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Response to *The University of Miami Independent System for Peer Review Review of Alaskan Harbor Seal Stock Assessment by* ***A. Rus Hoelzel***

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We respond to several suggestions made by the reviewer regarding our research on stock structure in harbor seals in Alaska. We restrict our comments to the primary points with which we have differing views to the Hoelzel review. We have provided a direct quotation of the point to which we are responding at the beginning of each response for the reader's convenience.

"… I would recommend further analysis of the available data towards a clearer assessment of possible stock boundaries. This should incorporate data based on both mtDNA and microsatellite analysis." (page 1, final paragraph)

Our response is divided into two sections, the first deals with the rationale behind our emphasis on mtDNA, the second summarizes our microsatellite findings to date

A. Justification for the analysis of mtDNA variation

Although we agree that additional analysis of bi-parentally inherited, nuclear DNA markers, such as microsatellites, could strengthen our understanding of population structure in Alaskan harbor seals, we fundamentally disagree with Dr. Hoelzel that inclusion of nuclear DNA is a necessary component to identifying stock structure and note a confusion between demographic isolation and reproductive isolation. We went to some length in the Administrative Report to pull language from agency guidelines on the definition of "stock" to avoid confusion on this issue. The salient sentences are "For the purposes of management under the MMPA, a stock is recognized as being a management unit that identifies a demographically isolated biological population." And "Demographic isolation means that the population dynamics of the affected group is more a consequence of births and deaths within the group (internal dynamics) rather than immigration or emigration (external dynamics). Thus, the exchange of individuals between population stocks is not great enough to prevent the depletion of one of the populations as a result of increased mortality or lower birth rates." And finally, "*Interbreed when mature* is acknowledged to include cases in which either: i) mating occurs primarily among members of the same demographically isolated group; or ii) the group migrates seasonally to a breeding ground where its members interbreed with members of the same group and with members of other demographically distinct groups that have migrated to the same breeding ground from other feeding areas (e.g., North Atlantic and central North Pacific humpback whales)." These regulatory definitions allow for stocks to be demographically isolated while not necessarily being reproductively isolated, as is the case for humpback whales. In such a case, the demographically isolated units would be identifiable through their mtDNA but not through nuclear DNA (a more detailed explanation follows in the next paragraph). Using these definitions, a positive finding of structure using mtDNA can never be negated by negative findings using nuclear DNA.

In the cases where populations are both demographically and reproductively isolated, nuclear DNA would strengthen results from mtDNA. Because limited tagging data indicate that adult male harbor seals appear not to disperse more frequently than females, it may be worthwhile to pursue nuclear DNA analyses, and we have begun such a study (see B below). However, we emphasize that this is not *necessary* given positive findings for population structure using mtDNA.

Many genetic markers have found application in the analysis of population structure and dispersal patterns in wildlife species, including blood proteins, enzymes, coding and non-coding segments of DNA, and nuclear and cytoplasmic genomes. The analysis of variation within mtDNA has special application to the resolution of demographic relationships among animal groupings, and has been widely used in the identification of units of conservation and management (Moritz, 1994; Avise, 1995). As the limiting sex, females define the reproductive potential of a population. Female dispersal, and not male dispersal, therefore defines the demographic relationships among groups of animals. Because of its strict maternal mode of inheritance, patterns of variation within mtDNA reflect the dispersive behavior of females over time, and thus the demographic relationships among groupings. By contrast, patterns of variation within bi-parentally inherited nuclear markers, including microsatellites, are influenced by both male and female patterns of dispersal. In many mammalian species, dispersal is biased towards males (Greenwood, 1980) such that differentiation may be minimal and often undetectable in nuclear markers even when females are highly philopatric. Considering the added effects of a much slower rate of genetic drift in nuclear markers due to a much larger effective population sizes (N_e), it is expected that differentiation will be much lower, and thus harder to detect in nuclear markers than in mtDNA in most mammal species. This has proven to be the case in a number of species where male-biased dispersal was documented independently (e.g., macaques, Melnick and Hoelzer, 1992).

The particular utility of mtDNA in the analysis of stock structure at the demographic level was the consensus opinion at an international workshop on Molecular Genetics of Marine Mammals (Dizon et al., 1997), and mtDNA has become the marker of choice in the identification of marine mammal stocks under the MMPA.

