WASSIP Advisory Panel Meeting
September 24, 2008
Hawthorne Suites
Anchorage, AK

Chair: Eric Volk

Call to order: 8:10 AM

Attendees:

Bruce Weir	Univ. of Wash.
Robin Waples	NMFS
Beth Stewart	AEB
Chuck McCallum	Lake and Pen. Bor.
Steve Honnold	ADF&G
Bob Dubey	YRDFA
Tim Baker	ADF&G
Sara Gilk	ADF&G
Eric Volk	ADF&G
Bill Templin	ADF&G
Chris Habicht	ADF&G
Jennifer Hooper	AVCP
Michael Smith	TCC
Art Nelson	BSFA
Steve Brown	CAMF
Pat Martin	CAMF
Loretta Bullard	Kawerak
Michael Sloan	Kawerak
Michael Link	BBSRI (LGL)
Jim Jasper	ADF&G
Dani Evensen	ADF&G

Preamble: These minutes follow the meeting agenda and are designed to summarize the main points from the meeting. For clarity, minutes are grouped by topic. This organization sometimes resulted in the summary of various communications that occurred during different times within the meeting into one section. Sentences or sentence fragments in **BOLD** within the minutes are action items or motions.

Meeting Agenda

1. Welcome and Introductions
2. Review and approval of agenda
3. Review of May, 2008 Technical Committee briefing by ADF&G
4. Technical Committee presentation of comments on ADF&G proposal to AYK SSI
5. ADF&G will present alternative timelines
6. Review and approval of minutes from April 2008 meeting – during the meeting, this item was merged with item #8

7. Technical issues – during the meeting, this item was merged with item #9
8. Issues over April 13, 2007 meeting minutes
9. Adequacy of the 2006 samples
10. Process formality
11. Budget issues
12. Frequency of meetings and next meeting
13. Adjourn

1. Welcome and Introductions

The Chair (Eric Volk) welcomed participants, especially members of the Technical Committee (TC) who were able to attend (Dr. Bruce Weir and Dr. Robin Waples). Several handouts were made available, including the agenda, minutes from the March 2008 meeting, the WASSIP semi-annual progress report submitted to NOAA/NMFS, WASSIP TC comments, and the WASSIP MOU revised March 2008. One handout contained an updated sample table provided by Kodiak staff concerning samples collected in the Westward Region.

2. Review and Approval of Agenda

Several items were added to the agenda per participants' suggestions, including items # 8 – 11 as outlined above in the updated agenda. The agenda was approved with these additions.

The Chair then reviewed specific items on the Agenda, and what to expect for each item. The Chair stressed the need for Advisory Panel (AP) members to know roughly what was presented to the TC at the May 2008 meeting, and the need for the AP to be heard regarding the comments made in the TC's document. He initiated a discussion on whether a court reporter might be useful for taking detailed meeting notes in the future. There was no support from the AP to have a court reporter. The Chair also expressed the desire to retain some continuity despite recent ADF&G staffing changes, and his intention to remain as chair.

3. Review of May 2008 Technical Committee briefing by ADF&G

Bill Templin gave a summary PowerPoint presentation reviewing what was presented to the TC in May 2008. The presentation gave reviews of methods, markers, and baseline status.

A discussion followed regarding the choice of marker types. Mike Smith expressed concern that the AP as a group had not yet decided to use SNPs, and that microsatellites should still be considered given their use and available baselines. He asked if there was any discussion at the Seattle meeting regarding marker choices. The Chair replied that this was one of the specific topics to be covered by the TC.

4. Technical Committee presentation of comments on ADF&G proposal to AYK SSI

Dr. Waples gave an overview of the main points of the TC comments on the ADF&G proposal to AYK SSI and associated technical issues. The TC felt that the proposal had a number of strengths, including experienced PIs, state of the art techniques, clear and attainable goals, an

ambitious sampling program, rigorous data protocols, and expectation of finding other useful information. However, some issues bear closer scrutiny. Although the TC felt that these were not deal breakers, they did feel that they could affect the likelihood that the project could accomplish the stated goals.

