

Center for Independent Experts (CIE)

Review of Harbor Seal Population Structure Analysis

Document reviewed:

“The Analysis of Population Genetic Structure in Alaska Harbor Seals, *Phoca vitulina*, as a Framework for the Identification of Management Stocks.”

by

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Executive Summary

This review was organized by the Center for Independent Experts (CIE) to review the document entitled “The Analysis of Population Genetic Structure in Alaska Harbor Seals, *Phoca vitulina*, as a Framework for the Identification of Management Stocks” by Greg M. O’Corry-Crowe, Karen K. Martien, and Barbara L. Taylor. The purpose was to review the scientific research reported in O’Corry Crowe *et al.*, however the motivation of the study was to provide guidance for harbor seal management.

The review document reported on the analysis of tissue samples collected from 881 harbor seals at 180 locations throughout the range of harbor seals in Alaska. The Boundary Rank (BR) analytical method used by O’Corry Crowe *et al.* was geographically constrained and used mitochondrial DNA (mtDNA) markers and haplotype frequency statistics to examine genetic relatedness of small initial units. Samples from particular areas were included or excluded based on an adjusted sample size determined by the number of non-unique haplotypes within the sample. The use of adjusted sample size resulted in exclusion of 26% of the original samples in the final analysis, and the elimination of some sample units entirely. This approach, while avoiding inappropriate conclusions based on too little data, left too many gaps in coverage to provide a realistic framework for implementable management decisions in some geographic regions. In the future it is desirable to develop a method that looks at more of the data, without eliminating highly diverse strata entirely.

The authors determined that mtDNA was the marker of choice for stock identification and based their analyses only on mtDNA. The Southwest Fisheries Science Center (SWFSC) laboratory has analyzed microsatellite data for about 400 Alaska seals; however, in-depth analyses of these data relative to stock structure have not been conducted. This is in part because genetics investigators believe that mtDNA is the most effective marker and also because staff time or funding have not been available to pursue this analysis. Even though mtDNA is the preferred overall method for examining stock structure, microsatellites may be a useful tool. In areas such as Kodiak and southeast Alaska where structure is not clear, an effort should be made to use microsatellite data to augment results of mtDNA analyses and to help refine understanding of population structure and boundaries.

The BR method used in the review document is based on geographically constrained comparisons of small initial units. In the harbor seal analysis, the initial units were generally made up of sample sites within 50 km of each other (or connected by other sites within 50 km). Although this approach was designed to minimize *a priori* assumptions about stock boundaries, it too required some subjective decisions about how sample sites would be combined. These decisions resulted in the elimination of some geographic areas entirely from the final analyses. For example, even though most of the Kodiak sites could be connected by the “50-km rule”, they were subdivided into 4 smaller initial units. Some of these small units were then excluded from the analysis due to the small adjusted sample size. While sensitivity testing including all Kodiak samples suggests that finer scale structure exists in this region, it is not possible to determine stock boundaries or the actual number of population units based on the existing approach and sample sizes.

Comparisons of several analytical methods (BR, UPGMA and NJ) strengthened the conclusions about stock definition for some geographic regions and highlighted areas of uncertainty for others. For example, under all three methods, sample sites in PWS, the east Kenai, and the

Copper River delta grouped very early. Similarly, Ugashik and Togiak in Bristol Bay grouped early with each other and much later with the Pribilofs. Ketchikan and Frederick Sound, both within inside waters of southeast Alaska, also grouped in all three analyses. In contrast, results for the Kodiak region and southeast Alaska were much more variable. Viewed in combination, this highlights the need for additional samples in areas of uncertainty and perhaps for additional analytical techniques or consideration of additional types of data.

The twelve population units proposed in O’Corry-Crowe *et al.* include five in southeast Alaska; yet more than half of the overall area, including hundreds of haulouts, thousands of seals and numerous communities that harvest seals, is excluded from these units. A similar situation exists in the Kodiak/lower Cook Inlet region, where three units are defined yet very large areas with many seals and many harvesting communities are excluded. Therefore, although the statistics applied in the BR analysis demonstrate 12 population units at the $p < 0.1$, the proposed scheme for population subdivision is not implementable in these regions. In contrast, the study also includes areas where geographic coverage and sample size are adequate and stock designations can be made. All lines of genetics evidence, as well as tagging information and population trend data, indicate that the PWS/CRD/east Kenai region is demographically isolated and should be treated as a separate stock. Furthermore, the population has been declining for almost 20 years, despite decline reversals in other areas of the Gulf of Alaska. Management actions for this region do not need to await further resolution of population structure in other areas. Data are similarly quite straightforward for Bristol Bay and for the Pribilofs.

The conclusion that the three current stocks of harbor seals in Alaska are too broadly defined is well-supported by the review document. The Gulf of Alaska stock as defined in NMFS SARs includes waters from Cape Suckling to Unimak Pass and throughout the Aleutian Islands, and includes both the Kodiak region and PWS/CRD. In all genetics approaches, PWS/CRD/east Kenai and seals from Kodiak clustered separately early and consistently. Other information also supports the conclusion that Kodiak and PWS are demographically isolated and represent different stocks. Data are insufficient to resolve how seals within the Kodiak region, Kamishak Bay and Cook Inlet should be treated. The relationship of seals along the south side of the Alaska Peninsula to those from Kodiak and the Aleutian Islands is completely unknown. The Bering Sea stock as defined in the SARs includes both Bristol Bay and the Pribilof Islands. All mtDNA genetics methods have identified Bristol Bay as a separate demographic unit. It clustered very late with the Pribilofs using BR and not at all with UPGMA and NJ, suggesting that the current Bering Sea stock includes at least two smaller management units, and perhaps more, when data from the Aleutians are included. The SARs define a southeast Alaska stock that extends from the Canadian border to Cape Suckling and includes Yakutat, Icy and Glacier bays as well as the rest of southeast Alaska. Large gaps in sample coverage occur for this region, but existing data suggest genetic differences at least between inside and outside waters. How many stocks are ultimately defined throughout Alaska will depend on obtaining adequate sample coverage for all regions, development of methods that can perhaps collapse unique haplotypes that are just 1 or 2 mutational steps apart, and/or incorporating other genetic evidence such as microsatellite data to clarify the overall picture.

Inadequate sample sizes and gaps in sample coverage limit the ability of genetics studies to identify meaningful management units throughout the state and many questions remain about effects of sample coverage on the final outcome. While the current analysis is very informative,

its application to actual conservation and management decisions is limited by the large areas that are omitted from the final conclusions.

Recommendations are as follows: 1) Additional samples should be obtained from areas such as Yakutat Bay, Icy Bay, the Aleutian Islands, and the Alaska Peninsula for which there is no information on stock structure from genetics or other types of studies and sample sizes are too low to be included in the BR analysis; 2) additional samples should be collected from geographic areas where stock definition is unclear or incomplete such as the Kodiak region and southeast Alaska; 3) if adequate samples are not available through the Alaska Native Harbor Seal Commission (ANHSC) biosampling program, then a directed sampling program should be initiated in areas where samples are of high priority; 4) funding should be made available to conduct analysis of currently available microsatellite data ($n = \pm 400$), and to conduct more microsatellite laboratory analyses, as necessary, to develop adequate sample sizes; 5) new analytical methods should be explored that will allow inclusion of more of the existing samples in a haplotype frequency analysis; 6) movement data from satellite tagging studies in Bristol Bay, Kodiak and southeast Alaska should be further reviewed to see whether this information can guide inclusion of currently excluded units in the final analysis; 7) PWS should be designated as a separate management unit as soon as possible so that appropriate management actions can be taken to address the ongoing decline; and 8) a workshop should be held in which presentations made by SWFSC for this review are also made to representatives of harbor seal hunting communities throughout Alaska, the ANHSC, and perhaps also to representatives of other interested groups.

Background

Harbor seals inhabit the coastal waters of Alaska from southeast Alaska north and west through the Gulf of Alaska, along the Aleutian Islands, and into the Bering Sea in Bristol Bay and the Pribilof Islands. They co-occur with commercial fisheries throughout their range in Alaska, and some incidental mortality occurs in conjunction to these fisheries (Angliss & Lodge 2002). Harbor seals are also subject to directed human-caused mortality in the form of subsistence hunting by Alaska Natives under an exemption to the moratorium on take imposed by the Marine Mammal Protection Act (MMPA). This subsistence harvest is managed through an agreement between the National Marine Fisheries Service (NMFS) and the Alaska Native Harbor Seal Commission (ANHSC), under which both parties agree to “promote the sustained health of harbor seals in order to protect the culture and way of life of Alaska Natives who rely on the harvest of harbor seals for subsistence uses” (ANHSC and NMFS 1999).

