Contents

Workshop for Genetic Research on Pollock Resources in the Central Bering Sea June 6-9, 2005 Seattle, Washington USA

1. Introductions and Election of Meeting Officials

1.1. The meeting nominated L. Low as meeting chair and I. Spies as rapporteur with assistance from each party.

1.2. The agenda was agreed upon by all parties.

2. Review of Current Information and Research

2.1. M. Canino (AFSC) put together a list of studies on Pollock genetic stock structure (WP-8). Earlier studies using allozymes and mtDNA may represent mixed stocks because they were not taken during spawning season. Studies of Atlantic cod have shown considerable genetic differentiation between spawning groups, and thus it would be worth while to re-examine stock structure of other gadids. Russian party inquired how many samples were used in they study and where the NCBS sample was collected (in September by a US catcher-processor, slightly northwest of the donut hole).

2.2. The Russian party presented 'Microsatellite analysis of population structure of Bering Sea Pollock' (WP-10). They found weak but significant structuring, using nine microsatellite loci. The North Kuril grouping appears genetically distinct, as do the East Bering Sea samples. There was some discussion on sampling and analysis with U.S. party and Japanese party.

2.3. Dr. Canino discussed his work on gene frequency stability over time using work on both microsatellite and pantophysin. More discussion centered on the question of genetic differentiation between year classes.

2.4. The Japanese party presented work they had done on walleye pollock (WP-13) by mtDNA sequencing of the mitochondrial control region, plus mtDNA, RFLP, and SNP analysis of the Calmodulin gene. Sampling locations ranged from Japan to Shelikov Strait. The mtDNA analyses found an east-west split, between Japan and the Bering Sea. SNP analysis of the Calmodulin gene indicated three major clusters: EBS, Japan, and WBS/Russia. Questions centered on desire for larger sample sizes in the future and historical explanations for observed broad scale genetic differentiation. The question of future work was brought up in order to ascertain with statistical confidence the population structure of walleye pollock and use the data for fisheries management.

2.5. Further discussion centered on the accuracy of genetic analysis and the desire to ascertain discrete population structure of walleye pollock, which to date has not been possible given various limitations (i.e., sample size, marker selection, funding, etc.).

3. Research Planning, Specimen Collection and Exchanges

3.1. The workshop began discussion on this agenda by first reviewing existing samples. The US scientists noted that they have genetic samples from spawning pollock aggregates from various locations including the Bering Sea dating back to 1997. These samples are available to any interested parties. The Russian scientists stated they have samples from the Western and Northern part of the Bering Sea and from the areas listed in WP-10, and that they are open to sharing samples. It was noted that 50-100 individuals should be collected from each spawning aggregation (or sampling location), in order for them to be representative. Any tissue will be sufficient (muscle/bones/fin clip), but fish should be sampled within a half an hour or so of death.

3.2. Sample preservation in ethanol can last typically for 3-5 years, but in some cases as long as 30 years. Preservation of DNA is best done in -80°C freezer. It was noted that samples preserved for more than 5 years, can result in "noise" in the microsatellite loci. Some genetic research has been successful on herring scale samples that were in storage for about 30 years. Many times scales are stored dry and that this can degrade the sample quality. It was noted that properly preserved otoliths could be used for genetic analysis as long as there is sufficient tissue left on the otolith and that microsatellite analysis is more robust than allozyme analysis because the sampling requirements are not as stringent. Fin clips are easy to take in the field and are good for DNA extraction. SNPs should work the same as nuclear analysis. The Poland scientist noted that they have some otoliths from the past fishery operations on pollock in the CBS, EBS, Aleutian Islands, and GOA since 1978. Similar collections are also likely available from Korean research cruises. The US party noted that they have opportunities to collect fin clips from fisherman but that such cooperative agreements usually require producing results fairly quickly (within several years).

3.3. Ichthyoplankton surveys should be pursued as another source of DNA (ensuring that the eggs/juveniles are pollock). The BASIS surveys may be a possible source of samples. The Japanese scientists noted that they may have juvenile pollock samples collected in recent years.

3.4. The need for a definitive study on pollock was highlighted. It was noted that a definitive study would entail having 20 good microsatellite markers and multi-year samples from spawning pollock collected in all the main areas.

3.5. The issue of using of muscle tissue and more easily collected samples (e.g., fin clips) was discussed at length. The Japanese scientists stated that they had some difficulties extracting DNA from the fin clips they received from the AFSC. They had no problems extracting DNA from muscle and thus would prefer this type of sample. The main advantage for using fin-clips is the ease of collection. While both tissue types can be used, where possible the workshop felt that innovative sampling approaches (e.g., simply taking muscle tissue with each otolith collection) should be pursued.

3.6. The issue on the need to focus attention on developing better markers arose. WP-8 states that small sample sizes and samples from non-spawning individuals were possible issues related to the lack of differentiation between Bogoslof pollock and that from surrounding areas. The lack of sufficient markers was listed as another possible issue. This question of criteria to use to determine if different stocks exist was raised. Evidence for genetic differentiation implies a self recruiting, genetically distinct stock. If one is able to provide evidence that genetic differentiation is constant over time, then that is evidence of genetic differentiation. Typically, however, discrete boundaries do not exist in marine fishes. Instead, there is often isolation by distance and some sort of cline in genetic characteristics. As an example, the AFSC recently completed a genetic analysis of Atka mackerel in the Aleutian Islands using nine microsatellite loci. Four locations were sample with results of a high degree of correct classification to region (76-80% certainty location of origin). This study suggests that since similar methods are being used for pollock, the genetic markers that were used should be adequate.

3.7. The issue on the ability of genetic research to address questions on the degree to which the Bogoslof population represents the entire Aleutian Basin pollock was discussed. It was pointed out that distinguishing Aleutian Basin pollock from EBS and other areas is largely a different issue than understanding the relative abundance between the Aleutian Basin stock and the Bogoslof portion of that stock during the winter spawning aggregations. Analysis of historical collections may provide some new insights on genetic discretion of the Bogoslof region as compared to the pollock from the Aleutian Basin and other regions.

4. Recommendations to the Scientific and Technical Committee

4.1. *Historical specimen availability.* The workshop recommends that parties to the Convention develop an inventory of historical samples. It is envisioned that these data will be presented and updated eventually to be available on a website. The data should include basic information such as geographic position, the storage method, date collected, and availability of subsidiary data (e.g., age, length, sex, gonad maturity, etc.).

4.2. *Current and planned research.* The workshop recommends that parties present a report on the ongoing and planned activities on genetics research related to pollock. This should be broken into three main areas: current sample collection and processing efforts, historical sample analysis, and research on developing new markers or methods. This will help guide areas where research is lacking and inform on activities around the region.

4.3. *Development of baseline research.* The workshop encouraged member countries to pursue research on improving genetic markers for pollock. The use of alternative markers will help improve the understanding of how stocks are related.

4.4. *Sample exchange.* The workshop recommends that sample-exchange among member parties be facilitated.