhaps nearby areas. Empirical tests of these hypotheses can be provided by collecting known genetic samples and assessing their relative assignment probability values to calculate bias factors. Although CWT data for fish with IA < 90% were limited to eight fish, results indicate that excluding them from the dataset was appropriate. Five fish with IA < 90% were incorrectly assigned (62%, n = 8), demonstrating that including these fish in stock mixture compositions would have incorrect results. Alternatively, genetic markers can be selected to increase resolution for these regions.

Genetic stock of origin estimates for fish with high probabilities were 100% consistent with CWT data (n = 31). While our mixture proportions calculation is based only on fish with high probabilities and may bias slightly high for the Klamath, these results provide the most conservative (in terms of our confidence in the stock identifications) measure for stocks originating from those regions. If a "weak stock" has potential to constrain a fishery, then policy may find the most conservative dataset more desirable because all conservative dataset assignments including those to the "weak stock" have high certainty.

The Klamath-Siskiyou region has a complex biogeographical history and is the site of numerous "species breaks" (Soltis et al. 1997, Bury and Pearl 1999, Bellinger et al. 2005, Miller et al. 2006). Only the highest elevations in the Klamath-Siskiyou range were glaciated during the Pleistocene, so lower elevation rivers in this area provided refugia during the last ice age. Within the Klamath basin, Banks (1999) documented substantial heterogeneity among Klamath River Chinook stocks. Currently the Klamath basin is represented by three stocks in the GAPS baseline v2: Klamath fall, Trinity fall, and Trinity spring. Stocks divergent from what is currently included in the GAPS baseline from the Klamath basin and similar stocks from adjacent rivers have the potential to either mis-assign or assign with low probabilities back to the Klamath. The California Coast is represented by two stocks, the Eel and Russian Rivers, and the Northern California/ South Oregon Coast is represented only by the Chetco. There are several rivers with Chinook populations in Southern Oregon and Northern California that have the potential to mis-assign to the Klamath or California/Oregon Coast. Further characterization of stocks within and adjacent to the Klamath basin are recommended to assess potential mis-assignment to this region.

Accurate assessment of Klamath basin and California's coastal stocks would likely benefit from better genetic characterization of fish in these regions. Statistical methods to reduce bias and increase accuracy are funded by the Pacific Salmon Commission and NOAA to support work by S. Kalinowski (personal communication). With improved stock characterization data and statistical methods we hope to increase our accuracy of estimates of mixture proportions and justify likely error rates with greater accuracy.

Stock mixture composition of Chinook salmon encountered off the Coast of Oregon is expected to vary throughout the season, by stock and life history types (ocean and stream), and by migration timing of adults returning to breed (Nicholas and Hankin 1988). Our results demonstrate the potential to detect differential use of habitat by Chinook salmon over relatively short time intervals (one week).

Using studies of this type we have the potential to detect short-term fluctuations in the distributions of adult Chinook salmon. The weeks when the Klamath and Rogue contributions were the greatest, 11.32% and 19.13% respectively (mixture proportions calculated with IA) were notable because they represent spikes well above the average. This could be related to timing of migration of these stocks through specific regions. CWT data would not be capable of discriminating spikes in migratory timing and oceanic distribution such as detected using genetic analyses, primarily because they are not present in a sufficiently large number of fish and do not provide spatial or temporal resolution.

Project CROOS represents application of genetic information to estimate stock distribution and behavior of fish in the ocean. Fish harvest locations and genetic stock identification coupled with unit fishing effort provided by fishermen has allowed us to compile and analyze detailed data. As this dataset grows over time it will allow us to address a wide range of management and science questions and provide a foundation to measure seasonal, regional, decadal, and global climate change on the distribution of salmon stocks.

Acknowledgements

Sampling for Project CROOS began June 4 with the in-kind contribution of volunteer fishermen participating on the steering committee, however official funding was not granted by Oregon's Watershed Enhancement Board until June 23, 2006. Clearly the success of this project relies upon the continued advice, participation and contributions from fishermen participating on the steering committee and project. Four members of the steering committee (Scott Boley, Jeff Feldner, Bob Kemp and Paul Merz) helped develop at-sea protocols and logistics of training fishermen, providing them with sampling materials, and recovering the samples and transporting them to the lab. We are especially grateful for these contributions and to the Oregon Watershed Enhancement Board for funding this research as well as the Pacific Salmon Commission's Chinook Technical Committee for funding the standardization of the GAPS microsatellite baseline.

Citations

Banks, M. A., E. Anderson, A. Antonovich, T. D. Beacham, M. R. Bellinger, S. M. Blankenship, M. Campbell, J. Candy, N. A. Decovich, J. C. Garza, C. M. Guthrie III, T. A. Lundrigen, P. Moran, S. R. Narum, Seeb, L. W., J. J. Stephenson, K. J. Supernault, D. J. Teel, W. D. Templin, K, Warheit, J. K. Wenburg, S. F. Young, and C. T. Smith. Power analysis of the GAPS baseline. In prep. for submission to Canadian Journal of Fisheries and Aquatic Science.

Banks, M. A. 2005. Stock identification for the conservation of threatened or endangered species. In: Stock identification methods Eds: Cadrin, S.X., K.D. Friedland and J.R. Waldman. Elsevier Press. pp 593-613.

Banks, M. A. and Jacobson, D. P. 2004. Which genetic markers and GSI methods are more appropriate for defining marine distribution and migration of salmon? North Pacific Anadramous Fish Commission Technical Report 5.

Banks, M.A. W. Eichert, and J.B. Olsen. 2003. Which genetic loci have greater population assignment power? *Bioinformatics* 19**:**1436-1438

Banks, M. A., V. K. Rashbrook, M. J. Calavetta, C. A. Dean, and D. Hedgecock. 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. Canadian Journal of Fisheries & Aquatic Sciences 57: 915-927.

Banks, M.A. and W. Eichert. 2000. WHICHRUN (version 3.2) a computer program for population assignment of individuals based on multilocus genotype data. *Journal of Heredity* 91**:**87-89.

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. Journal of Heredity 90: 281-288.

Baudouin, L, S. Piry, and J. M. Cornuet. 2004. Analytical Bayesian approach for assigning individuals to a population. Journal of Heredity 95:217-224.

Beacham, T. D., B. McIntosh, and C. MacConnachie. 2002. Microsatellite identification of individual sockeye salmon in Barkley Sound, British Columbia. Journal of Fish Biology 61:1021-1032.

Bellinger, M. R., T. Mullins, S. Haig and E. Forsman. 2005. Phylogenetic relationships among *Phenacomys* voles as inferred by Cytochrome-b. Journal of Mammalogy 86:201-210.

Bury, R. B. and C. A. Pearl. 1999. Klamath-Siskiyou herpetofauna: biogeographic patterns and conservation strategies. Natural Areas Journal 19:341-350.

Cairney M., J. B. Taggart, and B. Hoyheim. 2000. Atlantic salmon (Salmo salar L.) and cross-species amplification in other salmonids. Molecular Ecology 9: 2175- 2178.

Greig, C., D. P. Jacobson, and M. A. Banks. 2003. New tetranucleotide microsatellites for fine-scale discrimination among endangered Chinook salmon (Oncorhynchus tshawytscha). Molecular Ecology Notes 3: 376-379.