To ensure that human-caused mortality, whether incidental to fisheries or from directed subsistence harvest, does not cause harbor seals to decline beyond the point at which they cease to be a significant functioning element in the ecosystem of which they are a part or below their optimum sustainable population level (as specified in the MMPA) and occurs at levels that will promote sustained health of harbor seals (as specified by the ANHSC-NMFS agreement), it is necessary to understand and manage them according to stock structure as well as overall abundance.

In the 1995 Alaska Marine Mammal Stock Assessment Reports (SARs), NMFS recognized three separate management units for harbor seals in Alaska waters (see Angliss & Lodge 2002): 1) the

southeast Alaska stock; 2) the Gulf of Alaska stock, including the Aleutians; and 3) the Bering Sea stock. This designation was based primarily on differing population trends in the three areas, with seal numbers stable or increasing in southeast Alaska, declining in the Gulf of Alaska, and possibly declining in the Bering Sea. Results of satellite-tagging studies in southeast Alaska, Prince William Sound (PWS), and near Kodiak also suggested that harbor seals did not move between these three areas (Lowry *et al.* 2001; Small *et al.* in prep.; Hastings *et al.* submitted; Small and Ver Hoef 2001). No seal instrumented within southeast Alaska, the Gulf of Alaska or the Bering Sea traveled outside of the region where it was tagged. Tagging results indicated that most seals remained within 50 km of the tagging location throughout the period the tag functioned (1-12 months). Similar site fidelity was indicated by flipper tags recovered in PWS 1-4 yrs after tagging. NMFS recognized that additional information was needed about dispersal and movement patterns of Alaskan harbor seals, but nonetheless believed the existing information warranted the designation of three stocks.

When three stocks of Alaska harbor seals were initially defined, genetics techniques for stock identification were in the early stages of development and genetic differences were not the basis for stock designation. However, NMFS initiated genetics studies of harbor seal stock structure in fall 1994 and results of those studies indicate that the original designation of three stocks almost certainly underestimates the actual number of population units present for Alaska harbor seals (see Federal Register notice 67). Satellite-tagging data as well as population trend data also support the existence of more than three management units. For example, tagged seals do not move between PWS and Kodiak, or between inside and outside waters of southeast Alaska, even though PWS and Kodiak are both considered part of the Gulf of Alaska stock and all of southeast Alaska is classified as a single stock. Similarly, population trends differ between PWS and Kodiak, and between inside and outside waters of southeast Alaska (Small *et al.* 2003).

Results of NMFS genetics studies as they relate to harbor seal stock structure are presented in O’Corry-Crowe *et al.* (2003). The findings of O’Corry-Crowe *et al.* (2003) are the subject of this review.

Review Process

This review consisted of three parts: 1) review of a series of background papers that established a framework for the review, 2) a meeting at which a variety of presentations were made about stock definition, harbor seal biology, and genetic methods used to address the stock identification problem, and 3) preparation of individual written reports by each of the reviewers.

Papers

Ten background papers were provided to reviewers as part of the original statement of work and addressed either: 1) the management framework and definition of stocks for marine mammals, or 2) genetics methods used to define stocks for harbor seals and other continuously distributed marine mammals.

Management framework and stock definition

Background documents addressing stock definition and the management framework included sections of the Marine Mammal Protection Act (MMPA), NMFS guidelines for identifying population stocks, the agreement between the Alaska Native Harbor Seal Commission and NMFS regarding conservation and management of harbor seals, two manuscripts discussing definition of population units to be used in conservation (Taylor 1997 and 2003), and an August 2002 Federal Register notice regarding stock structure of Alaska harbor seals. These documents are summarized briefly below.

Section 2 of the MMPA as amended in 1994 states that marine mammal “species and population stocks should not be permitted to diminish beyond the point at which they cease to be a significant functioning element in the ecosystem of which they are a part, and, consistent with this major objective, they should not be permitted to diminish below their optimum sustainable population.” It adds that “marine mammals have proven themselves to be resources of great international significance, esthetic and recreational as well as economic, and it is the sense of the Congress that they should be protected and encouraged to develop to the greatest extent feasible commensurate with sound policies of resource management and that the primary objective of their management should be to maintain the health and stability of the marine ecosystem.” Section 3 of the MMPA defines a population stock as “...a group of marine mammals of the same species or smaller taxa in a common spatial arrangement that interbreed when mature.” MMPA Section 117 requires that NMFS publish stock assessment reports (SARs) for each such stock in which they describe the geographic range; estimate minimum population size, productivity rates, current trend, and human-caused mortality; describe commercial fisheries that interact with the stock; categorize the stock as non-strategic (human-caused mortality not likely to reduce population below its optimum sustainable level) or strategic; and estimate the potential biological removal level for the stock. This requires a functional definition of population stock.

Correct delineation of harbor seal stocks in Alaska is particularly important because harbor seals are an important subsistence resource to Alaska Natives and are hunted throughout much of their Alaska range. Section 101 of the MMPA specifies that Alaska Natives who dwell on the coast of the North Pacific Ocean or the Arctic Ocean of Alaska are exempted from the moratorium on take of marine mammals if take is for subsistence purposes or for the purpose of creating and selling authentic native articles of handicrafts and clothing, and in each case is not accomplished in a wasteful manner. Under Section 119 of the MMPA, NMFS signed a co-management agreement with the Alaska Native Harbor Seal Commission (ANHSC), a representative body for native subsistence users of harbor seals in Alaska, in April 1999. One of the purposes of this agreement was to “promote the sustained health of harbor seals in order to protect the culture and way of life of Alaska Natives who rely on the harvest of harbor seals for subsistence uses.” The agreement includes provisions for monitoring the harvest “to ensure that harbor seals are conserved for subsistence and other uses” and encourages the development of local and/or regional management plans that ensure harbor seals are used for subsistence in a sustainable manner. An understanding of stock definition is required both to ensure conservation for sustainable subsistence use and for the development of management plans.

In April 1996, NMFS convened a workshop to review stock assessment related processes, discuss ways of defining stocks for management under the MMPA, establish guidelines for identifying population stocks, review guidelines for preparing stock assessment reports, and

develop guidelines for the entire stock assessment process (Wade and Angliss 1997). The stock definition section of this report states:

The workshop participants recognized that there are many different ways to define stocks. The appropriate stock definition depends upon the management goal. It was therefore recognized that a stock is a management unit, and does not necessarily have an exact definition in the real world divorced from a management goal. Populations in the real world exhibit a broad continuum of various levels of differentiation, making it difficult to choose a single universal definition of a biological stock that will be meaningful for all species and populations. Stocks are often defined as a unit that will preserve genetic diversity, but there are other possible definitions. Under the MMPA there is a clear mandate to maintain populations as a functioning element of the ecosystem, but there is no language to suggest that distinct genetic units should be the management unit.

Many types of information can be used to identify stocks of a species: distribution and movements, population trends, morphological differences, genetic differences, contaminants and natural isotope loads, parasite differences, and oceanographic habitat differences. Evidence of morphological or genetic differences in animals from different geographic regions indicates that these populations are reproductively isolated. Reproductive isolation is proof of demographic isolation, and thus separate management is appropriate when such differences are found. Failure to detect differences experimentally, however, does not mean the opposite. Dispersal rates, though sufficiently high to homogenize morphological or genetic differences detectable experimentally between putative populations, may still be insufficient to deliver enough recruits from an unexploited population (source) to an adjacent exploited population (sink) so that the latter remains a functioning element of its ecosystem. Insufficient dispersal between populations where one bears the brunt of exploitation coupled with their inappropriate pooling for management could easily result in failure to meet MMPA objectives.

Therefore, careful consideration needs to be given to how stocks are defined. In particular, where mortality is greater than a PBR calculated from the abundance just within the oceanographic region where the human-caused mortality occurs, serious consideration should be given to defining an appropriate management unit in this region. In the absence of adequate information on stock structure and fisheries mortality, a species' range within an ocean should be divided into stocks that represent defensible management units.

Examples of such management units include distinct oceanographic regions, semi-isolated habitat areas, and areas of higher density of the species that are separated by relatively lower density areas. Such areas have often been found to represent true biological stocks where sufficient information is available. There is no intent to define stocks that are clearly too small to represent demographically isolated biological populations, but it is noted that for some species genetic and other biological information has confirmed the likely existence of stocks of relatively small spatial scale.

