December 16, 2004

The National Marine Fisheries Service (NOAA, NMFS) and the Alaska Native Harbor Seal Commission (ANHSC), through their joint Harbor Seal Co-management Committee, are pleased to make available the results of an independent scientific peer review of:

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.

The purpose of this independent review was to evaluate new science that has management implications. The American Institute of Biological Sciences, Scientific Peer Advisory and Review Service was contracted to identify and recruit reviewers, solicit reviews, and convene a panel meeting on October 12-14, 2004 in Juneau, Alaska. Five individuals were recruited to review the research, two of whom were requested to submit their reports anonymously. The other three reviewers participated in the panel meeting prior to completing their reports. At this meeting, the authors of the report gave an extensive presentation of the research, which was followed by an interactive question and answer period with the panel reviewers. The panel reviewers were Maria Kretzmann, Daniel Pike, and Steven Carr:

> Dr. Kretzmann (http://www.southampton.liunet.edu/person/faculty/kretzmann.html) received her Ph.D. in Biology from the University of California Berkeley and conducts research on mammalian reproductive processes the behavioral and physiological ecology of several species of seals and sea lions.
>
> Daniel Pike has a Master of Science from the University of Manitoba, Canada and was formerly director of wildlife management with the Nunavut Wildlife Management Board, Iqaluit. He is the Scientific Secretary of the North Atlantic Marine Mammal Commission (http://www.nammco.no/main.htm) and is based in Tromsø, Norway.
>
> Dr. Carr received (http://www.mun.ca/biology/people/faculty_frameset.html) his Ph.D. in Genetics from the University of California Berkeley and has research interests in molecular systematics and population genetics of vertebrate species, including terrestrial, freshwater, and marine fishes and mammals.

All three reviewers remarked at the panel meeting that the presentations and the interactive sessions greatly increased their understanding of the research and its management context. Reviewer Kretzmann, in particular, annotated her final report to make especially clear the way in which her views developed through this process.

Many of the reviewers' concerns pertained to the application of the Boundary Rank analysis, a new method the performance of which is not well known. The panel reviewers came to appreciate the overall result that there likely are many more stocks of harbors seals in Alaska than currently recognized. Much of their discussion with the authors centered on suggestions to make the presentation of the analysis more clear.

We sincerely hope that everyone with an interest in or responsibility for stewardship of harbor seals in Alaska will find this review helpful in our common purpose of establishing sound management practices based on the best information available.

Peter L. Boveng
NMFS Review Coordinator

Brendan P. Kelly
ANHSC Review Coordinator

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES REVIEW OF ADMINISTRATIVE REPORT LJ-03-08

SUBMITTED TO THE NATIONAL MARINE FISHERIES SERVICE/ALASKA NATIVE HARBOR SEAL COMMISSION ALASKA HARBOR SEAL CO-MANAGEMENT COMMITTEE

October 2004

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

By G.M. O'Corry-Crowe, K.K. Martien, and B.L. Taylor

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MEETING SUMMARY

Review Format and Process

The American Institute of Biological Sciences (AIBS) was tasked by the National Marine Fisheries Service/Alaska Native Harbor Seal Commission (NMFS/ANHSC) Alaska Harbor Seal Co-management Committee to organize a review of Administrative Report LJ-03-08, 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." The purpose of this independent review was to evaluate new science that has management implications. The process was intended to be more thorough than a typical journal review in that the research could be considered in greater depth, with interaction among reviewers.

AIBS recruited five individuals to review Administrative Report LJ-03-08. Two of the reviewers were requested to submit their critiques anonymously using a Form and Guide drafted by AIBS following the terms of reference of their contract with NMFS/ANHSC. The other three reviewers also submitted reports according to the same Form and Guide but in addition attended a meeting that convened on October 12-14, 2004, at the National Marine Fisheries Service in the Federal Building in Juneau, Alaska. At this meeting the authors of the report, Greg O'Corry-Crowe, Karen Martien, and Barbara Taylor, gave an extensive presentation that included considerable background, explanation of methods used, rationales employed in data analysis, as well as the report itself. This was followed by an equally extensive question and answer period by the on site reviewers. The on site reviewers were Maria Kretzmann, Daniel Pike, and Steven Carr.

This document contains: 1) this Meeting Summary outlining the salient points stemming from discussions between the on site reviewers and the authors, 2) a list of participants and attendees, 3) the Executive Summary from Report LJ-03-08 itself, 4) the individual reports from the on-site reviewers, revised after discussion at the meeting, 5) the individual reports from the anonymous reviewers who were not privy to the detailed presentation that took place in Juneau, and 6) a list of the references that were provided to all reviewers as supplementary material.