The following were items the TC felt could bear closer scrutiny:

1) The stated performance measures, e.g. estimates will be within 5% of the value 95% of the time were not clear.
2) The basic ability to discriminate between closely related chum salmon populations may not be possible.
3) Although the main goal is mixed stock analysis, other more general goals that aim to better understand the biology and population structure of salmon, identify conservation units, quantify gene flow between populations, etc. could be served, but that the methods to describe population structure was not outlined in enough detail in the proposal.
4) Although there is consensus in the scientific community that SNPs are the marker of the future, and will probably become more and more important while microsatellites will become less important, the decision on marker choice is also an economic, political, and management issue. If the goals change to include population structure, then ascertainment bias would be an issue.
5) The TC also expressed concerns regarding the temporal stability of baseline populations.

Dr. Weir then showed a few PowerPoint slides related to SNPs in human genetics. The main conclusion was that SNPs are here to stay because they are becoming so cheap and they are numerous. Much of the presentation dealt with problems in correctly scoring SNPs, but many of these issues are not issues with few loci (less than 100 SNPs) because you have time to look at genotype calls. You can do well in addressing goals with an appropriate number of SNPs.

Break

Marker choice

During various times at this meeting, the issue of marker choice was discussed.

Pat Martin voiced his concerns that the Department failed to consider some stakeholder's perspectives in discussions about choice of marker type and did not first ask in detail what the objectives of the stakeholders were. The way the Department handled the marker choice issue has rubbed some stakeholders the wrong way. He felt it would be helpful if the Department recognized that the decision of marker type had not been made by all stakeholders. Today we have the opportunity with Dr. Weir and Dr. Waples to make that decision.

Dr. Waples clarified that all the major issues that have been raised by the TC for the project apply regardless of marker type since the issues have more to do with sampling and biology than genetics. The TC didn't try to look at this issue in detail, and they do not have strong opinions on

the short term benefits of different markers although they said that there is a general transition to SNPs in the research community. They discussed the advantages of each marker type at this point in time.

Mike Smith voiced concerns over timeliness if the decision is to increase the number of SNPs and suggested that microsatellites could provide faster turnaround, although he said he understood that the current set of microsatellites and SNPs had comparable power.

There was some discussion on which marker type would more likely result in higher resolving power for populations that are genetically similar. Beth Stewart and Dr. Waples discussed the potential for SNPs to screen loci under selection while Dr. Weir pointed out that microsatellites have higher mutation rates. Despite these higher mutation rates, Dr. Weir said that SNPs provide more inherent variation (10-fold increase in signal among populations).

In the afternoon, a resolution was passed unanimously by the AP approving the use of SNPs for this project (see section 6).

PowerPoint presentation on statistical methods to increase power through pooling strata

Chris Habicht made a PowerPoint presentation outlining potential methods to pool across strata to increase statistical power to strive for the criteria desired by the AP (1% accuracy, 99% of the time). The best approach is a hierarchical Bayesian which allows non-symmetric confidence intervals around estimates and where strata inform each other.

Dr. Waples pointed out that when samples are pooled across strata, inferences can no longer be made about each stratum. Only inferences about fish captured in the fisheries is possible and you should not extrapolate to all fish in an area. The AP needs to decide if these are reasonable trade-offs. Care needs to be taken to ensure that the sampling is random.

Dr. Waples and Jim Jasper both discussed the potential for the information in the mixtures to strengthen the baseline. There were some concerns that adding information from multiple years of mixtures into the baseline may be dangerous.

Dr. Weir initiated a discussion about bootstapping of both the mixtures and the baseline. Dr. Weir thought the Bayesian methods are fine but it may be complicated to determine the assumptions.

Pat Martin urged staff to look for novel ways to present data so that they are understandable to the lay audience and the BOF, including 3-D surface plots.

LUNCH: 11:50 – 1:00pm
Continuation of item #4; Adequacy of temporal sampling – baseline.

We started off with a discussion about the adequacy of the temporal sampling for the baseline. The TC noted that some baseline collections for a population were collected 15 to 20 years apart for chum. They were concerned that the magnitude of the genetic variation within populations

across these lengths of time periods may be large in comparison to the amount of genetic variation among populations.