Gutierrez, C. M. 2006. Declaration concerning the Klamath River fall Chinook salmon fishery. The Secretary of Commerce. http://www.commerce.gov/opa/press/Secretary_Gutierrez/2006_Releases/July/Klamath.pdf

Hankin, D. G., J. H. Clark, R. B. Deriso, J. C. Garza, G. S. Morishima, B. E. Riddell, C. Schwarz, and J. B. Scott. 2005. Report of the Expert Panel on the future of the coded wire tag recovery program for Pacific salmon. Pacific Salmon Comm. Tech. Rep. No. 18: 230 p. Available at: http://www.psc.org/pubs/CWT/EPfinalreport.pdf

Kalinowski, ST. 2003. Genetic Mixture Analysis 1.0. Department of Ecology, Montana State University, Bozeman MT 59717. Available for download from http://www.montana.edu/kalinowski

Moran, P., Banks, M., Beacham, T., Garza, C., Narum, S., Powell, M., Campbell, M., Seeb, L., Wilmot, R., Young, S., Ardren, A., Wenburg, J. 2005. Interlaboratory Standardization of Coast-wide Chinook Salmon Genetic Data for International Harvest Management. A progress report from the Genetic Analysis of Pacific Salmonids (GAPS) consortium to the Chinook Technical Committee of the Pacific Salmon Commission, FY2004, FY2005, 20 October 2005

Milner,G. B., D. J. Tee1, F. M. Utter. 1983. Genetic stock identification study. Bonneville Power Administration, Agreement DE-A179-82BP28044MOOl), Coastal Zone and Estuarine Studies Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 101 electronic pages (BPA Report DOE/BP-I0002). http://www.efw.bpa.gov/cgi-bin/efw/FW/publications.cgi

Nicholas, J. and D. G. Hankin. 1988. Chinook salmon populations in Oregon's Coastal river basins: Description of life histories and assessment of recent trends in run strength. Oregon Department of Fish and Wildlife, Informational Report 88-1. 359 pp.

Olsen, J. B., P. Bentzen, and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7: 1083-1090.

Pacific Fishery Management Council. 2006. Review of 2005 Ocean Salmon Fisheries. (Document prepared for the Council and its advisory entities.) Pacific Fishery Management Council, 7700 NE Ambassador Place, Suite 200, Portland, Oregon 97220-1384.

Pella, J. and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fishery Bulletin 99:151-167.

Pella, J. and M. Masuda. 2006. The Gibbs and split-merge sampler for population mixture analysis from genetic data with incomplete baselines. Canadian Journal of Fisheries and Aquatic Science 63:576-596.

Rannala, B. and J. L. Mountain. 1997. Detecting immigration using multilocus genotypes. Proceedings of the National Academy of Science 94:9197-9291.

Rexroad, C. E., III, R. L. Coleman, A. M. Martin, W. K. Hershberger, and J. Killefer. 2001. Thirty-five polymorphic microsatellite markers for rainbow trout (Oncorhynchus mykiss). Animal Genetics 32: 317-319.

Reynolds J. H. and W.D. Templin. 2004. Detecting specific populations in mixtures. Environmental Biology of Fishes 69:233-243.

Seeb, L. W., A. Antonovich, M. A. Banks, T. D. Beacham, M. R. Bellinger, S. M. Blankenship, M. Campbell, N. A. Decovich, J. C. Garza, C. M. Guthrie III, T. A. Lundrigen, P. Moran, S. R. Narum, J. J. Stephenson, K. J. Supernault, D. J. Teel, W. D. Templin, J. K. Wenburg, S. F. Young, and C. T. Smith. Development of a standardized DNA database for Chinook salmon. Accepted, in revision, Fisheries.

Shaklee, J. B., T. D. Beacham, L. Seeb and B. A. White. 1999. Managing fisheries using genetic data: case studies from four species of Pacific salmon. Fisheries Research 43:45-78.

Soltis, D. E., M. A. Gitzendanner, D. D. Strenge and P. E. Soltis. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. Plant Systematics and Evolution 206:353-373.

Teel, D. J., P. A. Crane, C. M. Guthrie III, A. R. Marshall, D. M. VanDoornik, W. D. Templin, N. V. Varnavskaya, and L. W. Seeb. 1999. Comprehensive allozyme database discriminates chinook salmon around the Pacific Rim. (NPAFC document 440) 25p. Alaska Department of Fish and Game, Division of Commercial Fisheries, 333 Raspberry Road, Anchorage, Alaska USA 99518.

Table 1. Total number of samples collected during 2006 CROOS fishing season, total number processed by the Genetics laboratory, and total number fish assigned to the GAPS baseline.

Samples	Number	Number subtracted from dataset	Reason
Total Collected	4318	1221	Archived in laboratory (can be genotyped with additional funding)
Total processed	3097	530	Failed to amplify at 8 or more loci
Total assigned to baseline	2567		

Table 2. Genetic estimates of mixture proportions for fisheries samples collected off the Coast of Oregon during Project CROOS (2006). Mixed Stock Analysis (MSA) using the total dataset (n = 2567) was compared to results from Individual Assignment (IA) and by varying how individuals with <90% IA were treated. The difference between IA (all fish) and IA rigorous dataset is provided as a bias factor. Difference between MSA and stringent results are provided for comparison. Sample sizes for all regions are split by IA ≥ 90% and IA < 90%.

	MSA (all fish)	IA (all fish)	Stringent dataset (IA < 90% grouped)	Conservative dataset (IA ≥ 90%, n = 2097)	Proportion fish IA < 90% to total dataset (bias factor)	Difference stringent from MSA dataset (as %)	Sample size by region (all data)	Sample size rigorous (IA ≥ 90)	Sample size IA < 90
Central Valley fa/sp	59.08%	59.49%	57.62%	70.53%	1.87%	-1.46%	1527	1479	48
Rogue R.	7.61%	7.83%	4.40%	5.39%	3.43%	-3.21%	201	113	88
Mid Oregon Coast	7.11%	6.97%	3.31%	4.05%	3.66%	-3.80%	179	85	94
Klamath R.	6.58%	6.47%	5.53%	6.77%	0.93%	-1.05%	166	142	24
U Columbia R. su/fa	3.03%	3.00%	1.75%	2.15%	1.25%	-1.28%	77	45	32
N CA / S OR Coast	2.36%	2.38%	1.71%	2.10%	0.66%	-0.65%	61	44	17
California Coast	2.17%	2.22%	1.87%	2.29%	0.35%	-0.30%	57	48	9
N Oregon Coast	1.89%	1.87%	0.97%	1.19%	0.90%	-0.92%	48	25	23
L Columbia R. fa	1.78%	1.87%	0.74%	0.91%	1.13%	-1.04%	48	19	29
S Puget Sound	1.57%	1.64%	0.82%	1.00%	0.82%	-0.75%	42	21	21
L Fraser R.	1.11%	1.17%	0.86%	1.05%	0.31%	-0.25%	30	22	8
Mid Col. R. tule	1.05%	1.01%	0.86%	1.05%	0.16%	-0.19%	26	22	4
Hood Canal	0.82%	0.78%	0.23%	0.29%	0.55%	-0.59%	20	6	14
Deschutes R. fa	0.75%	0.55%	0.19%	0.24%	0.35%	-0.56%	14	5	9
L Columbia R. sp	0.74%	0.58%	0.16%	0.19%	0.43%	-0.58%	15	4	11
N Puget Sound	0.58%	0.43%	0.04%	0.05%	0.39%	-0.54%	11	1	10
SnakeR. fa	0.46%	0.31%	0.08%	0.10%	0.23%	-0.38%	8	2	6
S Thompson R.	0.37%	0.39%	0.19%	0.24%	0.19%	-0.18%	10	5	5
Washington Coast	0.25%	0.27%	0.04%	0.05%	0.23%	-0.21%	7	1	6
SSE Alaska	0.17%	0.23%	0.04%	0.05%	0.19%	-0.13%	6	1	5
Mid Fraser R.	0.14%	0.12%	0.08%	0.10%	0.04%	-0.06%	3	2	1
Willamette R.	0.12%	0.12%	0.08%	0.10%	0.04%	-0.04%	3	2	1
U Fraser R.	0.09%	0.08%	0.08%	0.10%	0.00%	-0.01%	2	2	0
W Vancouver Is.	0.07%	0.08%	0.04%	0.05%	0.04%	-0.03%	2	1	1
E Vancouver Is.	0.05%	0.08%	0.00%	0.00%	0.08%	-0.05%	2	0	2
Central BC Coast	0.03%	0.04%	0.00%	0.00%	0.04%	-0.03%	1	0	1
N Thompson R.	0.03%	0.04%	0.00%	0.00%	0.04%	-0.03%	1	0	1
Unknown (IA < 90)			18.31%	n/a			n/a	470	
Total	100%	100%	100%	100%	18.31%	18.31%	2567	2567	470