Taylor (1997) noted that any marine mammal management scheme must incorporate stock structure for it to be successful, and discussed the consequences of failing to correctly identify such structure when a group of animals is subject to human-caused mortality. She noted that “population stock” (or simply stock) carries a double meaning and may refer to either: 1) groups delineated by a very low rate of genetic exchange, or 2) groups of animals that are demographically separate and experience differential risk and should therefore be managed

separately. She presented a model in which two population stocks connected by dispersal were managed as a single unit. Abundance was based on the combined size of the two stocks and human-caused mortality concentrated on only one stock. Taylor's model indicated that when dispersal rates were less than a few percent per year and population structure ignored, there is a danger of reducing the human-impacted population below management objectives, and in fact eliminating it.

Taylor (2003) discussed differences between maintaining populations at certain population levels (OSP) versus maintaining them as functioning elements of ecosystem, both of which the MMPA requires. She referred to her 1997 definition of stocks and discussed the need to define stocks in a manner that incorporates scientific uncertainty in a precautionary manner. She reiterated that "population stock" carries a double meaning and included 1) groups delineated by genetic differences, or 2) groups that are essentially demographically separate, where demographic separation means that if a population declines, immigration from another area would not be sufficient to reverse the decline over a period of decades. Taylor noted that whether or not stock definition is straight-forward, NMFS must nonetheless estimate population boundaries and implement management decisions based on these boundaries. Stock definition can err by either lumping or splitting groups of animals incorrectly. Splitting can result in unnecessarily conservative estimates of allowable take, while lumping runs the risk of failing to maintain a group of animals as "functioning elements of the ecosystem" in parts of their range. Taylor pointed out that the scientific challenges regarding stock definition are to obtain adequate data; develop methods to minimize errors in stock definition; and develop better methods to integrate a variety of data types including genetics, distribution, abundance, etc. For managers, the challenge is to develop a definition of stocks that incorporates scientific uncertainty in a precautionary manner. Since data are often insufficient to make probabilistic statements about risk, it is necessary to incorporate elements that will allow good definition in the face of such uncertainty, i.e. to 1) use all available data on scale of population structure from areas with sufficient data; 2) provide an incentive to gather the needed data; and 3) be precautionary in step with the degree of risk faced by not making correct stock definition decisions.

On 26 August 2002, NOAA published a Federal Register notice regarding new information about fine-scale stock structure for harbor seals in Alaska. The purposes of the notice were to: 1) inform constituents that several lines of evidence indicate finer-scale stock structure for Alaska harbor seals than current Stock Assessment Reports indicate; 2) advise the public that NMFS and the ANHSC are evaluating harbor seal stock structure through a co-management process; and 3) solicit additional information that the public would like considered during this evaluation. The notice summarized recent scientific studies relevant to the stock structure question, including genetics analyses that identified twelve genetically and demographically independent groups of seals, satellite telemetry studies, and population trend analysis.

Genetics studies and methods

Westlake & O'Corry-Crowe (2002) examined mitochondrial DNA from 778 seals sampled at 161 locations from Japan to Alaska. They used two main approaches to estimate genetic differentiation: 1) estimating Φ -statistics that incorporate information on the number of mutational steps among individual haplotypes plus differences in haplotype frequency, and 2) using conventional F-statistics and haplotype frequencies only. They found substantial levels of

population subdivision over spatial scales of 600-800 km, and for 5 geographic centers of distribution in Alaska: southeast Alaska, Prince William Sound (PWS), Kodiak Archipelago, Bristol Bay, and the Pribilof Islands. A neighbor-joining tree placed Bristol Bay and the Pribilofs on the same branch and Southeast Alaska, Prince William Sound and Kodiak on another. Additional differentiation between Kodiak and PWS seals, and between Bristol Bay and the Pribilofs, indicated that the current three management stocks listed in the stock assessment reports are inappropriate.

Martien & Taylor (2003) discussed the limitations of a traditional hypothesis-testing approach for defining management units or stocks in continuously distributed species where the lack of distributional gaps makes structure difficult to detect. Hypothesis testing requires *a priori* definition of hypothesized units, which are then tested to see if significant genetic differences exist. Martien and Taylor used a simulation model based on isolation-by-distance (a stepping-stone model where genetic differentiation is controlled by the dispersal rate between adjacent populations) to evaluate the ability of hypothesis testing to correctly identify population structure. They found that such an approach generally identifies fewer units than are necessary to adequately manage local populations and protect them from over-exploitation.

Martien *et al.* (submitted) presented a new analytical method called Boundary Rank (BR) for examining population structure in bottlenecked dolphins. The BR method uses hierarchical clustering to group samples into management units based on their genetic similarity, unlike most genetic methods which require *a priori* definition of such units. Martien *et al.* compared the performance of BR to another genetic clustering method called SAMOVA, and to results of long-term observational and photo-identification studies in the study area. They found that BR results, unlike SAMOVA, were consistent with observational data and concluded that BR is likely to be a reliable tool for evaluating population structure in continuously distributed species such as bottlenecked dolphins.

O’Corry-Crowe *et al.* (2003) (the subject of this review) analyzed mitochondrial DNA (mtDNA) from 881 harbor seals sampled from 180 sites throughout Alaska. They used a geographically constrained cluster analysis (Boundary Rank) described by Martien *et al.* to analyze the genetics data, and compared the results to two classical distance-based clustering and phylogeny reconstruction analyses (Unweighted Pair Group Method using Arithmetic averages/UPGMA and neighbor joining/NJ) and a traditional hypothesis testing approach. They identified 12 statistically different ($p < 0.1$) clusters of sampling sites based on analyses of mitochondrial DNA (mtDNA) and estimated that dispersal among these areas is very low. They concluded that the current designation of three Alaska harbor seal stocks is not adequate to meet the management objective of maintaining population stocks as functioning elements of their ecosystem. They further noted that even though sample coverage is still incomplete, they believe the conclusion of multiple small population units that should be managed separately is robust and is unlikely to change.

Review Meeting

The review took place on 16-18 March 2004 at the Southwest Fisheries Science Center (SWFSC) in La Jolla, California. It included three reviewers (myself/Kathryn Frost, Brent Stewart and Russ Hoelzel) and three genetics experts from the Southwest Fisheries Science Center genetics staff (Barb Taylor, Greg O’Corry-Crowe, and Karen Martien). Presentations were made on Day 1 on stock definition, harbor seal biology, genetics methods, and the study

being reviewed (O’Corry-Crowe *et al.* 2003). These presentations were extremely professional, well-organized, and helpful for understanding rationale, methods, results and interpretation of the study under review. Non-participating observers were present from SWFSC, the US Marine Mammal Commission, and the National Marine Mammal Laboratory during these presentations. During Day 2 and part of Day 3, reviewers met with genetics investigators to discuss particular aspects of the study methods, results, and conclusions, and to request some additional analyses demonstrating the effects of different sample sizes or lumping strategies on the genetics model outcomes. Reviewers met among themselves during the early afternoon of Day 3 and then began drafting their individual reports. The SWFSC staff were extremely well-prepared, cooperative, and helpful. They responded quickly to reviewer requests for additional analyses and willingly addressed any questions they were asked by reviewers.

Formal presentations included the following:

- I. Harbor Seal study rationale (Barb Taylor)
- II. Introduction to the Marine Mammal Protection Act (Barb Taylor)
- III. Importance of stock definition to the PBR process (Barb Taylor)
- IV. Genetics primer
 - a. Dynamics of genetics and statistical power (Barb Taylor)
 - b. Marker choice and comparing frequencies (Greg O’Corry-Crowe)
- V. Harbor seal biology (Greg O’Corry-Crowe)
- VI. Genetic analyses used for harbor seals (Karen Martien)
 - a. Hypothesis testing
 - b. Parameter estimation (clustering methods, dispersal rate estimation)
- VII. The study
 - a. Initial units (Greg O’Corry-Crowe)
 - b. Hypothesis testing (Greg O’Corry-Crowe)
 - c. Clustering methods (Karen Martien)
 - d. Dispersal rates (Karen Martien)
- VIII. Conclusions
 - a. Current stock too broadly defined (Greg O’Corry-Crowe)
 - b. Identified units appropriate, sample coverage limiting, existence of small units unlikely to change (Barb Taylor)

Summary of Findings

Genetic samples and data

Were the methods of selecting, collecting, and handling samples adequate relative to the conclusions drawn?

Tissue samples were collected from 881 harbor seals at 180 locations throughout the range of harbor seals in Alaska. Sample distribution was not uniform among regions and was not proportional to the abundance of seals in an area, largely because samples were obtained on an opportunistic basis.