B. The analysis of variation within 11 microsatellite loci

We initiated a study a number of years ago to examine variation within several microsatellite markers in harbor seals in Alaska in order to attain a complete picture of population structure in this species. The analysis of variation within nuclear and mtDNA markers allows examination of male dispersal and breeding behavior as well as female dispersal patterns, and thus provides insights into the reproductive as well as demographic relationships among strata. Analyzing more genetic markers is also potentially of use to the study of the epidemiology of diseases (e.g., epizootics) and the estimation of genetic diversity.

Research is ongoing but some preliminary results are available. To date, 340 seals have been analyzed for variation at 11 loci (D. Campbell and G.O'Corry-Crowe, unpubl.). The level of polymorphism varies greatly among loci. Furthermore, some loci are more informative than others at documenting population subdivision. There are indications that different loci are informative at different spatial, and thus perhaps temporal, scales. This may be related to the mode and rate of mutation at each locus. Our preliminary analysis of population structure was conducted along the lines of the Westlake and O'Corry-Crowe (2002) analysis of macro-geographic subdivision in North Pacific harbor seals as revealed by mtDNA. Differentiation within microsatellites has been observed in Alaska across distances on the order of 600km to 1,000km, indicating that both male and female dispersal is low across this geographic scale. We have not yet investigated finer-scale differentiation in Alaskan harbor seals using nuclear loci. However, similar studies of microsatellite variation have documented population subdivision on the order of 300-500km in European harbor seals (Goodman, 1998) and > 600km in harbor seals from British Columbia, southeast Alaska and the Gulf of Alaska (Burg et al., 1999). We found lower levels of differentiation, on average, for microsatellite markers compared to mtDNA suggesting higher male-mediated gene flow. Caution, however, is needed here as differences in levels of heterogeneity between nuclear and cytoplasmic markers are likely also influenced by differences in rates of genetic drift due to different N_e. More samples, and possibly more markers, need to be screened for variation within microsatellite markers.

REVIEW OF METHODOLOGY

"[The small geographic size of the initial units used in the clustering analyses] can lead to two problems. First, especially in social species, local sampling may bias unit samples with the inclusion of close kin... Second, small samples risk large errors in the estimation of allele frequencies, ... [which] could lead to erroneous patterns of clustering." (page 4, second paragraph)

The reviewer notes that the small geographic size of the initial units used in the clustering analyses can lead to the inclusion of close kin within the units and risks large errors in the estimation of allele frequencies, which could lead to erroneous patterns of clustering. The reviewer is correct in this assessment. We were very aware of these potential sources of bias when conducting our analyses; it was because of these concerns that we chose to exclude initial units from which sample sizes were small relative to diversity. We used the summary statistic n_a to assess the sample size within each initial unit relative to its haplotypic diversity, giving us an indication of which units' allele frequencies were likely to be biased by small sample size. By excluding these units, we prevented them from influencing the clustering patterns. Exclusion of poorly sampled units also helps to reduce the impact of possible sampling of close kin on the results of the clustering analyses, as the inclusion of a pair of closely related individuals will have far less influence on the estimated haplotype frequencies in a large sample than it will in a small sample.

From the perspective of analyzing mtDNA, close kin share a common mtDNA haplotype. Thus, if a small initial unit contained several closely related animals, then a large proportion of the samples from that initial unit would share a single haplotype. Our data show the opposite pattern; in our smallest initial units, nearly every sample possessed a unique haplotype. This suggests that our analyses are not being biased by the inclusion of close kin.

REVIEW OF RESULTS AND INTERPRETATION

"A trial to test [the effect of sample size on the clustering analyses] was undertaken during the site visit, whereby units defined by larger sample sizes (N > 50) were re-sampled for 20 individuals at random. This changed the structure of the resulting dendogram." (page 6, first partial paragraph)

The reviewer noted that a trial undertaken during the review, in which initial units containing 50 or more samples were sub-sampled at random down to only 20 samples, resulted in a change in the clustering results. This result again illustrates why we chose to exclude poorly sampled sites. Of the 16 initial units we included in our analyses, all but 2 had sample sizes greater than 20. In contrast, 5 of the 15 initial units that we excluded had sample sizes of 20 or more. Thus, the re-sampled sites were not representative of those included in the study, but rather were more representative of those that we excluded due to inadequate sample size. We would expect that reducing the sample size in several of the initial units to such low levels would greatly increase the chances of the clustering methods being misled by sampling errors.

The results of the sub-sampling exercise illustrate the importance of eliminating poorly sampled areas from the analyses, as we did in our study. The only changes between the original dendrogram and that resulting from the re-sampling was in the clustering order for the initial units around PWS, an area for which the re-sampling reduced the total sample size by 65%, from 196 to only 70. That such a dramatic reduction in sample size, which rendered PWS one of the most poorly sampled areas in the re-sampled analysis, should have an impact on the results simply reinforces the importance of excluding poorly sampled areas from the analysis in order to avoid a known source of bias.