BC = British Columbia, CA = California, Col. = Columbia, E = East, L = Lower, N = North, OR = Oregon, R = River, S = South, W = West,

Table 3. Total number of fish that did not assign to a region of origin with ≥ 90% probability and the proportion, by region, expressed as a percentage of the total "unknown" dataset.

	n < 90% probabilities	n ≥ 90% probabilities	Total n	Proportion by region of IA < 90% fish to all < 90 %
Mid Oregon Coast	94	84	178	20.00%
Rogue R.	88	113	201	18.72%
Central Valley	48	1479	1527	10.21%
U Columbia R. su/fa	32	45	77	6.81%
L Columbia R. fa	29	19	48	6.17%
Klamath R.	24	142	166	5.11%
N Oregon Coast	23	25	48	4.89%
S Puget Sound	21	21	42	4.47%
N CA / S OR Coast	17	44	61	3.62%
Hood Canal	14	6	20	2.98%
L Columbia R. sp	11	4	15	2.34%
N Puget Sound	10	1	11	2.13%
California Coast	9	48	57	1.91%
Deschutes R.fa	9	5	14	1.91%
L Fraser R.	8	22	30	1.70%
Snake R. fa	6	2	8	1.28%
Washington Coast	6	1	7	1.28%
SSE Alaska	5	1	6	1.06%
S Thompson R.	5	6	11	1.06%
Mid Columbia R. tule	4	22	26	0.85%
E Vancouver Is.	2	0	2	0.43%
Central BC Coast	1	0	1	0.21%
Mid Frase rR.	1	2	3	0.21%
N Thompson R.	1	0	1	0.21%
Willamette R.	1	2	3	0.21%
W Vancouver Is.	1	1	2	0.21%
U Fraser R.	0	2	2	0 %
Total	470	2097	2567	100%

* hypothetical calculation based on all "incorrect" fish actually assigning correctly

Table 4. Weekly and average over all week genetic estimates of stock mixture proportions (MSA) in weeks with sample sizes over 100 fish IA ≥ 90% harvested during openers in July, August and September in 2006. The date listed was the first day of weekly opener.

Reporting_Unit	July 9	July 16	July 30	Aug 1	Sept 17	Sept 24	Oct 22	Average
Central Valley fa/sp	58.88%	71.26%	66.08%	71.49%	50.46%	65.00%	43.91%	61.01%
Mid Oregon Coast	4.72%	5.39%	4.90%	4.68%	7.79%	12.14%	12.61%	7.46%
Rogue R.	4.49%	2.99%	2.45%	1.70%	15.03%	5.00%	19.13%	7.26%
Klamath R.	3.82%	4.19%	6.99%	4.26%	11.32%	4.29%	10.43%	6.47%
U Columbia R. su/fa	4.94%	2.99%	4.55%	2.98%	0.74%	0.71%	1.30%	2.60%
N CA / S OR Coast	1.12%	0.60%	1.40%	2.13%	5.75%	2.14%	2.61%	2.25%
California Coast	0.67%	1.20%	0.70%	2.13%	5.38%	1.43%	3.91%	2.20%
L Columbia R. fa	4.49%	2.40%	1.75%	2.55%	0.74%	0.71%	0.00%	1.81%
S Puget Sound	4.04%	1.20%	3.15%	1.28%	0.19%	0.71%	0.00%	1.51%
N Oregon Coast	0.22%	0.60%	0.70%	0.00%	0.93%	5.71%	2.17%	1.48%
Mid Columbia R. tule	1.57%	2.40%	1.05%	2.55%	0.00%	0.36%	0.00%	1.13%
L Fraser R.	2.47%	0.00%	2.10%	1.28%	0.19%	0.00%	1.74%	1.11%
Hood Canal	2.92%	1.20%	0.70%	0.43%	0.00%	0.00%	0.43%	0.81%
Deschutes R.fa	0.67%	1.20%	0.35%	1.28%	0.37%	0.36%	0.00%	0.60%
L Columbia R. sp	0.90%	0.60%	0.35%	0.43%	0.19%	0.36%	0.87%	0.53%
S ThompsonR.	1.12%	0.60%	0.70%	0.00%	0.00%	0.36%	0.00%	0.40%
N PugetSound	0.90%	0.60%	0.35%	0.43%	0.19%	0.00%	0.00%	0.35%
Snake R.fa	0.67%	0.60%	0.35%	0.00%	0.00%	0.00%	0.00%	0.23%
Washington Coast	0.22%	0.00%	0.00%	0.00%	0.37%	0.36%	0.43%	0.20%
SSE Alaska	0.22%	0.00%	0.00%	0.43%	0.19%	0.00%	0.43%	0.18%
W Vancouver Is.	0.00%	0.00%	0.70%	0.00%	0.00%	0.00%	0.00%	0.10%
U Fraser R.	0.22%	0.00%	0.00%	0.00%	0.00%	0.36%	0.00%	0.08%
E Vancouver Is.	0.22%	0.00%	0.35%	0.00%	0.00%	0.00%	0.00%	0.08%
Willamette R.	0.22%	0.00%	0.35%	0.00%	0.00%	0.00%	0.00%	0.08%
Mid Fraser R.	0.22%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.03%
N ThompsonR.	0.00%	0.00%	0.00%	0.00%	0.19%	0.00%	0.00%	0.03%
Grand Total	445	167	286	235	539	280	230	

Table 5. Results from coded-wire tag (CWT) blind test: the Oregon State University Marine Fisheries Genetics Laboratory provided population assignments for individual fish to the Oregon Department of Fish and Wildlife prior to CWT data availability. ODFW assembled this comparison of known-origin fish to OSU's genetic assignment. 100% of fish (n = 31) with probabilities ≥90% were assigned correctly.