All 881 samples were used to determine overall haplotype diversity. However, in examining genetic differentiation among regions, the authors did not use all of the samples that were

analyzed. The clustering methods examined in O’Corry Crowe *et al.* (2003) required that samples be divided into initial units. They found it was impractical to treat each of the 180 sampling sites as an initial unit since some were represented by only 1-2 samples and other sites were so close together that they didn’t warrant separate treatment (*e.g.* neighboring beaches in the same bay). Therefore, they grouped nearby sites into initial units. Studies of harbor seal movements in the Atlantic and Pacific have indicated that most harbor seals stay within 50 km of their capture site during the period of observation. For this reason, O’Corry Crowe *et al.* grouped together all sampling sites within 50 km of each. Sites were grouped sequentially which meant that sites at either of a unit might be more than 50 km apart. Habitat differentiation and gaps in haulout distribution were also used to refine delineation of units. This resulted in the delineation of 31 initial units. In this process, 27 samples from 14 sites were excluded since sample size was low and because the sites were not within 50 km of other sample sites with which they could be combined.

The genetic differentiation methods used by O’Corry Crowe *et al.* were based on haplotype frequency. For such frequency-based analyses, where genetic differentiation is estimated by comparing haplotype frequency, small sample sizes and high haplotype diversity can result in a bias that causes poorly sampled sites to appear less differentiated than they actually are. To be cautious and avoid this type of bias, O’Corry Crowe *et al.* used what they called an adjusted sample size, n_a . This n_a is the number of samples from a site that have a haplotype that is represented more than once at that site. Unique haplotypes were not included since they do not contribute any information to frequency-based comparisons.

The use of adjusted sample size resulted in exclusion of a substantial number of the original samples from the final analyses since any initial unit with $n_a \leq 4$ was dropped. This eliminated 172 samples and 14 of the initial units. In addition, 29 samples from an east Kodiak unit were excluded due to uncertainty in where to place the unit boundaries. This process reduced the overall sample size to 653.

While the goal to avoid bias was desirable, the elimination of 26% of the original samples resulted in the complete elimination of some areas from the analyses (for example, the Aleutians, the Alaska Peninsula, Yakutat Bay and Icy Bay) even though some had sample sizes > 20 . In other regions, such as Kodiak and southeast Alaska, it resulted in patchy coverage and large gaps between the provisional management units identified by the analysis. For example, samples from all of northern and eastern Kodiak (almost 40% of the total Kodiak samples) were excluded from the analysis. A similar but much less extreme situation occurred for southeast Alaska where 12% of the samples were excluded. The resulting management units, while certainly indicative of considerable population structure, leave too many gaps in coverage to provide a realistic basis for implementable management decisions.

During the review meeting, reviewers asked SWFSC personnel to conduct a modified BR analysis in which they constrained sample size. For any initial unit with a sample size ≥ 50 (south and east PWS, south Kodiak and Vixen-Sitka), sample size was limited to 20 unless $n_a \leq 4$, in which case it was increased to 25 (south Kodiak). The limitation of sample size for these areas resulted in some changes to the proposed management units at both the $p < 0.1$ and $p < 0.05$ levels. While units for the Pribilofs, Bristol Bay, and most of southeast remained the similar when sample size was limited, there were differences in the way Kodiak, Kenai, PWS and Glacier Bay sites clustered. This modified analysis was not intended to suggest that the new groupings were likely, but to point out the effect that small sample sizes might have on BR

results. The changes in unit groupings caused by limiting sample size emphasize the need for additional samples in areas where coverage is fragmented and /or sample sizes are small.

Were limitations of the sampling scheme and data adequately acknowledge and considered?

The authors of the review document fully acknowledge the limitations in sample size in both the document and in the review process. Although the sample size (>800) appears large, it is fact less than 1% of the population. This is aggravated by high haplotypic diversity in harbor seals, and by the huge geographic extent of the study area. Investigators were very conservative about including data from areas where sample coverage was poor. While this was good because it reduced the potential for biasing the results and making areas appear similar or dissimilar when they may not have been, it resulted in large gaps between the population units identified. This is not problematic in a research context, but it is more difficult to deal with when trying to design a management scheme.

Analytical methods

Were the laboratory analyses appropriate and applied correctly?

The state of genetics research is rapidly changing. As more data become available for more and different types of animal populations, there is a need for new and better analytical methods that produce results that are useful in a management context. The authors of this report took advantage of new laboratory techniques which allowed them to extract mtDNA not only from skin and other soft tissue, but also from molted hair. Discussions with the investigators indicate they are also developing methods to analyze other hard tissue such as teeth and bone. This will expand the potential sample base to include samples from agency and museum archives.

Genetics studies to date have used two main genetic markers, mitochondrial DNA and microsatellites, to examine population structure. The study under review used differences within the control region of the mitochondrial genome (mtDNA) to examine population structure for Alaskan harbor seals. The mtDNA control region is thought to be selectively neutral and therefore evolves much more rapidly than regions that are subject to selection. It is maternally inherited, and the offspring inherits a single allele from its mother. The rapid evolution and maternal mode of inheritance of mtDNA make it useful for studying dispersal patterns and identifying demographically discrete population units. It is clear from the Alaska harbor seal study that mtDNA is informative of population structure and is very useful in identifying population sub-units. However, with the current sample sizes, it is also clear that mtDNA by itself is not adequate to clearly resolve stock structure questions.

Microsatellites are also used as genetic markers in studies of evolutionary relationships, interbreeding, kinship etc. Microsatellites (nDNA) are biparentally inherited, are part of the nuclear genome, and change less rapidly than mtDNA. During the review process, presenters demonstrated why microsatellites may be less useful than mtDNA for studying demographic relationships under certain circumstances. This is especially true when male dispersal is high but female dispersal low (high female philopatry), resulting in low differentiation for nDNA but high differentiation for mtDNA. The effect size for frequency statistics based on mtDNA may be as much as four times greater than for nDNA because mtDNA is haploid and based only on the effective number of females.

Nonetheless, microsatellites have been successfully used to identify population structure for harbor seals in Europe. Goodman (1998) found patterns of population differentiation derived from microsatellites were similar to those from mtDNA analysis and suggested that philopatry in harbor seals operates over scales of 300–500 km. He found that differentiation was greater over equivalent distances where the distribution was discontinuous, such as along coasts where haulouts were separated by large distances or by stretches of open sea.

The genetics program at SWFSC has analyzed microsatellites from more than 400 Alaska harbor seals (the same samples for which mtDNA was analyzed), although this analysis was not included as part of O’Corry-Crowe *et al.* (2003). In presentations to reviewers, SWFSC staff indicated that they have examined 11 microsatellites in nuclear DNA of Alaska harbor seals and that some indicated large regional differences while others did not. Mean F_{st} values depended on the choice of microsatellite markers and differed greatly depending on the markers chosen. Even though the laboratory analysis has been completed for a substantial number of harbor seal samples, there has not, to date, been a detailed analysis of these microsatellite data for purposes of examining stock structure of Alaska harbor seals. There are several reasons for this, including: 1) no dedicated funding for the harbor seal genetics program to cover staff time or sample analysis has been received since 2001; 2) researchers strongly believe more samples should be processed before such an analysis is undertaken; and 3) researchers at SWFSC believe mtDNA is a more powerful tool for examining the stock structure question.

Even though mtDNA is the preferred overall method for examining stock structure, microsatellites may prove useful for augmenting the mtDNA analyses. In particular, in areas such as Kodiak and southeast Alaska where structure is not clear, microsatellite data may add to the picture and help to refine understanding of what sort of structure exists and where boundaries occur. Funding should be provided for SWFSC to conduct additional laboratory analyses as necessary and to undertake a more complete analysis of the utility of microsatellite data in helping to clarify the findings from mtDNA studies.

Were the statistical analyses appropriate and applied correctly? Were the novel methods used in the study developed and tested in a scientifically sound manner?

Stock definition is particularly challenging in species such as harbor seals which appear to be continuously distributed yet exhibit demographic differences among regions in abundance trends and movements. There may be no obvious indicators of where boundaries between demographic units are located. The investigators in this study have taken the initiative to evaluate existing methods for genetic analysis and to develop new methods responsive to management needs relative to stock structure. They did extensive comparisons among methods which were very informative for interpreting the results.

Traditional hypothesis testing, as generally applied to genetics analyses, requires an *a priori* definition of provisional stock boundaries to be used in clustering of samples (Martien and Taylor 2003). Because this method starts “large” with the presumed actual stock structure, it is not well-suited to finding the true boundaries, fine tuning estimated boundaries, or detecting additional boundaries nested within the hypothetical stocks. Finding significant differences between strata identifies structure, but it provides no guidance about whether the boundary is in the right place. Using hypothesis testing it is more likely to define too few than too many stocks,

and the consequences of this underestimation can be substantial and detrimental to the unidentified stocks.