"The large number of apparent migrants between Frederick Sound and Ketchikan is consistent with the lack of support for this putative population division based on F_{st} and Φ_{st}." (page 6, second full paragraph)

The reviewer cites the "large number of apparent migrants between Frederick Sound and Ketchikan" as evidence of lack of support for these as separate units. However, while the estimated number of dispersers moving from Frederick Sound to Ketchikan (34 per generation with 95% confidence limits of 17.6-49.6) is high from an evolutionary perspective, the annual dispersal rate (0.03% per year with 95% confidence limits of 0.016% to 0.044%) is miniscule. The annual rate from Ketchikan to Frederick

Sound is similarly small (0.02% with confidence limits of 0.018% to 0.025%). Because the PBR Guidelines defines stocks on the basis of demographic independence, not evolutionary independence, it is the annual dispersal rate that is relevant. The rates estimated between Frederick Sound and Ketchikan provide strong evidence of the demographic independence of these areas and are far below the level at which the two areas could be safely managed as a single stock under the MMPA.

RECOMMENDATIONS

"I would ... recommend that ... spatial autocorrelation, assignment tests and STRUCTURE be employed on existing data to help assess putative stocks." (page 8, first full paragraph)

The reviewer recommended that spatial autocorrelation analysis, assignment tests and the clustering method STRUCTURE be used to evaluate population structure, particularly in the Kodiak Archipelago and Southeast Alaska. We agree that application of some of these methods may be an interesting way of further investigating the molecular ecology and evolution of Alaskan harbor seals and plan to pursue such analyses as time and resources allow. However, the objective of this research was to identify population structure at the stock level (demographically independent units) and we feel these methods are unlikely to be illuminating at this level. Therefore, we have given them lower priority than expansion and further analysis of the mtDNA for two reasons. First, these methods rely on nuclear DNA. We discuss our preference for mtDNA over nuclear DNA elsewhere (items A and B above), but briefly, nuclear DNA is expected to have lower statistical power to detect population structure and may lead to false conclusions of panmixia in cases where males disperse but females do not. Although results from nuclear DNA could strengthen population structure findings from mtDNA, they can never negate them.

Second, the two methods suggested by the reviewer are unlikely to be helpful in further elucidating stock structure. Spatial autocorrelation analysis is a useful tool for describing the overall pattern of population structure within a region. For example, the Mantel test used by Westlake and O'Corry-Crowe (2002) revealed that Alaskan harbor seals do conform to an isolation-by-distance model. However, there are no spatial autocorrelation techniques currently available that are able to identify either the number or location of population boundaries within the study region. While the method employed by Cassens et al. (2000), to which the reviewer refers, does have the advantage of being individual-based rather than requiring the *a priori* stratification typical of a traditional Mantel test, it is still capable only of determining the proportion of the genetic variation that is attributable to isolation-by-distance, not of identifying likely population boundaries.

Similarly, most assignment tests are only useful for testing the population membership of a sample once the populations have already been defined by some other means. STRUCTURE is the only assignment test available that is also capable of actually identifying populations. However, the authors of STRUCTURE caution against

the use of the method in this capacity, noting that they "do not claim (or believe) that [STRUCTURE] provides a quantitatively accurate estimate" of the number of populations present in a region and that the results "may not always have a clear biological interpretation" (Pritchard et al., 2000). Furthermore, STRUCTURE was designed for addressing evolutionary questions for which dispersal rates are substantially lower than those of interest under the MMPA. Thus, it is unlikely that the method will perform well when used to address the management questions relevant to Alaskan harbor seal stock definition.

'The comparison of sample-sets from different time periods should be undertaken, where possible.' (page 8, second full paragraph)

Although most samples used in the analysis were collected in the 1990s, a number of samples were collected in the 1970s. Comparing genetic profiles over time could help determine whether the genetic differences observed among areas were due to underlying structure or just sampling effects. None of the original 31 strata, however, had sufficient sample numbers from both time periods to allow a comparison. Only by combining the three initial strata from Prince William Sound were we able to assess potential changes in genetic composition of this region over time. Although a loss in haplotypic diversity was detected between the 1970s and 1990s, no differentiation was found between time periods within Prince William Sound (Westlake and O'Corry-Crowe, 2002), indicating that the differentiation observed among geographic strata in the current study were not due to sampling effects.