Barcode	Snout ID	Hatchery of Origin (CWT)	Stock of Origin (CWT)	Regional Genetic Assignment	Regional Probability	Accuracy of Genetic Assignment
6432	06J3385	MATTOLE SAL. GP. HAT	MATTOLE RIVER	California Coast	1	Correct
7748	06J3364	COLEMAN NFH	COLEMAN NFH	Central Valley fa	1	Correct
7730	06J3366	COLEMAN NFH	COLEMAN NFH	Central Valley fa	0.97	Correct
6424	06J3386	COLEMAN NFH	COLEMAN NFH	Central Valley fa	1	Correct
7465	06J3395	COLEMAN NFH	COLEMAN NFH	Central Valley fa	1	Correct
7704	06J3368	FEATHER R HATCHERY	FEATHER RIVER	Central Valley fa	0.93	Correct
8170	06J3379	FEATHER R HATCHERY	FEATHER RIVER	Central Valley fa	1	Correct
7469	06J3394	FEATHER R HATCHERY	FEATHER RIVER	Central Valley fa	0.97	Correct
910	06J6503	FEATHER R HATCHERY	FEATHER RIVER	Central Valley fa	0.99	Correct
7706	06J3367	FEATHER R HATCHERY	FEATHER RIVER	Central Valley fa/sp	1	Correct
4924	06J3378	FEATHER R HATCHERY	FEATHER RIVER	Central Valley sp	1	Correct
7487	06J3397	FEATHER R HATCHERY	FEATHER RIVER	Central Valley sp	0.97	Correct
9018	06J3381	COLEMAN NFH	COLEMAN NFH	Central Valley sp/fa	1	Correct
3341	06J3399	FEATHER R HATCHERY	FEATHER RIVER	Central Valley sp/fa	1	Correct
913	06J6513	FEATHER R HATCHERY	FEATHER RIVER	Central Valley sp/fa	1	Correct
4859	06J6510	COLEMAN NFH	COLEMAN NFH	Central Valley fa	1	Correct
3781	06J2497	FEATHER R HATCHERY	FEATHER RIVER	Central Valley fa/sp	1	Correct
6175	06J5416	FEATHER R HATCHERY	FEATHER RIVER	Central Valley fa/sp	1	Correct
6409	06J3387	IRON GATE HATCHERY	KLAMATH RIVER	Klamath River	1	Correct
9991805	06J3353	TRINITY R HATCHERY	TRINITY RIVER	Klamath River	1	Correct
6426	06J3357	TRINITY R HATCHERY	TRINITY RIVER	Klamath River	1	Correct
7471	06J3392	TRINITY R HATCHERY	TRINITY RIVER	Klamath River	0.99	Correct
7488	06J3396	TRINITY R HATCHERY	TRINITY RIVER	Klamath River	1	Correct
3331	06J6400	COWLITZ SALMON HATCH	COWLITZ R 26.0002	L Columbia fa	0.92	Correct
6357	06J3358	H-CHEHALIS R	S-HARRISON R	L Fraser R.	0.99	Correct
7454	06J3393	ELK R HATCHERY	ELK R (ELK R HT)	Mid Oregon Coast	0.99	Correct
904	06J6405	ELK R HATCHERY	ELK R (ELK R HT)	Mid Oregon Coast	1	Correct
921	06J6514	SIUSLAW NATURAL PRODUCTION TAG	SIUSLAW R	N Oregon Coast	0.96	Correct
848	06J2804	ELK R HATCHERY	CHETCO R	N California/ S Oregon Coast	0.99	Correct
4403	06J2816	COLE RIVERS HATCHERY	COLE RIVERS HATCHERY	Rogue	1	Correct
6246	06J6403	COLE RIVERS HATCHERY	COLE RIVERS HATCHERY	Rogue	0.99	Correct
9447	06J3391	N/A	N/A	Rogue	0.98	TAG READ WRONG
919	06J6512	FEATHER R HATCHERY	FEATHER RIVER	Central Valley fa	0.64	n/a (<90%); correct
7746	06J3365	ROCK CREEK	UMPQUA R (ROCK CR HT)	Mid Oregon Coast	0.8	n/a (<90%); correct
9015	06J3382	COLE RIVERS HATCHERY	COLE RIVERS HATCHERY	Rogue	0.63	n/a (<90%); correct

(Table 5, Cont.)

Barcode	SNOUT ID	HATCHERY of Origin (CWT)	STOCK OF ORIGIN (CWT)	REGIONAL GENETIC ASSIGNMENT	REGIONAL PROB-ABILITY	ACCURACY OF GENETIC ASSIGNM.
1516	06J3321	GROVERS CR HATCHERY	GROVERS CR 15.0299	Mid Oregon Coast	0.66	n/a (<90%); incorrect
3747	06J3359	COLE RIVERS HATCHERY	COOS R - PUBLIC	Mid Columbia tule	0.62	n/a (<90%); incorrect
3731	06J3363	TRINITY R HATCHERY	TRINITY RIVER	Rogue	0.48	n/a (<90%); incorrect
1031	06J2495	KALAMA FALLS HATCHRY	KALAMA R 27.0002	Mid Oregon Coast	0.42	n/a (<90%); incorrect
6232	06J6402	ELK RIVER	ELK R (ELK R HT)	N California/S Oregon Coast	0.81	n/a (<90%); incorrect
6152	06J5415	BANDON HATCHERY	COOS R - PUBLIC			amplification failure
1823	06J2806	CARLTON REARING POND	METHOW & OKANOGAN			amplification failure
3185	06J6401	COLEMAN NFH	COLEMAN NFH			amplification failure
7772	06J6500	COLEMAN NFH	COLEMAN NFH			amplification failure
9720	06J6508	COWLITZ SALMON HATCH	COWLITZ R 26.0002			amplification failure
1024	06J2496	ELK R HATCHERY	ELK R (ELK R HT)			amplification failure
1016	06J3348	FEATHER R HATCHERY	FEATHER RIVER			amplification failure
7703	06J3369	FEATHER R HATCHERY	FEATHER RIVER			amplification failure
9009	06J3380	FEATHER R HATCHERY	FEATHER RIVER			amplification failure
9717	06J6509	FEATHER R HATCHERY	FEATHER RIVER			amplification failure
9374	06J2814	TRINITY R HATCHERY	TRINITY RIVER			amplification failure
3197	06J3398	NO TAG				
4360	06J3388	NO TAG				
4373	06J3389	NO TAG				
9446	06j3390	NO TAG				
846	06J2805	NO TAG				

Figure 1. Reporting regions (numbers) and populations (letters) for GAPS baseline v1 (population latitudes/longitudes not available for v2) used for Project CROOS genetic stock identification (Key to numbers and populations listed in Appendix 1).

