

Response to *The University of Miami Independent System for Peer Review*
Review of Alaskan Harbor Seal Stock Assessment by Kathy Frost

by Greg O’Corry Crowe, Karen Martien & Barbara L. Taylor
Southwest Fisheries Science Center

Major point #1: *Exclusion of so many samples and resulting gaps in analysis make results unimplementable*

The reviewer argues that the exclusion of 228 of the sequenced samples from our final analyses, and the resulting geographic gaps in the results, render the results of the study ‘unimplementable’ in a management context. We disagree with this assessment. From a scientific viewpoint, the exclusion of these samples is not only justifiable, but is required in order to avoid producing results that are biased and potentially misleading.

As we explain in Appendix 3 of the reviewed document, if the sample size from an area is small relative to the haplotypic diversity of the area, as is the case for the initial units we excluded from our analyses, then any frequency-based measure of genetic differentiation between that area and another will be negatively biased. In other words, the two areas will appear to be more genetically similar to each other than they actually are. If we had included such poorly sampled areas in our analyses, they would have tended to cluster together early in each of the three clustering analyses, suggesting that they are more genetically similar to each other than to better sampled strata. This result would have been corroborated by the hypothesis-testing analysis, which would have shown these poorly-sampled units to be not significantly differentiated. However, this apparently robust result would not have been a true reflection of population structure, but rather a result of a known bias in the statistical measure used to assess differentiation. By excluding these areas, we were able to largely eliminate this source of bias and produce results that, though less comprehensive, are more accurate.

By excluding poorly sampled areas we were also able to avoid creating the impression that genetic data were able to provide useful information in areas from which sampling is simply inadequate to do so. The Alaskan Peninsula and Aleutian Islands are an excellent example. Though this region accounts for a quarter of the initial units defined in our analyses, it contains only 6% of the total samples sequenced for the study. With only 54 samples distributed along more than 2,100 kilometers of coastline, it would clearly be inappropriate to make management decisions in this area on the basis of the available genetic data. By excluding this and other poorly-sampled areas of the state from our analyses, we were able to highlight areas from which further genetic sampling and/or the analysis of other types of data will be necessary before stock boundaries can be drawn.

The criticism that our results are ‘unimplementable’ may stem from a misunderstanding of the goals of our study and the steps we feel must be taken before our results can be incorporated into a management decision regarding the exact number and

placement of stock boundaries. The goal of this study was to determine what the available genetic data can tell us about stock structure in Alaskan harbor seals. The goal was not to determine the exact number and placement of stock boundaries. The reviewer is right: our genetic results alone cannot be used to immediately implement a revised stock structure for Alaskan harbor seals; the gaps in coverage of genetic samples prevent this. However, our results can be used as a framework for developing a final stock definition. In other words, the twelve demographically-independent units identified in our study should serve as a starting point for stock definition. Our analyses indicate that the dispersal rate between these units is low enough that each unit needs to be managed independently from the others. Thus, the final stock definition must place each of the 12 units we identified in a separate stock.

Decisions regarding how to incorporate areas not included in our analyses into the final stock definition will have to be made on the basis of other types of data. The appropriate stock structure in these areas cannot be determined from the available genetic data; that is why they were excluded from our genetic analyses. In many of these areas, non-genetic data, such as tagging, trends in abundance, traditional ecological knowledge, distribution and habitat, are available to help guide the placement of stock boundaries. In other areas, few such data are available and decisions regarding the definition of stocks will be far more subjective. The integration of all of the available data into a final stock definition is a task that will require the input of many people and will ultimately fall to managers. An example of this integration step can be seen in the Federal Register Notice produced by the Alaska Region (AKR)(NOAA 2002). In that notice, AKR presents a set of proposed stocks for harbor seals that are based on the results of our genetic study but use non-genetic data to determine the precise number and location of stock boundaries. The notice illustrates the method by which the results of our study can be ‘implemented’ in a management context. A similar case with sampling gaps was encountered in identifying stock structure for harbor porpoise along the coasts of California, Oregon and Washington. A paper was published (Chivers et al. 2002) that presented the genetic results (as far as they went) and the stock assessment reports used these data together with data on distribution to refine stock boundaries.

Major Point 2. In areas where structure is unclear, microsatellites should be used.

“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.”

We agree that although our first priority should be expanding the mtDNA study by sequencing more samples from poorly represented areas, that as a secondary future level of research, microsatellite data may improve analyses. For example, new Bayesian methods have just been published that allow assignment tests for multiple samples taken together of unknown “origin” (in this case stock). Doing such analyses is not trivial, since good genetic representations of potential stocks is necessary. However, if

resolution is not forthcoming through adequate sampling (the preferred solution) then some areas may be salvaged through this type of approach.

Major point #3: *Tagging data should be incorporated more explicitly into the definition of initial units in order to allow the inclusion of more samples in the clustering analyses*

The reviewer felt that tagging data should be incorporated more explicitly into the definition of initial units. For example, she argued that the tagging data showing a high degree of movement between Port Moller and Port Heiden, both on the northern side of the Alaskan Peninsula, could be used as justification to combine these two areas into a single initial unit, despite the fact that they are more than 50km apart. Combining these areas would increase sample size enough to allow their inclusion in the genetic analyses.