Because of the limitations of traditional hypothesis testing for defining stock boundaries in a continuously distributed species such as harbor seals, O’Corry-Crowe *et al.* (2003) used the BR method described by Martien *et al.* (submitted) in an attempt to get around the problem of arbitrary *a priori* definition of stock boundaries. They did so by defining small initial units and clustering them based on genetic similarity between adjacent units (constrained by a connectivity matrix). At each clustering step the two most similar units were merged. BR identified twelve demographic units at $p < 0.1$ and at nine at $p < 0.1$. Because BR starts small and gradually adds units, O’Corry-Crowe *et al.* (2003) think it is more likely to detect actual boundaries and not to underestimate the overall number of stocks. However BR, like hypothesis testing, does require some *a priori* stratification of the units to be compared. Even this stratification, although at a much finer geographic scale, is somewhat subjective and can introduce uncertainty into the analysis. In the harbor seal analysis, difficulties in deciding how to stratify the initial units resulted in the elimination of east Kodiak ($n = 29$ and $n_a = 9$) from the final analysis. Similarly, other decisions to lump or split sample sites affected whether adjusted sample sizes for initial units were large enough to be included in the analysis. Samples for Port Moller and Port Heiden were not included because they were more than 50 km from inner Bristol Bay sites and had small n_a , yet satellite tagging data clearly indicate movements between inner Bristol Bay and westward along the Alaska Peninsula to these areas. Additional samples will help to address the appropriate designation of initial units, but in the mean time other ways to get more information out of the samples at hand should be explored.

Using haplotype frequencies to compare adjacent areas is a useful approach. However, when haplotype diversity is great as it is in Alaska harbor seals, there are often sample groups that end up having almost no haplotypes in common. For 881 harbor seals sampled in this study there were 243 unique haplotypes, with 145 of them represented by a single seal. Haplotypes represented by single individuals contribute no frequency information for regional comparisons. Thus, only 40% of the total haplotypes contributed information that could be used in defining stock structure. In this study, site clusters with n 's of 5-27 resulted in useful sample sizes of 0-4. Three sites with n 's of 8-9 had no haplotypes in common, and contributed no information about haplotype frequency. For the BR approach to be most informative, larger sample sizes are needed from the areas with high haplotype diversity so that these areas can be included in the analysis.

The approach to n_a merits some additional attention. Obtaining good sample coverage is one of the greatest challenges of the harbor seal genetics program. It is unfortunate when a substantial proportion of the collected samples, and sample sites, cannot be used because they have unique haplotypes. Since haplotype diversity is so high, it would be desirable to explore analytical methods which would allow combination of haplotypes that are genetically close, perhaps 1 base pair apart. This “pruning” would increase the usable sample size and allow inclusion of more site groups in the comparisons. It is desirable to develop a method that looks at more of the data, without eliminating highly diverse strata entirely.

Direct comparisons of BR to several conventional distance-based clustering and phylogeny reconstruction analyses (UPGMA and NJ) and a hypothesis testing approach were extremely useful. BR comparisons were geographically constrained by a connectivity matrix and genetic differentiation was recalculated whenever two groups were combined. In contrast, UPGMA and

NJ were not geographically constrained. UPGMA links two units with the smallest genetic distance first, followed by successively more distant units or groups. Genetic distance of a group is calculated as the mean distance of all the units in that group to the other units or groups. NJ uses distance data to reconstruct evolutionary trees. For both of these methods, non-adjacent sites could group together.

The results of BR, UPGMA and NJ methods were quite similar, lending strength to the overall conclusions of the analyses. Under all three methods, PWS, the east Kenai, and the Copper River delta grouped early. Similarly, Ugashik and Togiak in Bristol Bay grouped early with each other and much later with the Pribilofs. Ketchikan and Frederick Sound, both within waters of southeast Alaska, also grouped in all three analyses. The other southeast Alaska groups and Kodiak/Kamishak varied among analyses.

O’Corry-Crowe *et al.* (2003) performed some sensitivity analyses which they referred to but did not show in detail in their report. During the review meeting, reviewers requested some additional analyses in which they included all of the 31 initial units, and combined some initial units to see what effect changing the initial groupings might have. In one iteration, the 31 initial units were collapsed into 18 units and in another to 11 units. Most resulting clusters were similar to those in the initial analysis at the $p < 0.05$ level. PWS and the CRD, and northern and southern Bristol Bay clustered early. Samples from the Aleutians and the Alaska Peninsula were included in these additional analyses and clustered with the Pribilofs. Ketchikan and Frederick Sound units, whether or not they were enlarged to include adjacent sites, grouped early in all analyses. Outer southeast Alaska sites (Grand Island and Sitka) grouped with other southeast Alaska units in all analyses, but much later in the clustering. All southeast sites were clearly separate from more northern sites. Kenai, Kodiak and Kamishak Bay sites clustered separately from PWS/CRD, except that east Kenai clustered with PWS in the O’Corry-Crowe *et al.* analysis and with Kodiak sites in the review meeting iterations. When Yakutat and Icy bays were included, they clustered with either Glacier Bay or inside SE Alaska sites. During the review process, SWFSC personnel presented results of sensitivity for the Kenai/Kodiak/Kamishak region. In those analyses, samples from southern and eastern Kodiak always clustered separately from sites in northern and western Kodiak. Similarly, in the analyses requested by the reviewers, southern or southeast Kodiak clustered separately from the rest of this region until late in the analysis.

In combination, these different iterations of the BR analysis, as well as UPGMA and NJ analyses, demonstrate that, as stated in O’Corry-Crowe *et al.*, the initial definition of three stocks of Alaska harbor seals as presented in the SARs (southeast, Gulf of Alaska and Bering Sea) captured some but not all of the major population structure for Alaska harbor seals and missed some finer-scale structure entirely. On a very large scale, all analyses confirm that southeast Alaska, the northern Gulf of Alaska, and seals in the Bering Sea (Bristol Bay and the Pribilofs) are genetically distinct separate groups. However, they also indicate additional structure, in some cases more clearly than others. Westlake and O’Corry-Crowe (2002) reached the same conclusion.

Resolution of stock structure around Kodiak and lower Cook Inlet is complex. UPGMA and NJ analyses place seals from these sites in different and geographically disjunct clusters. All iterations of BR analyses, including simulations that SWFSC staff presented to the reviewers and additional iterations requested by the reviewers, cluster sites in Cook Inlet and northern/western Kodiak separately from sites in southern and eastern Kodiak. However, this is clearly a situation

where additional samples and additional analytical approaches are needed to better understand the underlying population structure and identify boundaries. Harbor seals in the southern Kodiak region (Tugidak Island) underwent an 85% decline during 1976-1988. It is unknown how such a large decline may have affected haplotype diversity within this region and thus comparisons among different clusters of sites.

Discussion and interpretation of other studies

Was the interpretation of other, non-genetic evidence relevant to harbor seal population structure logical and appropriate?

The authors presented information about trends in abundance and data from satellite-tagging studies of harbor seals in Alaska. This information was used to help make decisions about composition of initial analytical units for BR, and discussed in the context of the 12 clusters identified by the BR analysis.

To date, tagging studies have been remarkably consistent with results of genetics analyses, and confirm that harbor seals in Alaska show strong site fidelity, at least on the scale of tag longevity. However, sample sizes for both genetics studies and tagging studies are still small. For genetics studies, sample sizes in most broad regions are usually no more than 100-200 seals and sometimes less. For tagging studies, sample sizes are generally between 25-75 individuals, out of populations of thousands.

In some areas, the consideration of tagging and survey results helped to clarify interpretation of genetics results. In PWS, for example, different genetics methods placed PWS and the east Kenai in the same or different clusters. PWS tagging results indicate that both non-pup and pup seals from southern PWS do make at least short duration movements to the east Kenai region (Lowry *et al.* 2001; Small *et al.* in prep.). While this does not confirm interbreeding between the areas, it at least makes the genetic association of the two regions more plausible. Similarly, the movement of one of the tagged PWS seals to Yakutat points out the need for more samples from that area, and for comparisons to seals from the PWS region. No seal satellite tagged in PWS or on Kodiak has traveled to the other area in almost 10 years of tagging. Population trends, based on aerial surveys, are very different in Kodiak and PWS. At the time the initial three stocks were defined in the NMFS SARs, both Kodiak and PWS were declining. Currently however, seal numbers are increasing steadily in Kodiak while they continue to decline in PWS (Frost *et al.* 1999; Small *et al.* 2003).