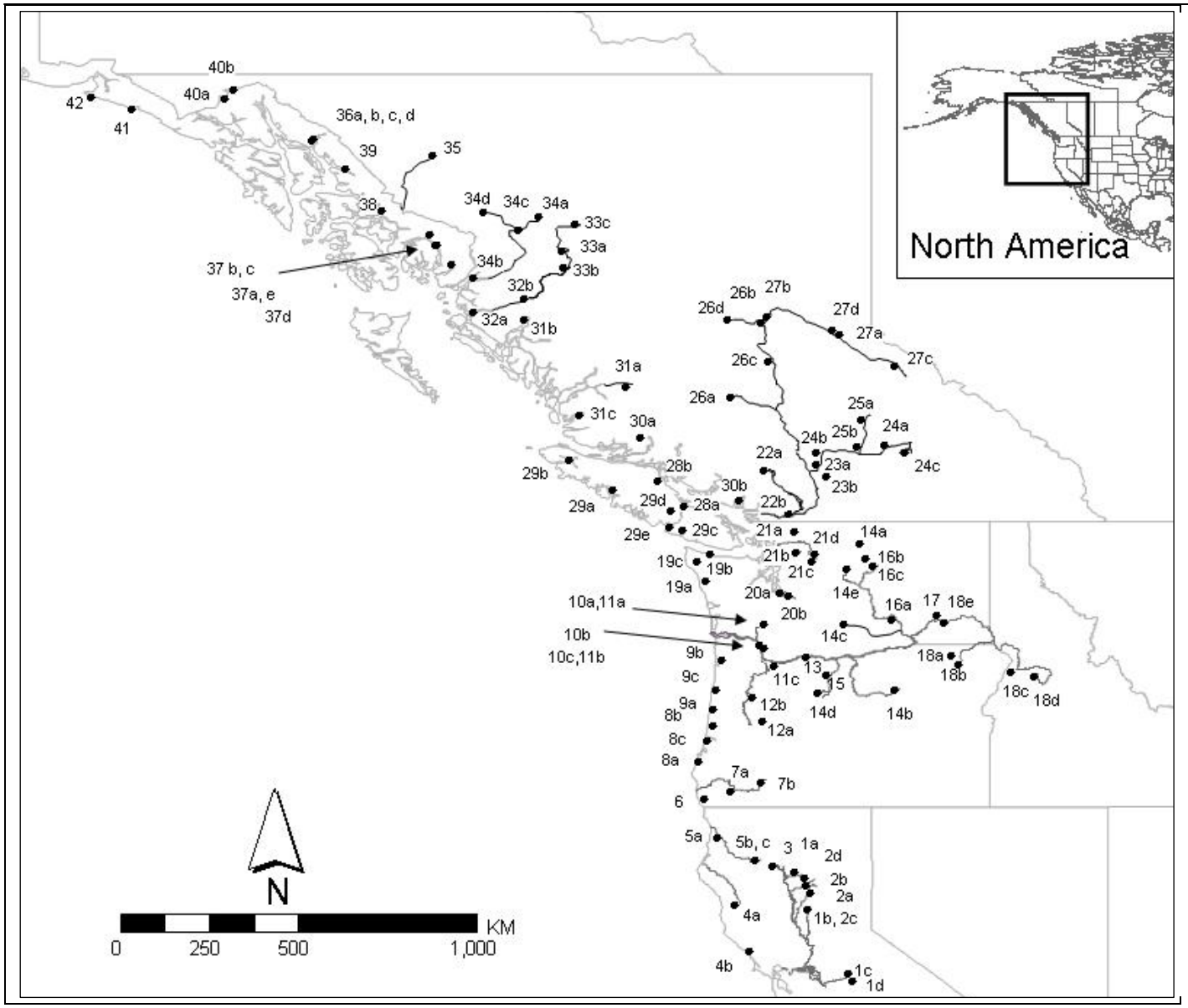

Figure 2. Fishing days open on weekly basis ("opener") and harvest limits for commercial fisheries conducted from Cape Falcon south to Humbug Mountain during 2006.

JUNE

S	M	T	W	T	F	S	
				1	2	3	
4	5	6	7	8	9	10	Weekly harvest limit 75 fish
11	12	13	14	15	16	17	
18	19	20	21	22	23	24	
25	26	27	28	29	30		

JULY

S	M	T	W	T	F	S	
						1	
2	3	4	5	6	7	8	
9	10	11	12	13	14	15	Weekly harvest limit 75 fish
16	17	18	19	20	21	22	
23	24	25	26	27	28	29	
30	31						

AUGUST

S	M	T	W	T	F	S	
		1	2	3	4	5	Weekly harvest limit 75 fish
6	7	8	9	10	11	12	
13	14	15	16	17	18	19	
20	21	22	23	24	25	26	
27	28	29	30	31			

SEPTEMBER

S	M	T	W	T	F	S	
					1	2	
3	4	5	6	7	8	9	
10	11	12	13	14	15	16	
17	18	19	20	21	22	23	Weekly harvest limit 50 fish
24	25	26	27	28	29	30	

OCTOBER

S	M	T	W	T	F	S	
1	2	3	4	5	6	7	
8	9	10	11	12	13	14	
15	16	17	18	19	20	21	
22	23	24	25	26	27	28	Weekly harvest limit 50 fish
29	30	31					

Figure 3. Samples received, genotyped, analyzed for stock mixture analysis, and number with individual assignment probability ≥ 90% during 2006.

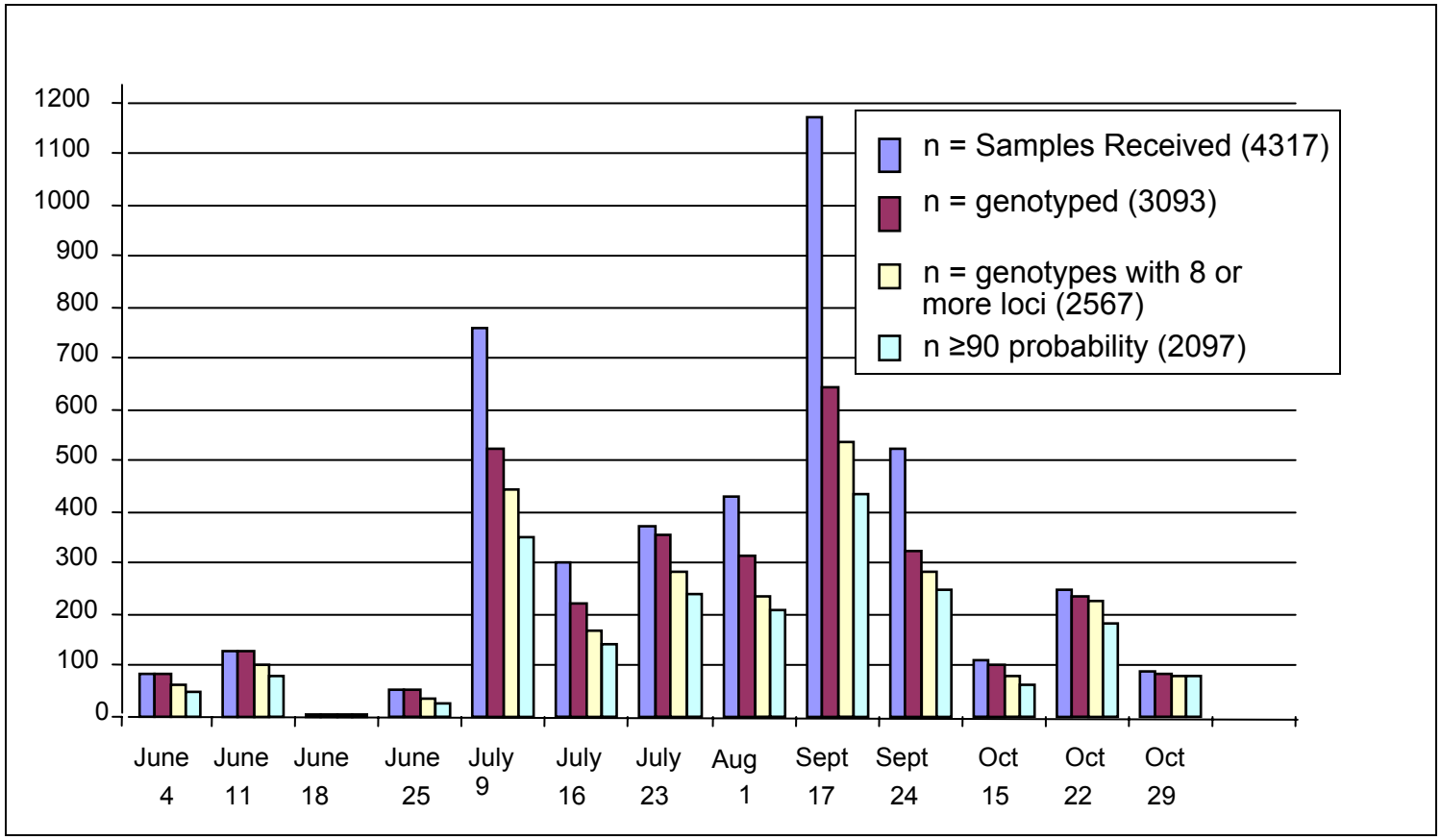

Figure 4. Genetic estimates of stock mixture proportions of Chinook salmon (n = 2567) harvested off the coast of Oregon during the 2006 Project CROOS pilot study. Mixture proportions were estimated using the GAPS (Genetic Analysis of Pacific Salmonids) standardized microsatellite baseline v2 with 166 populations combined into 44 reporting regions and program GMA (Kalinowski 2003).