Bill Templin outlined what the laboratory was doing to address this problem and pointed out that re-sampling all the populations would be very expensive - sites are remote. Dr. Waples said that it is not essential to have multiple samples for every collection; instead there is a need to do **quantitative analysis from the current samples to look at the relative variation among years and between populations**. He proposed that **ADF&G could also test to see if temporal variation within the baseline is a problem by using baseline from different years to see if known mixtures allocate the same way.**

Adequacy of sample size – baseline.

Next, a discussion about the adequacy of the baseline population sample size issues ensued. Pat Martin pointed out that in the AYKSSI proposal, the population size for some collections was low (Franks Lagoon was 18 fish) and that the results in a CTC progress report by Kalinowski et al. on high resolution GSI said that the baseline sampling error was usually the largest error. Bill Templin (a co-author of the report) said that the result was unexpected and that they are still trying to figure it out. Dr. Waples and Bill Templin provided a list of variables which could contribute to the proportion of the error due to the baseline including the baseline sample sizes and how well differentiated the stocks are.

Pat Martin asked why there were generally fewer individuals in the SNP baseline than there were in the allozyme baseline. Bill Templin said that it was a tradeoff between geographic distribution and maximizing number of fish for each collection. For the same cost, you could not have both. In addition, the reason that most populations have 95 fish instead of 100 is due to efficiency available, because the analysis is based on a plate size of 96 wells.

Next the discussion moved to the effect of this decreased sample size. In the TC report, there were concerns raised regarding the effect of sample size on the tree diagram based on Cavali-Sforza and Edward (CSE) distances. Both Drs. Waples and Weir agreed that this is only an issue for tree diagram using CSE (not for mixture analyses) and suggested using Weir and Cockerham distances for drawing trees.

Finally, a discussion ensued regarding the distribution of baseline relative to the distribution of spawning aggregates. Pat Martin noted that there was an order of magnitude difference in the distribution of these for chum salmon. Bill Templin agreed that this could affect the mixed-stock analyses and said that ADF&G is rectifying the problem by adding baseline and testing the issue using 100% simulations.

Testing of the baseline

A discussion ensued about the appropriateness of the 100% simulation methods used to test the baseline as a result of comments made by the TC. Dr. Waples said that there was nothing really wrong, but that mixtures do not look like that generally, so better to do simulations with other

mixtures – more realistic ones. These 100% simulations represent a worst-case scenario and are important to make sure that the model is doing what is needed.

Ascertainment issues

In the morning, Dr. Weir asked for clarification on how the chum salmon SNPs were developed. Bill Templin described the process. Ascertainment was heavily weighted towards western Alaska stocks. One question posed by Loretta Bullard related to the use of a single chum salmon for sequence data and one by Pat Martin regarding where all the ascertainment samples came from for the various marker development periods. Bill Templin explained that the Department has contributed more populations to the ascertainment samples and that many more fish are being used during the new stages of marker discovery. Pat Martin asked for and **Bill Templin offered to provide a list of the ascertainment samples being used during the next phase in marker development.**

In the afternoon, Michael Link asked for clarification on how sockeye salmon SNPs were developed and Chris Habicht described the process. Ascertainment samples were spread out throughout the species range.

Dr. Waples did not see any reason to expect that ascertainment bias would bias stock composition. The ascertainment concerns that the TC had were related to understanding population structure and evolutionary questions.

Attaining estimates with adequate precision

In the morning, several AP members (Loretta Bullard, Pat Martin, Beth Stewart) wanted to hear from the TC what they thought of the ability of the baseline and of the mixture sample sizes to meet the "99%, 1%" level of accuracy and precision that the AP would like the Department to strive to achieve. Dr. Waples outlined data gaps that prevented the TC from determining whether the target was attainable. Among these were: 1) the underlying biological differentiation among populations; 2) how this structure matches up with management issues; 3) statistical issues related to the number of fish it will take in a sample to get estimates in the range that you want; and 4) defined performance measures, specifically 5% and 1%.

Bill Templin clarified that the performance measures are related to fixed percentages – i.e. +/- 5%, means that if the estimate is 10% then the CI would be from 5% to 15%. Dr. Waples and Bill Templin both pointed out that even with perfect identifiably, sample size will limit the accuracy and precision of the estimate due to sampling error.