Within Kodiak region, satellite tagging and aerial survey results do little to clarify the genetics picture. Genetics results suggest genetic differentiation between seals in south and west Kodiak. However, genetics data for eastern Kodiak were not included in the original O'Corry-Crowe *et al.* analysis. Movement data indicate that satellite-tagged pups from south Kodiak use both the east and west sides of Kodiak, although most eventually returned to the tagging location (Small *et al.* in prep). Non-pup seals tagged on the east, west and south sides generally stayed near the tagging location or moved to another region and subsequently returned (R. J. Small, personal communication). Although these are short-term studies (< 1 yr) with small sample sizes, they do indicate strong short-term site-fidelity. There are no aerial survey trend sites on the north and west sides of Kodiak; thus, it is not possible to look for differing population trends. More genetics samples are needed from Kodiak, particularly from areas that were excluded from the

BR analysis due to inadequate sample sizes. Additional tagging in what appear to be boundary regions would also be informative.

In southeast Alaska, O’Corry-Crowe *et al.* excluded several initial units from the BR analysis because sample sizes were small or they were > 50 km from adjacent sites. However, satellite tagging data indicate that seals tagged in inside waters of southeast Alaska in Frederick Sound traveled north to Haines and south almost to the Ketchikan region (ADF&G, unpubl. data), and suggests that Ketchikan and Frederick Sound may not be demographically separate. Genetics information is not conclusive since these sites did not differ at the $p < 0.05$ level. Tagging data suggests that intermediate areas with small n_a could be included with other samples from inside waters in the overall analysis. This would result in fewer voids in sample coverage for southeast.

Different population trends for Ketchikan and Sitka suggest there may be demographic isolation between harbor seals in inside and outside waters of southeast Alaska. However, trend data are not available for other southeast Alaska regions such as Frederick Sound or Grand Island.

Tagging data indicate that seals tagged in the inside waters of Frederick sound moved extensively north and south but did not travel to the outer coast. Similar demographic isolation has been documented for harbor seals in inside and outside waters of Washington and Oregon (Lamont *et al.* 1996).

In Bristol Bay, satellite tagging data indicate substantial movement of seals among haulouts along the north side of the Alaska Peninsula as well as within inner Bristol Bay. These data could have been used by the authors to justify inclusion of samples from Port Moller and Port Heiden in the BR analysis and provide a more extensive picture of stock boundaries.

O’Corry-Crowe *et al.* discuss information about harbor seal diet in the context of demographic structure. This does not appear to be an appropriate tool for use in identifying population units or in clarifying interpretation of genetics data regarding stock definition. It is to be expected that seals in different regions, as well as those feeding in different habitats within the same geographic region, will have different diets depending on prey availability. For example, seals in PWS from haulouts only a few kilometers apart had different fatty acid signatures indicating different diets (Iverson *et al.* 1997). Diet may also vary by age, gender or individual preference.

Conclusions

Were the conclusions sound and derived logically from the results? Specifically, are the twelve population units described in the report consistent with the definition of stocks, as provided in the Marine Mammal Protection Act (MMPA) and as implemented by NMFS (see reference 4, Wade and Angliss, 1997)?

The research findings presented in the report appear to be sound and logically derived using methods that were refined and tested with management applications in mind. The authors are clearly working hard to develop methods that facilitate management decisions and to choose “a scale that will allow management to meet the objectives of maintaining the range while avoiding management units that are so small that demographic independence is not plausible.” The BR method employed by O’Corry-Crowe *et al.* was specifically designed and tested to identify stock boundaries in continuously distributed species such as harbor seals. Nonetheless, current limitations in sample size, coupled with a haplotype frequency approach that results in elimination of some existing samples from the analysis, result in some large geographic gaps

between the twelve proposed stocks. Questions remain about effects of sample coverage on the final outcome of the analysis. Therefore, although the statistics applied in the BR analysis demonstrate 12 population units at the $p < 0.1$, I think it is premature to seek a management approach based on all of these 12 units.

As stated in the report of the Guidelines for Assessing Marine Mammal Stocks (Wade and Angliss 1997) “the concept of stock or management unit is a human construct, and these units should be designed in a way to facilitate management.” Furthermore, the report states that “It was concluded that splitting was to be preferred in situations where incorrect lumping could lead to the depletion of a stock,” but by the same token that “...stocks should not be split into unreasonable small units.” Any management scheme in Alaska must be soundly based as well as defensible and understandable to the people it affects. It is essential that management be precautionary to ensure that harbor seals remain “functioning elements of their ecosystem” as required by the MMPA and are conserved for subsistence and other uses as required by the co-management agreement between NMFS and the ANHSC. However, management must also be implementable.

The 12 population units proposed in O’Corry-Crowe *et al.* include five in southeast Alaska, yet more than half of the overall area, including hundreds of haulouts, thousands of seals and numerous communities that harvest seals, is excluded from these units. A similar situation exists in the Kodiak/lower Cook Inlet region where three units are defined yet very large areas with many seals and many harvesting communities are excluded. The proposed scheme for population subdivision is not implementable in these regions. Clearly, additional samples are needed, as well as additional genetics methods and other types of biological information.

Although there are problems with devising a management scheme based on all 12 proposed population units, there are clearly areas where geographic coverage and sample size are adequate. For example, all lines of genetics evidence, as well as tagging information and population trend data, indicate that the PWS/CRD/east Kenai region is demographically isolated from adjacent areas and that the population is declining. There is also significant human-caused mortality in this region in the form of subsistence hunting. Regardless of whether or not stock definition is clear in southeast Alaska or near Kodiak, the data indicate that the PWS region should be managed as a separate stock. Management actions for this region do not need to await further resolution of population structure in other areas. Data are similarly quite straightforward and adequate for Bristol Bay and for the Pribilofs.

Taylor (2003) stated that “inconsistent definitions do not necessarily mean that management will be inconsistent.” She pointed out that where populations are healthy, there may be no need for active management, and stock definition boundaries may have few consequences. In contrast, in areas where human-caused mortality is significant and populations are declining, inadequate management based on inappropriate stock definition boundaries may have serious consequences. This appears to be the case in Alaska. For some regions, such as southeast where populations are stable or increasing (except Glacier Bay), there may not be an immediate need for active management. There is time to collect additional samples, refine analytical techniques, and pursue new lines of evidence. In others, such as PWS, the existing data are adequate, and there is an immediate need to address stock identification and determine what actions can be taken to reverse the ongoing decline. For some regions, such as Glacier Bay, abundance is declining rapidly yet evidence for stock structure is not entirely clear when a variety of methods are considered. This area may be one where inappropriate lumping and failure to identify stock

structure may have deleterious consequences. Areas such as this should be an immediate priority for additional sample collection, as well as the application of other information and techniques (such as microsatellite analysis) that might clarify the situation.

These findings indicate that current stocks of harbor seals in Alaska are too broadly defined to meet the management objectives of the MMPA of maintaining population stocks as functioning elements of their ecosystem.

This conclusion that current stocks of harbor seals in Alaska are too broadly defined is well-supported by the review document. The authors of the review document based their conclusions on a new approach termed Boundary Rank (BR) that was developed specifically to address stock identification in a management context. However, BR results were also compared to several other analytical methods and statistical approaches. These comparisons strengthened the conclusions and also highlighted areas where additional information is needed. The conclusion that current stocks of harbor seals in Alaska are too broadly defined is also supported by the analyses of Westlake and O’Corry-Crowe (2002) indicating additional structure within the currently defined Gulf of Alaska and Bering Sea stocks.

Regardless of method and despite some uncertainty in the specifics of stock definition and boundaries, additional population structure beyond the three stocks defined in the Alaska harbor seal SARs (Angliss and Lodge 2002) is clearly indicated.

Gulf of Alaska stock - The Gulf of Alaska stock as defined in NMFS SARs includes waters from Cape Suckling (< 100 km east of the CRD) to Unimak Pass (along the south side of the Alaska Peninsula) and throughout the Aleutian Islands. As currently defined, this stock includes seals in Kodiak as well as PWS/CRD. In all genetics approaches, PWS/CRD/east Kenai and seals from Kodiak clustered on different dendrogram branches early and consistently in the analyses. Only when sample size was constrained in an exploratory BR iteration requested by reviewers did the PWS sites not sort out cleanly from all other regions, suggesting not that stock definition was questionable but that sample size is critical in obtaining an accurate picture.

Other biological information (movement data from tagging studies; population trend data) also supports the conclusion that Kodiak and PWS represent separate management units. Despite ecological conditions that have allowed abundance in the Kodiak region to increase for almost a decade, abundance in PWS continues to decline. The review document, particularly in combination with accompanying background documents that demonstrate the danger of defining too few stocks when one is declining, makes a convincing case that Kodiak and PWS/CRD/east Kenai should be managed as separate stocks in order to meet objectives of the MMPA to maintain population stocks as functioning elements of their ecosystem and of the co-management agreement between the Alaska Native Harbor Seal Commission and NMFS to ensure that harbor seals are conserved for subsistence and other uses.