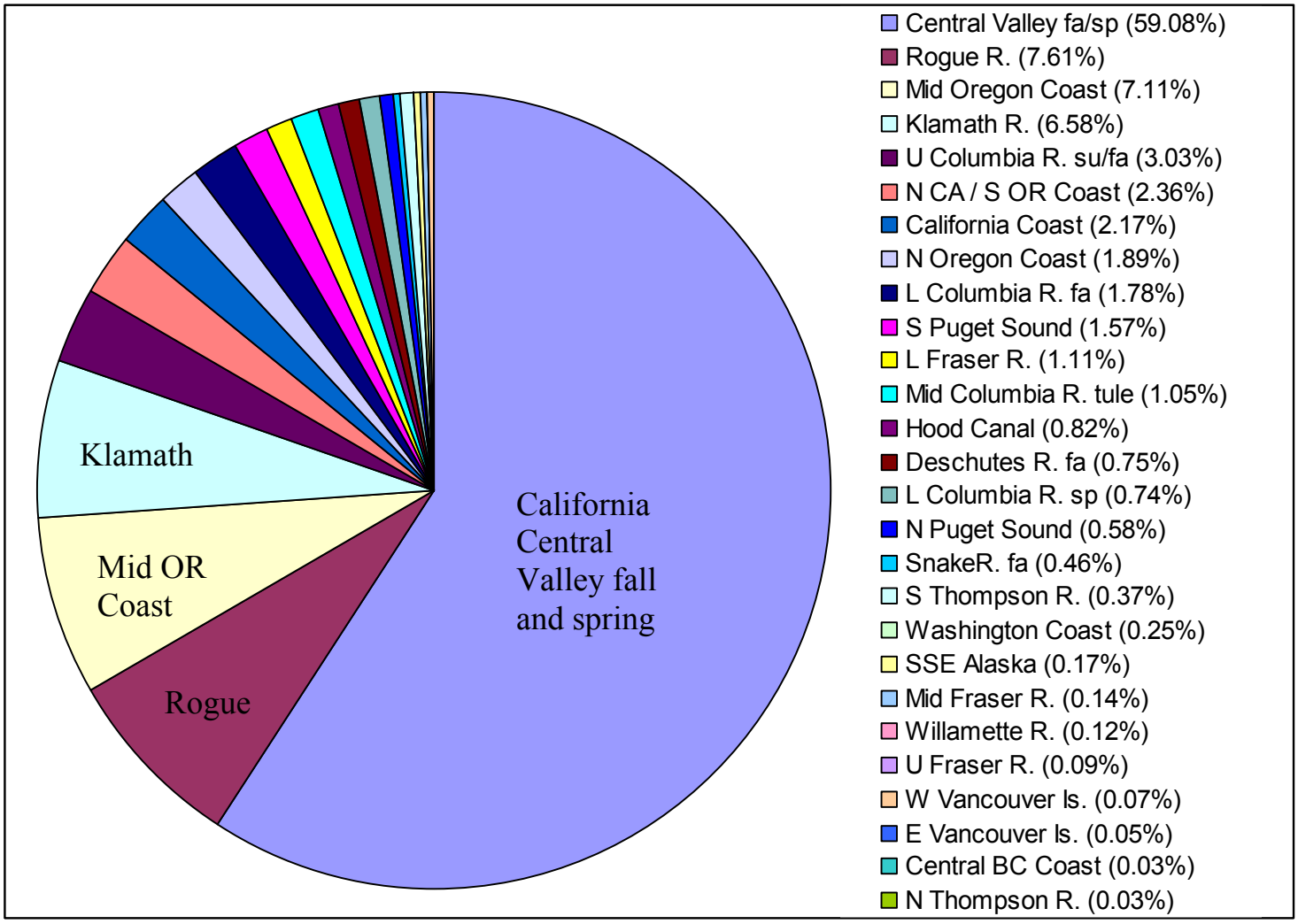

Figure 5. Comparison of four different treatments of genetic data to estimate stock mixture proportions (excluding California Central Valley stocks). MSA and IA (all fish) include all fish in the dataset; stringent dataset groups fish with low probabilities (<90%) into "uncertain" category, and the conservative dataset only includes fish with IA ≥ 90% to calculate stock mixture proportions (see text for details).

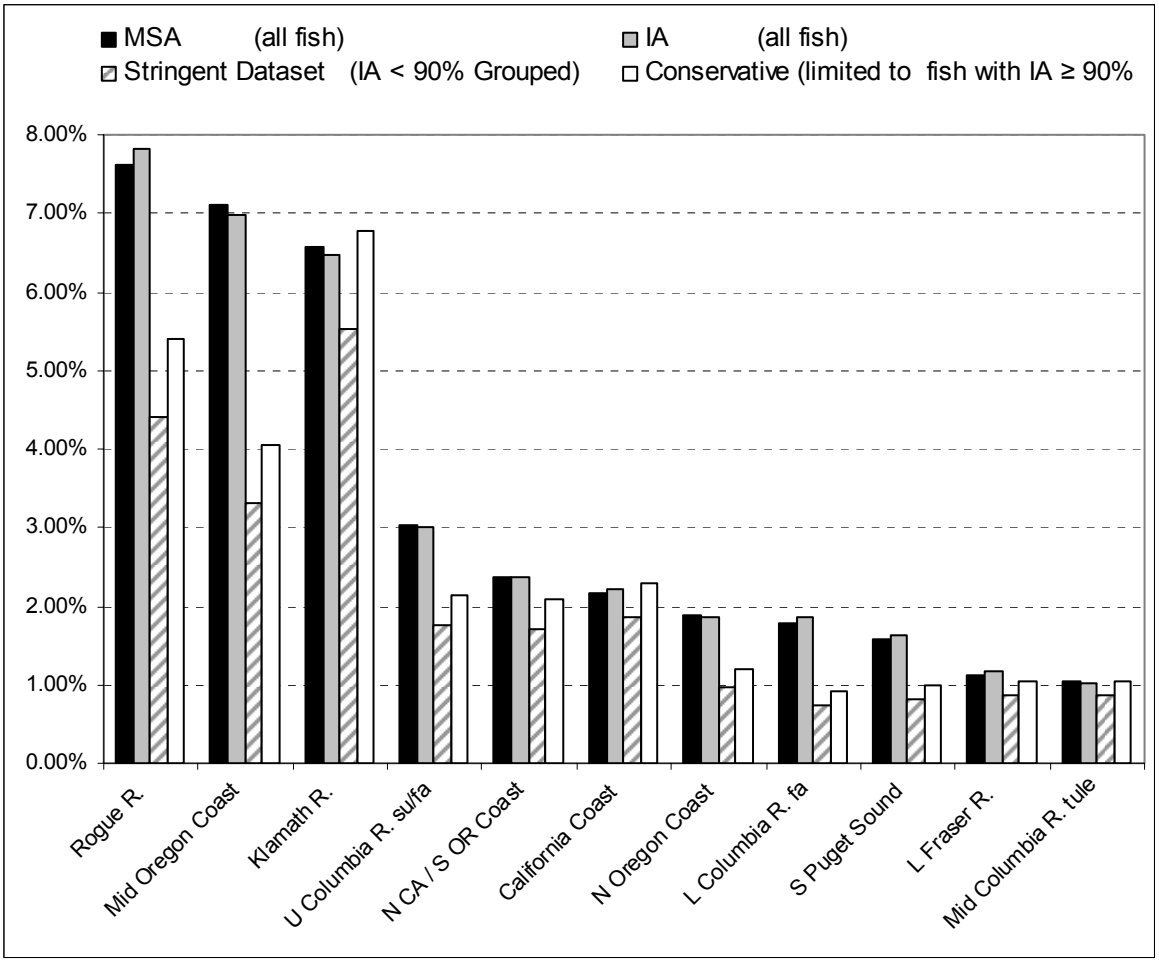

Figure 6. Genetic estimates of stock mixture proportions for Klamath River, California Coast, and Northern California/Southern Oregon stocks of Chinook salmon (weeks with n > 100 fish, MSA) during the 2006 CROOS pilot study. The date listed is the first day of the weekly opener.