'Final interpretation of results should incorporate a comparative analysis in the context of previous population genetic studies for the species.' (page 8, fourth full paragraph)

A number of other molecular genetic studies have been conducted on population structure in harbor seals (Lamont et al., 1996; Stanley et al., 1996; Goodman, 1998; Burg et al., 1999; Westlake and O'Corry-Crowe, 2002). However, meaningful comparisons among studies are limited by differences in study objectives, study design, sample sizes and marker choice. In contrast to the current study, the emphasis of these other works was primarily on describing patterns of population structure in the absence of specific management objectives such as defining stocks. Study objectives ranged from investigating the genetic basis for geographic differences in reproductive timing (LaMont et al., 1996), describing the broad hierarchy of population structure across the entire species range (Stanley, et. al., 1996), measuring gene flow (male and female) patterns among 11 regions in the northeast Atlantic Ocean (Goodman, 1998), and documenting population subdivision in the northeast Pacific Ocean (Burg et al. (1999). These investigations took a more traditional approach to the *a priori* stratification of samples, which tended to be determined to a large degree by the availability of samples. Furthermore, population structure was examined primarily from an evolutionary rather than demographic perspective through phylogeny reconstruction and statistical hypothesis testing. For these reasons, we used our earlier study by Westlake and

O'Corry-Crowe (2002) on macro-geographic patterns of subdivision in the North Pacific as the more appropriate opportunity for comparison among studies.

Nevertheless, all these studies bare some comparison to the present work. The geographic scales over which we documented population structure within Alaska are similar to, and in some cases larger than, the distances over which subdivision has been found in other studies. Both mtDNA (Stanley et al., 1996) and microsatellite (Goodman, 1998) analyses found substantial population subdivision in the Northeast Atlantic Ocean over 300-500km. The former study also recorded differentiation among regions 150km apart. Lamont et al. (1996) found mtDNA differences along the west coast of the US on the order of 360-830km, while Burg et al. (1999) documented substantial mtDNA differentiation in parts of British Columbia over distances as small as 150km. In most studies, limitations in sample coverage and number prevented analysis of population structure over shorter distances.

"It is expected that new stock subdivisions will be recognized on a finer geographic scale than the current stock divisions, and that further analysis of existing data, together with the inclusion of some further samples from poorly sampled regions, will be required to define these new stock boundaries." (page 8, fifth full paragraph)

We concur with the reviewer that more samples are required in order to further refine stock boundaries in a number of areas. We must, however, stress that the findings presented in this report represent an extensive body of work that give clear direction to re-defining stocks of harbor seals in Alaska. Much of the harbor seal's Alaskan range has been well-sampled and the resulting genetic data has been extensively analyzed with respect to resolving population structure, female dispersal patterns and demographic relationships among harbor seal groupings.

REFERENCES

Avise, J. C. 1995. Mitochondrial DNA polymorphism and the connection between genetics and demography of relevance to conservation. Conservation Biology 9:686-690.

Burg, T.M., A.W. Trites, and M.J. Smith. 1999. Mitochondrila and microsatellite DNA analyses of harbour seal population structure in the northeast Pacific Ocean. Canadian Journal of Zoology. 77:930-943.

Dizon, A.E., W.F. Perrin et al. 1997. Report of the Workshop. Molecular Genetics of Marine Mammals. A.E. Dizon, S.J. Chivers and W.F. Perrin, eds. The Society of marine Mammalogy, SpecialPpublication 3:3-48.

Goodman, S. J. 1998. Patterns of extensive genetic differentiation and variation among European harbour seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorphisms. Molecular Biology and Evolution 15:104-118.

Greenwood, P.J. 1980. mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28:1140-1162.

Lamont, M. M. et al. 1996. Genetic substructure of the Pacific harbor seal (*Phoca vitulina richardsi*) off Washington, Oregon, and California. Marine Mammal Science 12:402-413.

Melnick, D.J. and G.A. Hoelzer. 1992. Differences in male and female macaque dispersal lead to contrasting distributions of nuclear and mitochondrial DNA variation. International Journal of Primatology 13:379-393.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecology 3:401-411.

Stanley, H. F., S. Casey, J. M. Carnahan, S. Goodman, J. Harwood, and R. K. Wayne 1996. Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). Molecular Biology and Evolution 13:369-382.

Westlake, R.l., and G.M. O'Corry-Crowe. 2002. Macrogegraphic structure and patterns of genetic diversity in harbor seals (*Phoca vitulina*) from Alaska to Japan. Journal of Mammalogy, 83(4): 1111-1126.