Data are insufficient to resolve how seals within the Kodiak region, Kamishak Bay, and Cook Inlet should be treated. Sample size is too small for some sites to have been included in the BR analysis. For others, different methods produced different relationships on genetic dendrograms. For all methods and in sensitivity testing conducted by O’Corry-Crowe *et al.* (described to reviewers but not included in their report) south and west Kodiak clustered in different groups, strongly suggesting population structure within this region. However, limited sample coverage

in some areas and the resulting omission of large areas from the final analysis make BR results of limited use in the management context. Because abundance has been increasing in this region for almost a decade, it is less critical that stock structure be immediately resolved.

The relationship of seals along the south side of the Alaska Peninsula to those from Kodiak and the Aleutian Islands is completely unknown. Sample size was too small for these regions to have been included in the O’Corry-Crowe *et al.* analyses. Exploratory analyses conducted as part of the review process suggested that these sites would cluster with the Pribilof Islands, and very late in the BR analysis with Bristol Bay, but not with other Gulf of Alaska sites. However nothing definitive can be implied from these explorations except that many more samples are needed.

Bering Sea stock – The Bering Sea stock as defined in the NMFS SARs includes both Bristol Bay and the Pribilof Islands. O’Corry-Crowe *et al.*’s BR analysis, as well as results of other analyses they compared to BR, indicated that north and south Bristol Bay seals clustered together very early. The Bristol Bay conclusions would have been more useful if they had also included seals at adjacent sites along the north side of the Alaska Peninsula. Exploratory analyses placing these sites with south Bristol Bay in an initial unit did indicate early clustering between this unit and north Bristol Bay, and not with other units. Bristol Bay seals clustered with Pribilof Island seals only very late in the BR analysis and not at all using UPGMA and NJ. Thus, O’Corry-Crowe *et al.*’s conclusions that stocks are too broadly defined seems clearly to be the case for the Bering Sea stock.

Southeast Alaska – NMFS SARs define a southeast Alaska stock that extends from the Canadian border to Cape Suckling. This broad region includes Yakutat and Icy bays, which were not included in the O’Corry-Crowe *et al.* analysis due to small adjusted sample size, as well as sites in what is more traditionally considered southeast. Exploratory analyses that lumped Yakutat and Icy Bay suggested they were more closely related to southeast Alaska sites than to PWS. For the remainder of southeast, small sample sizes for some initial units resulted in large gaps in the final BR analysis. BR clustered only two of the initial units (Frederick Sound and Ketchikan) at $p < 0.05$. UPGMA and NJ also clustered these initial units and tagging data suggest substantial movement within inside waters, lending strength to the conclusion that these sites constitute a demographically isolated unit. For other southeast units, clustering by different methods was less consistent, even though each unit was differentiated at $p < 0.05$ using BR. In combination, the analyses presented by O’Corry-Crowe *et al.* strongly suggest additional structure within the existing “southeast Alaska stock” but many questions remain about the actual number of units and their boundaries. The investigators should be encouraged to consider the analysis of microsatellite data, particularly for regions where sample size is limited and sites were excluded due to high haplotype diversity and resulting low n_a ’s. Microsatellite data may be useful in determining with which groups these excluded sites belong.

These findings also provide a framework for the identification of more meaningful management stocks and highlight the need for a re-appraisal of other information of relevance to stock structure including the interpretation of information on distribution, movement patterns, trends in abundance and foraging ecology as well as the incorporation of traditional ecological knowledge.

The findings in O’Corry-Crowe *et al.* do provide a framework for identification of stocks that will be more useful for making management decisions and guiding actions related to human-

caused mortality. This is particularly true for PWS and Bristol Bay where evidence for demographic independence is quite clear and consistent with movements and abundance data. For PWS, where abundance has been declining for almost 20 years, this information should be immediately applicable to management decisions whether or not stocks have been officially redefined. For other areas, limitations imposed by small sample size and large gaps in sample coverage limit the immediate utility of this analysis for management decisions. This is particularly true in regions like southeast Alaska and Kodiak where different analytical methods suggested somewhat different versions of structure and large gaps occur between areas identified as separate management units.

While some reappraisal of existing information relative to stock structure may be useful (for example using movements data to support inclusion of north Alaska Peninsula sites and some omitted sites in southeast Alaska in the analyses), there is a greater need for additional analytical methods that can take advantage of a larger proportion of the samples that have been collected. Targeted tagging in hypothesized boundary regions, particularly if it incorporates methods that extend data collection beyond a single year, may help to clarify genetics results. Similarly, additional trend data for some areas may be useful. The greatest need, however, is for more samples collected in a targeted manner from areas where boundary definitions are unclear.

The genetic study is still limited by sample coverage. Substantial gaps exist in areas of high conservation concern (see the non-circled areas in Figure ES-3), including the Aleutian Islands, the Alaska Peninsula, the northeastern Gulf of Alaska and parts of Southeast Alaska and the Kodiak Archipelago. Active collaboration with Alaska Native subsistence hunters and directed sampling is necessary if these important areas are to be sampled.

Inadequate sample sizes and gaps in sample coverage are strongly limiting the ability of genetics studies to identify meaningful management units throughout the state. While the current analysis is very informative, its application to actual conservation and management decisions is limited by the large areas that are omitted from the final conclusions. The power of sufficient sample size is evident for PWS where sampling has been geographically and temporally extensive and about 6% of the population has been sampled. Over 30% of the samples included in the final BR analysis were from PWS. As a result, conclusions about PWS stock structure were consistent and robust across all analytical methods.

In contrast, less than 1% of the population was sampled in other areas. It is essential that additional samples be obtained from these regions. In recent years, subsistence hunters in southeast Alaska (excluding Yakutat) have harvested more than 1,000 harbor seals per year (Wolfe *et al.* 1999, 2002), yet the total sample size for the southeast is just over 200. The annual harvest in Yakutat normally exceeds 200, but there are only 21 genetics samples from Yakutat. A concerted effort needs to be made to obtain samples from these harvests. If biosampling programs do not provide adequate samples in the very near future, or if there are boundary questions in areas where subsistence hunting does not occur, then directed field programs should be undertaken to provide these samples.

Although further sampling is needed to refine stock boundaries, the conclusion that there are multiple small units that need to be managed as separate stocks is not likely to change.

The BR analysis presented in O’Corry-Crowe *et al.*, as well as other genetics analyses presented for comparative purposes, present convincing evidence that there are more than three stocks of harbor seals in Alaska. How many stocks are ultimately defined will depend on obtaining adequate sample coverage for all regions, development of methods that can perhaps collapse unique haplotypes that are just 1 or 2 mutational steps apart, and/or incorporating other genetic evidence such as microsatellite data to clarify the overall picture.

Recommendations

- 1) Additional samples should be obtained from areas such as Yakutat Bay, Icy Bay, the Aleutian Islands and the Alaska Peninsula. These areas represent large sections of the Alaska coastline containing thousands of seals for which there is no information on stock structure from genetics or other types of studies. Currently, adjusted sample sizes are too low for sites to be included in the BR analysis. For example, even though more than 10,000 seals occur along the north side of the Alaska Peninsula (Small *et al.* 2003), this region was excluded from the BR analysis because there were too few samples. The exclusion of such large areas from the 12 demographic clusters identified by O’Corry-Crowe *et al.* limits the management applications of these findings.
- 2) Additional samples should be collected from geographic areas where stock definition is unclear or incomplete. Although there is considerable genetic evidence of finer population structure for harbor seals within the Kodiak region and southeast Alaska, there are substantial gaps in sample coverage which make the current BR analysis incomplete. For example, northern and eastern Kodiak are omitted entirely from the BR analysis due to inadequate sample size, yet more than 3,000 seals were counted there during trend count surveys (Small *et al.* 2001). Similarly, large areas were not included in the five clusters identified by BR in southeast Alaska. Information for Kodiak and southeast is particularly important since harbor seals are an important subsistence resource in these areas. Information about stock structure is necessary for determining sustainable levels of take on these and other regions. Additional samples are particularly needed from areas such as Glacier Bay where abundance is declining and conservation concerns may exist.
- 3) If adequate samples are not available through the ANHSC biosampling program, then a directed sampling program should be initiated in these areas. This could be done through a combination of the following: a) live capture and sample as many seals as possible in key areas, b) collect scat, and c) search museum and/or agency archives for hard tissue samples (such as claws and teeth) which might be analyzed using newly developed techniques.
- 4) Funding should be made available to conduct a thorough analysis of available microsatellite data, particularly as they relate to stock structure within southeast Alaska and near Kodiak. As necessary, additional laboratory analyses should be conducted to ensure adequate sample sizes. No method is likely to be perfect for answering the stock structure question, but multiple lines of evidence from different analyses is likely to help refine understanding of true population units. Laboratory analysis for microsatellites has been completed for more

than 400 seals for which there are also mtDNA data. Staff should be provided the time and resources to integrate analyses of these samples with other genetics results.