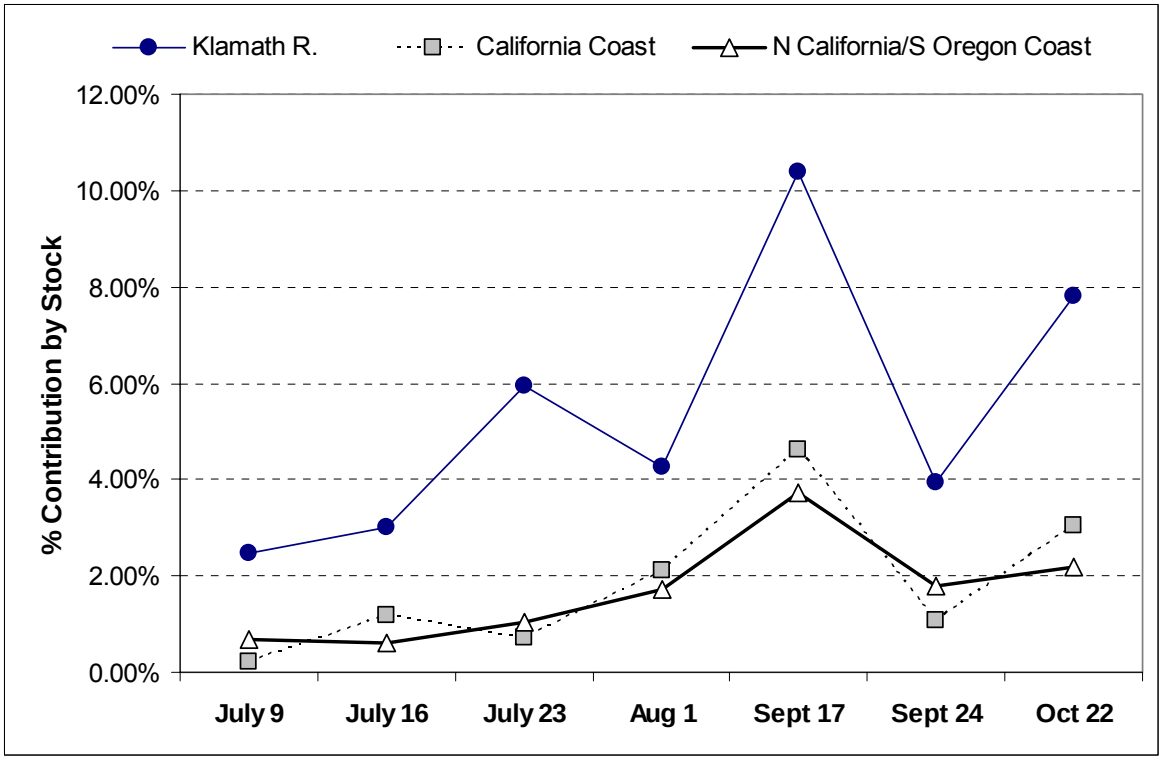

Figure 7. Contribution of Klamath fish as a percent of the total dataset for weeks with n > 100 and IA ≥90% probabilities during the 2006 CROOS pilot study. Three methods of stock mixture proportions are shown below for comparative purposes of accuracy and bias. The first (all) dataset is calculated using traditional MSA techniques; the second (stringent) dataset is calculated using individual assignment with fish having assignments < 90% pooled, and the third dataset (conservative) is calculated only using individual assignments for fish with ≥ 90% probabilities. The date listed is the first day of the weekly opener.

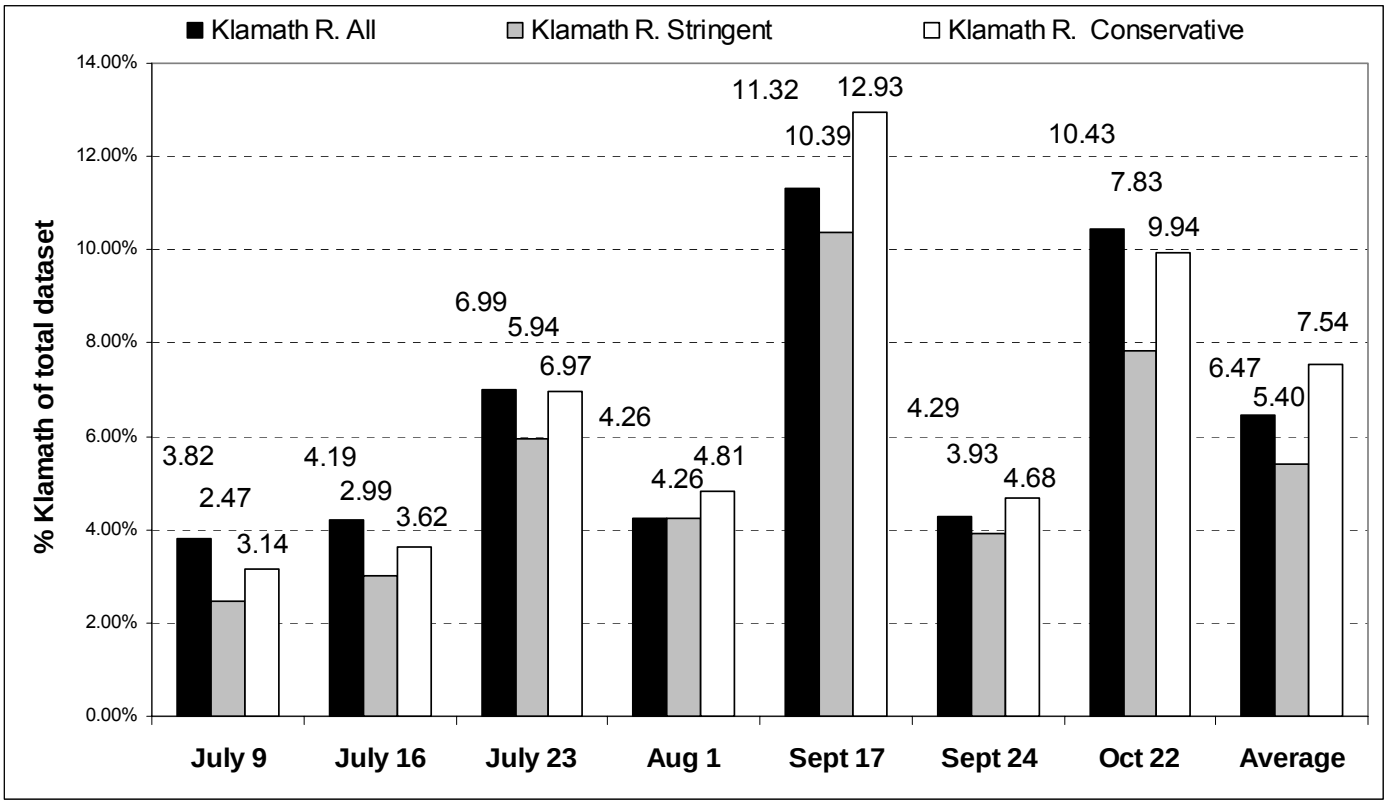

Figure 8. Time series for fish harvested off the Coast of Oregon during two weeks in 2006. The week of September 17 - 23 (A) yielded 1173 fish sampled with 539 usable genotypes. The following week (B), September 24-30, provided 521 fish samples, of which 280 provided sufficient genotypic data. Fish that assigned to the Klamath basin are highlighted in red.