Much of the afternoon was spent on issues related to the ability of the baseline and of the mixture sample sizes to meet the "99%, 1%" level of accuracy and precision that the AP would like the Department to strive to achieve. Most of this discussion was focused on methods to reach this goal. These discussions can be broken down into four parts: 1) Analyzing samples collected, but not slated for analysis, to increase the number of fish within some strata; 2) Pooling populations into larger reporting groups; 3) Pooling strata to increase the sample size; and 4) Developing markers to increase genetic identifiably.

Need for precision

There was a short discussion about how relaxing the precision requirements would make the goal more attainable. Pat Martin asked if it would help to bring the criteria down to 90%, 1%. Bill Templin and the TC agreed that relaxing the precision requirement would reduce the number of samples required in the mixture and reduce the amount of among-reporting group genetic differentiation needed.

Ways to attain precision

Analyzing tissues that were collected, but were not slated for analysis.

Pat Martin pointed out that we have received more samples than are slated for analysis and that the AP still has the opportunity to design the project to fit its needs rather than follow the default. In order to move forward, demonstrations of accuracy will need to be done before we can design the project. Three-quarters of the strata have been significantly over-sampled. He posed the question: How do we decide what samples to use? The discussion continued with how these extra samples would be paid for. Pat Martin suggested that two years with full data might be worth more than three with less data, but other AP members did not agree. Bill Templin suggested that trade-offs will need to be made unless additional funding is secured and that the AP should be involved in these trade-off decisions.

Pooling populations into larger reporting groups

At different times during the meeting, Dr. Waples and Chris Habicht brought up the idea of reducing the reporting groups by assigning more populations to each reporting group. This method would increase the proportions of fish from mixtures that are assigned to each reporting group which would allow for increased detectability. Reporting groups can also be adjusted for each fishery depending on the expected stocks present. The idea is to minimize the number of reporting groups with very low expected proportions. AP input will be important to determining what reporting groups are important for which fisheries.

Pooling strata to increase the sample size

During the PowerPoint presentation in the morning, Chris Habicht explored options for pooling strata to increase statistical power. These include pooling strata to maximize the number of fish contributing to the pooled estimate while keeping the estimate proportional to the commercial catch that the samples represent, taking advantage of other strata, and not assuming symmetrical distribution of the error. **The next step is to look at the baseline and see how these types of methods will affect the accuracy and precision of the estimates.**

Dr. Waples agreed that pooling would decrease confidence intervals but pointed out that there are many ways to pool these data, that these methods have trade-offs regarding the questions that can be answered, and that stakeholders need to be involved so that they can have input into what strata are pooled. Dr. Weir and Tim Baker suggested that testing among strata could be done to determine which strata could be pooled. Michael Link said that there will be times when

individual strata will be important for stock composition estimates. Pat Martin also thought that analyses on unpooled strata and pooled strata could be used side-by-side to answer different questions.

Pat Martin was surprised that we had only allocated six months to finish getting the methods figured out. These are issues that people have been working on for a long time and are not resolved.

A discussion followed about how this information would be incorporated into decision making. Two points emerged: 1) The AP would be involved in making these decisions; 2) The DNA extraction phase could begin because we could always go back and extract a few additional samples if the study design changed due to AP decisions.

Developing markers to increase genetic identifiably

In the morning there was some discussion on the number of SNPs developed for chum salmon. Bill Templin explained that the technology at that time and experience with Chinook salmon largely determined the number of SNPs proposed (48). Pat Martin pointed out the life history and evolutionary history of chum and sockeye salmon in western Alaska are different from Chinook salmon and that 45 – 48 SNPs was not adequate for either species.

The project includes development of new markers for chum salmon and the budget and timeline includes this component. Bill Templin pointed out that the entire transcriptome for chum salmon is now available and that the UW was looking for SNPs. The discussion regarding the development of new markers for sockeye salmon to increase the genetic identifiability of reporting groups centered over three aspects: 1) need for new markers, 2) time and cost required to complete this task, and 3) how new markers would be developed.

Dr. Weir asked how it would be decided if it is worth increasing the number of markers. There was general agreement that the AP would be involved in this decision and that they would need some information about the ability of the current baseline to provide estimates of stock composition given that there will be methods to pool strata and consolidate reporting groups. Bill Templin pointed out that if it was decided that more markers are needed, adding another 48 would be the most efficient due to the logistics of the current methods. Pat Martin said that he did not see any way around going after 96 markers, but other members of the AP did not feel that they had enough information to make that decision.