We considered this approach when discussing how to define initial units. However, we concluded that our study would be of greater use to managers if we focused on what genetics alone can tell us about population structure. This approach allows us to compare our results to those obtained from other types of studies, lending greater confidence in areas where our results agree with others, and perhaps new insight in areas of disagreement. For instance, the fact that all four Prince William Sound and Copper River Delta initial units cluster together early in our analysis corroborates the results of tagging studies that show a high degree of movement between these areas.

We agree that tagging data will be extremely valuable in clarifying stock structure, particularly in areas from which genetic data are sparse. However, we feel that this integrative step is best performed subsequent to the genetic study when final stock designations are being made. In this way, it can be made clear which data are being used to justify the location of each boundary. For instance, currently available genetic data are not sufficient to say that Port Moller and Port Heiden belong in the same stock, nor that they both belong in the same stock as northern Bristol Bay. However, if managers agree that the tagging data are sufficient to draw that conclusion, they should draw stock boundaries accordingly and cite the tagging data, not the genetic data, as justification for doing so.

Major point #4: *New methods should be developed to reduce haplotypic diversity and allow inclusion of more samples*

We agree, and have been working on such a method for several years. We have tried available methods to identify and remove the highly mutable base pairs that contribute to the problems engendered by high diversity, but none were effective for the harbor seal case. A new method (SiteSnipper developed by B.L.Taylor) is now in the phase of bias testing. This method sequentially removes the base pairs according to their contribution to the number of unique haplotypes (haplotypes found in only a single individual). Although the method is promising, it will take a minimum of several years to pass peer review and reach publication. The harbor seal study has already been

delayed for the peer review and publication step for the new method of Boundary Rank. Acceptance of new methods is a time consuming effort with an unpredictable outcome. Although we will continue our efforts to complete and publish SiteSnipper, we note that such an approach constitutes an attempt to ‘make the most’ out of an inadequate sample size, and that increasing sample sizes where possible so as to avoid the need for such a method is preferable.

Minor point #1: *Limited sample coverage in Cook Inlet and Kodiak Archipelago rendered the results of the clustering analyses of limited use in the management context*

The reviewer asserts that the limited sample coverage in Cook Inlet and the Kodiak Archipelago render the results of the clustering analyses in this area of limited use in a management context. We agree that genetic data alone are insufficient to fully resolve stock structure in this area. However, we disagree that these limitations render our results ‘of limited use.’ All of the genetic analyses, including the hypothesis testing, dispersal rate estimation, and all three clustering approaches, show that Kamishak Bay in lower Cook Inlet, West Kodiak and South Kodiak are demographically independent, with an estimated dispersal rate of fewer than 2 dispersers per year. This demographic independence is sufficient to conclude that these three areas should be managed separately. This result is of considerable value to managers attempting to define stocks in this area.

Nonetheless, as is true in many poorly-sampled areas of the state, the incorporation of non-genetic data and/or additional genetic sampling are needed in order to determine exactly where the boundaries separating these three areas should be placed and whether additional stocks are necessary in Cook Inlet, where genetic data are very sparse. A considerable amount of tagging data is available from the Kodiak Archipelago, primarily from the southern islands. These data should be helpful in clarifying stock structure along the southeastern portion of the archipelago. Furthermore, in the months between the completion of the CIE review meeting and receipt of the resultant reviews, we have received and analyzed additional samples from the eastern side of the Kodiak Archipelago. These samples will be included in the manuscript we are currently preparing for publication, and may help to clarify boundary placement within the Kodiak Archipelago.

Minor point #2: *Despite significant differentiation in the BR analysis, the fact that southeast units clustered differently using different methods raises questions about the actual number of units and their exact boundaries in this area*

We agree that, like most areas of the state, the exact number and location of boundaries in Southeast Alaska cannot be determined based on the genetic data alone, due to the fact that many parts of this region are unsampled or inadequately sampled. However, we disagree with the suggestion that the different clustering order from the different analyses is cause for concern. All pairwise comparisons of initial units from Southeast were significantly differentiated at the level of $\alpha = 0.05$ in the hypothesis-testing analysis except for Grand Island/Glacier Bay and Frederick Sound/Ketchikan.

Both of these comparisons were significant at $\alpha = 0.1$. Grand Island and Glacier Bay are not spatially contiguous and do not cluster together in any of the clustering analyses (including the two that do not impose a geographic constraint). Frederick Sound and Ketchikan were found to exchange dispersers at a low rate. Thus, the results of all of the genetic analyses combined suggest that these five areas represent five demographically independent units. Though the higher level relationships between these units may be unclear, as evinced by the different clustering results, the fact that they are all significantly differentiated (at $\alpha = 0.1$) and exchange few dispersers indicates that they should be managed separately. The hierarchical genetic relationships between these demographically independent units are simply not relevant to management. Thus, the only remaining question in Southeast Alaska is where the surrounding poorly sampled or unsampled areas should be placed in the final stock delineation.

Note that the reviewer was mistaken in stating that ‘significant differentiation’ was detected between units in Southeast Alaska in the Boundary Rank analysis, as none of the clustering analyses make any attempt to assess significance; only hypothesis-testing and dispersal rate estimation were used to assess the statistical or biological significance of any result.

References:

Chivers, S. J., A. E. Dizon, P. Gearin, and K. M. Robertson. 2002. Small-scale population structure of eastern North Pacific harbor porpoise, *Phocoena phocoena*, indicated by molecular genetic analyses. *Journal of Cetacean Research and Management* 4(2):111-122.

NOAA. 2002. New information indicates fine-scaled stock structure for harbor seals in Alaska. *Federal Register notice* 67[165]:54792-54794.