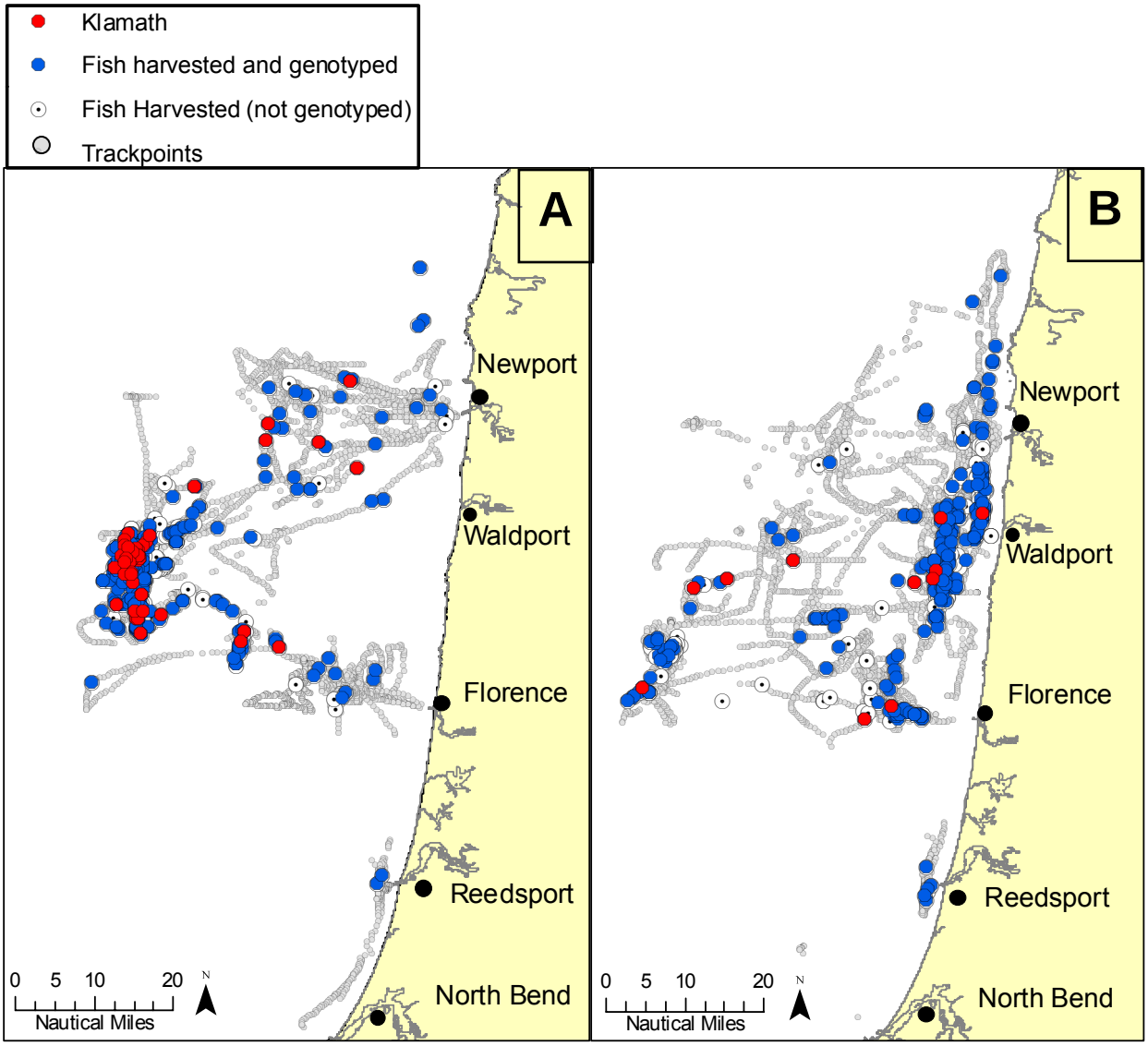

Figure 9. Genetic stock mixture analysis and fish harvested per unit effort in two sections of ocean fished during weeks of September 17-23 and 24-30th (see text for details). Fish assigning to the Klamath basin are highlighted in red.

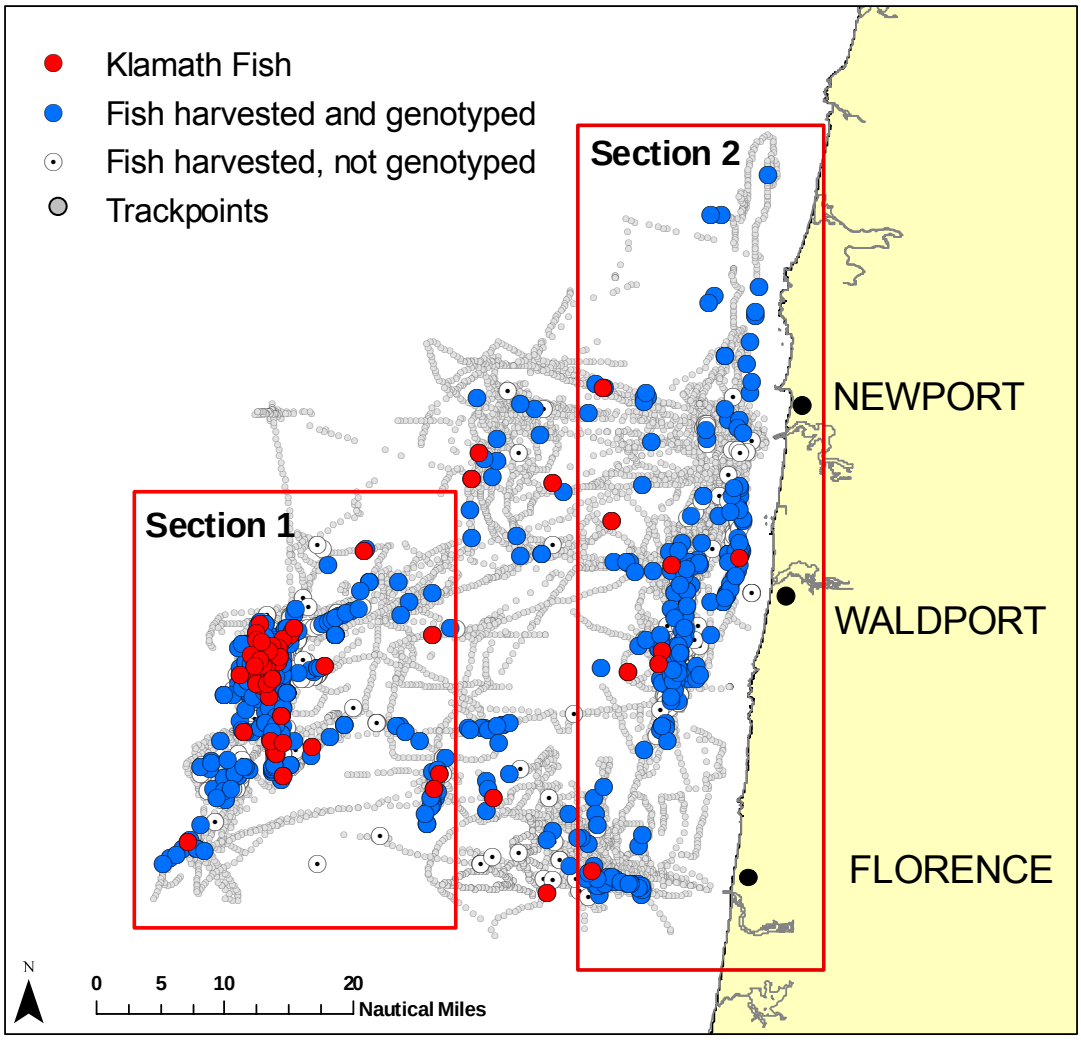

Figure 10. Genetic estimate of stock composition for regions that contributed at least 5% to the total mixture in Sections 1 and 2 (n = 469, 19 respectively) during September 17 – 23 and September 24-30th , 2006 (Section 1, 2; n = 35, 192, respectively) detailed in Figure 9.