There was discussion about the how adding this component would change the timeline for the project. Bill Templin presented an alternative time line that showed the final report being pushed back by a year. Pat Martin said that he thought he could get the Board of Fish (BOF) to push back their meeting that would utilize the information obtained through this project, but there was not consensus among the AP members that the BOF scheduling was easy to influence.

The Chair pointed out that given that the project does not contain funding for additional marker discovery and screening in sockeye salmon for either the baseline or the mixture samples, funding is an issue. Bill Templin provided an estimate of $300,000 to $500,000 for screening

the baseline for an additional 48 SNPs and pointed out that this would be a cut-rate price. A discussion ensued about ways to accommodate this cost within the current project by reducing the number of strata analyzed.

5. Presentation of ADF&G outline plan to commence analyses

Bill Templin provided a short presentation with two timelines – one without and one with addition sockeye salmon markers. Hard copies of the timelines were passed out to members of the AP and TC. Discussion on the different portions of the timelines ensued.

BREAK 3:00-3:10pm

Continuation of the TC comments:

A discussion ensued on continued participation of the TC in this process. There was a general desire expressed by several AP members for continued participation by the TC and a general agreement that the TC had been helpful. Both Dr. Weir and Dr. Waples said they were interested in staying involved as long as their time is well used; it would not make sense to come to all AP meetings. Dr. Waples and Pat Martin felt that Dr. Quinn has a lot to offer with his complementary background.

The Chair suggested that we could provide documents for comments from the TC. Pat Martin thought that the TC and other AP members might benefit by looking through the Cook Inlet report. **Bill Templin said that this report went through peer review process and that he would be willing to make the reviews available to the AP**.

Beth Stewart thought that the TC would be useful in discussion of harvest rate calculations. The Chair suggested that Drs. Terry Quinn and Milo Adkinson might be better equipped for these questions and that whomever is involved should come in at the beginning of the process rather than at the end. **The Chair said he would contact Terry and see if he wants to be on the TC.**

Advisory passed the following motion to allow the Department to begin analyses:

After the departure of Drs. Weir and Waples, The Chair asked if there was a motion to, in general terms, approve the timeline as it is laid out, proceed with the baseline analyses and extractions of sockeye and chum, and develop SNP markers.

Beth Steward made the motion and a discussion ensued to finalize a motion. The motion that was voted on contained the following language: "**I move that we approve the proposed relative timeline as outlined in the three-colored chart (option 1), that we specifically recommend that the department proceed with the sampling methodology and evaluation of the new GSI methods and baseline analyses and proceed with the DNA extractions for sockeye and chum samples and that the markers to be used in these analyses will be SNPs."**

After a discussion that resulted in some modifications of the first draft of the motion and general agreement on this final version, the Chair called for the question and asked if anyone opposed to the motion. Hearing none the motion was adopted unanimously.

6. ***Review and approval of minutes from April, 2008 meeting and # 8. Issues over the 2007 meeting minutes***

The process to approve the minutes for the 2008 meeting led to a lengthy discussion regarding the Page 2 language about the motion that was passed during the April 2007 meeting. Pat Martin felt that this motion should include language that made it apply to both the preproposals and the WASSIP goals and that the motion should have included the evaluation of the tradeoffs of pooling samples. There was general agreement to add the tradeoff of pooling samples portion but not to include the WASSIP goals (MOA) as a target of the motion – in other words that the motion was only relative to the pre-proposals. There was discussion about how to get these changes noted and it was decided that the change should occur in the March 24, 2008 minutes because the April 2007 minutes had already been approved.

The Chair suggested the following language should go into the March 2008 minutes: "Clarification of the April 2007 minutes should be amended as follows: "**both the sockeye and chum preproposal should include specific language that the Department will strive to achieve 99% accuracy / 1% error and will evaluate the tradeoffs in pooling samples to achieve that goal."**

Michael Link asked that following verbiage be struck on the last page, in closing paragraph: "We went to the mat"

The Chair motioned to adopt, Bob Dubey seconded, and all were in favor.