- 5) New analytical methods should be explored that will include more of the existing samples in a haplotype frequency analyses. For example, is there a way to combine haplotypes that are separated by only 1-2 mutational steps? Because haplotype diversity and the incidence of unique haplotypes is so high in Alaska harbor seals, current analyses using F_{st} statistics exclude about 25% of the total samples from the final analysis.
- 6) Movements data from satellite tagging studies in Bristol Bay, Kodiak and southeast Alaska should be further reviewed to see whether this information can guide inclusion of currently excluded units in the final analysis (for example, north Aleutian sites with Bristol Bay; Red Bay and Wrangell in southeast).
- 7) The presentations made to reviewers were outstanding. They were well-organized, clearly presented and led reviewers through all steps of the analytical process. They included the legal framework under the MMPA and the co-management agreement, the conceptual framework for the concept of stock identification, a discussion on genetics basics, the basics of harbor seal biology, and then the specifics of the study under review. A workshop should be held in which these presentations are made to representatives of harbor seal hunting communities throughout Alaska, the ANHSC, and perhaps also to representatives of the fishing and tourist industries. This issue is extremely important to hunters and others who want to conserve this important resource. The material is complex and hard to research and integrate without the help of experts. One of the most important contributions that could be made to the co-management process would be presenting this information to the hunters themselves so that they understand the complexity and consequences of the decisions that are made and the need for samples and the way in which they are used.

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Appendix I.

STATEMENT OF WORK

Background

In the 1995 Alaska Marine Mammal Stock Assessment Reports, the National Marine Fisheries Service (NMFS) defined three stocks of harbor seal in Alaska, based primarily on broad-scale geographic differences in trends in abundance. NMFS, however, recognized that considerable uncertainty about Alaskan harbor seal stock structure remained and in the fall of 1994 initiated genetic studies of harbor seal stock structure in Alaska. The report resulting from these studies, "The Analysis of Population Genetic Structure in Alaskan Harbor Seals, *Phoca vitulina*, as a Framework for the Identification of Management Stocks," is the subject of this review.

The format of this review will include an interactive panel to ensure a thorough presentation of the science as well as the management context. Further, the best way to obtain review and scientific recommendations from the panel is to establish a process that allows reviewers with different expertise both to interact with one another and to interact with the scientists responsible for the research being reviewed. These interactive presentations and discussions may require up to two full days of the panel's time. A third day should be planned for the review panel to provide feedback to the authors and to begin to draft the review. Although this review is for scientific research, the motivation for the research was to provide guidance for resource management. The management context is summarized in the report to be reviewed.

Reviewer Responsibilities

Expertise needed to review this analysis will include the following expertise: (1) knowledge of harbor seal biology, especially expertise in behavior and movements; (2) knowledge of population genetics, including statistical analysis of genetic data to detect/delineate population structure; (3) knowledge of conservation genetics including the different uses of mitochondrial and nuclear DNA in a conservation context; and (4) general knowledge of marine-mammal biology, bearing on population structure including basic population dynamics and an understanding of metapopulation dynamics.

Documents supplied to the consultant shall consist of draft manuscripts and a number of background papers (relevant publications and reports). The consultant shall become familiar with the ten references (see Appendix I), focusing on references 1, 3, and 10. Reference 10 provides the details needed to address the novel method referred to in Task 3B, described below. The consultant's duties shall not exceed a maximum total of three weeks, including one week to read all relevant documents, three days to attend a meeting with scientists at the NMFS La Jolla Laboratory, in San Diego, California, and several days to produce individual written reports comprised of the consultant's comments and recommendations. It is expected that the consultant's report shall reflect that his/her area(s) of expertise; therefore, no consensus opinion (or report) will be required.

Specific Reviewer Tasks and Schedule

1. Read and become familiar with the relevant documents provided in advance of the panel meeting.
2. Discuss relevant documents with scientists at the NMFS La Jolla Laboratory, in San Diego, CA, for three days, March 16-18, 2004.
3. Specifically address the following points (at a minimum):
 - A) Genetic samples and data:

Were the methods of selecting, collecting, and handling samples adequate relative to the conclusions drawn?

Were limitations of the sampling scheme and data adequately acknowledged and considered?
 - B) Analytical methods:

Were the laboratory analyses appropriate and applied correctly?

Were the statistical analyses appropriate and applied correctly?

Were the novel methods used in the study developed and tested in a scientifically sound manner?
 - C) Discussion and interpretation of other studies:

Was the interpretation of other, non-genetic evidence relevant to harbor seal population structure logical and appropriate?
 - D) Conclusions:

Were the conclusions sound and derived logically from the results? Specifically, are the twelve population units described in the report consistent with the definition of stocks, as provided in the Marine Mammal Protection Act (MMPA) and as implemented by NMFS (see reference 4, Wade and Angliss, 1997)?
4. Address the primary conclusions as stated in the executive summary of reference 1. Specifically, state whether each of the following conclusions is scientifically sound, and provide justifications for each of their assessments.
 - A) These findings indicate that current stocks of harbor seals in Alaska are too broadly defined to meet the management objectives of the MMPA of maintaining population stocks as functioning elements of their ecosystem.
 - B) These findings also provide a framework for the identification of more meaningful management stocks and highlight the need for a re-appraisal of other information of relevance to stock structure including the interpretation of information on distribution, movement patterns, trends in abundance and foraging ecology as well as the incorporation of traditional ecological knowledge.

C) The genetic study is still limited by sample coverage. Substantial gaps exist in areas of high conservation concern (see the non-circled areas in Figure ES-3), including the Aleutian Islands, the Alaska Peninsula, the northeastern Gulf of Alaska and parts of Southeast Alaska and the Kodiak Archipelago. Active collaboration with Alaska Native subsistence hunters and directed sampling is necessary if these important areas are to be sampled.

D) Although further sampling is needed to refine stock boundaries, the conclusion that there are multiple small units that need to be managed as separate stocks is not likely to change.

5. No later than April 1, 2004, submit a written report of findings, analysis, and conclusions (see Annex 1). The report should be addressed to the University of Miami Independent System for Peer Reviews, and sent to David Die, UM/RSMAS, 4600 via email to ddie@rsmas.miami.edu, and to Mr. Manoj Shivlani via email to mshivlani@rsmas.miami.edu.

Appendix II: Background material.

1. O’Corry-Crowe, G. M., K. K. Martien, and B. L. Taylor. 2003. The analysis of population genetic structure in Alaskan harbor seals, *Phoca vitulina*, as a framework for the identification of management stocks. Administrative Report LJ-03-08. Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA, 92037.
2. The Marine Mammal Protection Act (MMPA; specifically Sections 2 [findings and declaration of policy], 3(11) [definition of population stock], and 117 [stock assessments]).
3. Taylor, B.L. 1997. Defining populations to meet management objectives for marine mammals. pp. 49-65 in Molecular Genetics of Marine Mammals (A.E. Dizon, S.J. Chivers, and W.F. Perrin, eds.) Special Publication 3. Society of Marine Mammalogy, Lawrence, Kansas.
4. NMFS' guidelines for identifying population stocks (Wade, P.R., and R.A. Angliss. 1997. Report of the GAMMS Workshop, April 3-5, Seattle, Washington. NOAA Technical Memorandum NMFS-OPR-12. National Marine Fisheries Service, Office of Protected Resources, Silver Spring, MD. 69 pp.).
5. ANHSC and NMFS. 1999. Agreement between the Alaska Native Harbor Seal Commission and the National Marine Fisheries Service, for conservation and management of harbor seals. 12 pp.
6. Westlake, R. L., and G.M. O’Corry-Crowe. 2002. Macrogeographic structure and patterns of genetic diversity in harbor seals (*Phoca vitulina*) from Alaska to Japan. *Journal of Mammalogy* 83(4): 1111-1126.
7. Federal Register notice 67[165]:54792-54794.
8. Martien, K. K., and B. L. Taylor. 2003. Limitations of hypothesis-testing in defining management units for continuously distributed species. *Journal of Cetacean Research and Management*.
9. Taylor, B. L. 2003. Determining Units to Conserve. Unpublished manuscript presented at the Marine Mammal Commission Workshop on Future Directions in Marine Mammal Research, August, 2003, Portland, OR.
10. Martien, K. K., A. B. Sellas, P. E. Rosel, B. L. Taylor, and R. S. Wells. Submitted. Defining management units in Gulf of Mexico bottlenose dolphins in the absence of long-term observational data.