7. ***Technical issues and # 9. Adequacy of the sampling to date, especially chum salmon in 2006***

At different times during the meeting, the question about whether the collection efforts were adequate, especially for chum salmon in 2006 and 2008 where discussed. This is a summary of all these discussions. Beth Stewart and Pat Martin both felt strongly that without samples from the Y1 fisheries (marine Yukon River mouth), the information from this project would be severely compromised.

Two questions emerged: 1) Can we process and/or publish each species separately? 2) If no fish is captured in fisheries should there be a test fishery to gather samples?

Beth Stewart felt that the analysis of the two species could be decoupled, but the release of results (publication) should happen at the same time so that the sockeye would not dominate.

Beth Stewart felt that the Y1 samples are so important, that if there is no fishery, samples should still be obtained anyway. A discussion ensued with Tim Baker and Michael Link arguing that WASSIP was designed to find out what was captured in commercial and subsistence fisheries

and therefore test fisheries were not appropriate because they may not catch the same fish stocks. The Chair said that there was a similar discussion last March and the decision was to go ahead and do test fishing. Beth Stewart said that it could easily be fished as the commercial fleet does and offered to help as needed to make it happen.

The Chair said that he drafted the CIP request (that was due that day) to sample and analyze chum salmon taken in 2009 with the idea that if the 2006 samples are OK to use, then we would have four years worth of data, and if they were not OK to use, then we would have three years worth of data. The CIP request was for $1.25M. Pat Martin felt strongly that the 2006 chum salmon samples are too flawed to analyze and therefore the CIP request should have been only to sample 2009 and the analysis costs would come out of the existing funding (shift from 2006 to 2009).

Loretta Bullard pointed out, and Bill Templin agreed, that if sampling for chum salmon was done in 2009, that incorporating these samples in the analysis should not result in any push back in timing of the final report.

There was some discussion on ways to deal with the hole in the 2006 chum salmon collections if the funding for the CIP does not materialize. One option discussed was to take funds out of the current project to sample in 2009 but that some analyses would have to be given up to pay for this extra cost. There was a general agreement that if sampling was done in 2009, it should be the full-blown sampling similar to 2007 or 2008 (all fisheries, both species) and not just sampling in the Y1 area. There was also general consensus that analyzing the 2006, 2007 and 2008 for sockeye salmon and 2007, 2008 and 2009 for chum salmon would be acceptable.

10. Process formality issues

Michael Link made a motion to limit communications of substantive issues between the AP and the Department, to the AP meetings.

11. Budget issues

There was a lot of discussion regarding influence the AP has over the budget for this project. There was a consensus among AP members that they wanted to see how the money was going to be spent ("see the budget"). Mike Smith wanted to go further and give the AP power to approve personnel and contracts and felt that he had gone to bat for the budget and was frustrated that he had no say in the management of it. Beth Stewart pointed out that when this project was originally started, there were plans to hire an executive director and manage the budget – that did not materialize and that the CIP rules do not allow a random group to direct the budget. Loretta Bullard pointed out that the MOA says that the AP will have influence over the budget. It was recognized that this "influence" could be interpreted broadly or narrowly. The Chair pointed out the funding for this project has changed and that the budgetary oversight has changed along with it. Both The Chair and Bill Templin pointed out that the direction the AP wants to proceed will have a large influence on how the budget is constructed. **After much discussion, Bill Templin offered to show the AP the CIP budgets as they went out and a proposed budget for the project as it sits now with the recognition that as AP objectives change, so will the budget.**

12. Meeting frequency

The discussions on how often these meetings should occur and when the next one would occur took place at different times during the meeting and are summarized here. General consensus was that meeting should be more frequent than they have been. Loretta Bullard suggested that the meeting frequency be dictated by when decisions were needed from the AP. The general consensus was that the meetings should occur every three to six months, depending on what is going on within the project. The Chair agreed and set the next meeting to occur sometime at the end of February when the AP could review progress made on the pooling methodology and decisions were needed on which fish to analyze. The Chair said he would send out an e-mail to get feedback on the best time.

Adjournment at 17:46

The Chair made a motion to adjourn, Beth Stewart so moved and Bob Dubey seconded.