General Comments by Reviewers

All three on site reviewers agreed that the presentation greatly clarified the initial report, and that it should be emphasized to readers of this document that the anonymous reviewers did not have the benefit of the presentation and discussion.

D. Pike felt that the presentation and the report represent an excellent body of work that answered many, but not all of his questions. Likewise, S. Carr stated that the presentation filled in many of the gaps in the initial report.

M. Kretzmann stated that due to the interaction with the authors at the on site review; most of her questions were addressed. She also stated that she feels that this report presents the best available science to inform management decisions regarding harbor seals in Alaska. One thing in particular, the very narrow assessment of stock structure under the Marine Mammal Protection Act (MMPA), and the narrow definition of demographic independence among populations, became clear at the on site review.

Each on site reviewer revised his or her initial critique as a result of the extended presentation and ensuing discussion. Please refer to the next section and the individual reports of these reviewers for more details.

Specific Comments by Reviewers

S. Carr felt that a revised report including specific figures from the presentation would add greatly to the clarity of the study, as well as revisions to existing figures. Some specific suggestions included: genetic data and movement data should be included together in one figure; Figure 5, showing the defined initial units would be clearer if the names as well as the numbers of the units were included; and the cluster diagrams should include both the name and number of the initial units.

Some of the discussion centered on the rationale for excluding samples from the statistical analyses. The authors originally chose a somewhat arbitrary cutoff point for exclusion as an adjusted number of samples ($N_a = 4$) or lower (Table 1). The question remains a point whether the data from Kamishak Bay ($N_a = 5$) introduces bias or not. M. Kretzmann recommended a reworking of the analyses excluding Kamishak Bay, because this site was poorly sampled relative to population size and genetic diversity, and could therefore be excluded based on an objective criterion. The next lowest N_a was 6 for the Pribilof Islands, which were relatively well sampled given population size and genetic diversity.

D. Pike recommended that the authors consider each source of potential identified bias and discuss its probable magnitude and direction. This has to do with the sources of sampling bias and their ramifications for the conclusions. For example, there was heavy spatial and temporal bias in the sampling, and hunters, who sometimes provide samples, do not shoot seals randomly. These types of sampling bias could influence the conclusions and a summary of them would be useful. It appeared that most identified biases would contribute to a lack of power in discriminating stocks.

S. Carr felt that, from an alternative analysis of the data, the Glacier Bay and Kamishak Bay could be construed as extreme ends of a cline. The authors feel this is unlikely because in all methods of analysis the Prince William Sound samples cluster first and this cluster is significantly different from either the Glacier Bay or the Kamishak Bay samples. In two types of analyses, Neighbor Joining (NJ) and Unweighted Pair Group Method with Arithmetic averaging (UPGMA), Kamishak Bay clusters with Glacier Bay. This result is at odds with the observed stepping stone aspect of harbor seal populations, hence the boundary rank cluster determinations are more likely to represent the biological reality. The authors cautioned that assuming too few stocks might result in a scenario where if the Glacier Bay population declined, it might be expected that it would be repopulated from Prince William Sound. If defining the stock this way is incorrect, then the Glacier Bay population could be depleted or lost. M. Kretzmann pointed out that demographic independence is the relevant parameter to be estimated if the goal is to prevent any population from declining if it might not be recolonized from nearby areas. Demographic independence (i.e., low migration rates) among populations is supported by smaller genetic differences than reproductive independence or longer-term isolation of populations would be.

D. Pike felt that the authors should explicitly address the question of whether or not the dispersal rate pattern revealed by genetics is what prevails today, not 20 or 50 years ago. In addition S. Carr raised the possibility that the statistical analyses may be picking up old structure, and made the analogy to human populations in Newfoundland where old structure of Northern European populations is still in evidence. The authors responded that they are looking at the structure and haplotypic distributions as they reflect current movement patterns, and are not addressing questions about haplotypic origins,

genetic history, or geographical origins although this information may be forthcoming and would be a goal for future studies.

D. Pike raised the point that mitochondrial DNA analysis does not really give information about interbreeding of seal populations. Thus, in one important sense, the 12 population units defined are not consistent with the definition of stocks provided in the MMPA. The MMPA defines a stock as "a group of marine mammals of the same species or smaller taxa in a common spatial arrangement, that interbreed when mature." The defined 12 population units are based on maternally inherited mitochondrial DNA, and this does not provide explicit evidence that interbreeding among the units does *not* occur. It is highly probable that bi-parentally inherited DNA, such as microsatellites, would not show the degree of differentiation seen for mitochondrial DNA, because higher rates of dispersal among males would prevent differences from developing.

S. Carr thought that more information should be included regarding the MIGRATE program used to calculate dispersal rate. He suggested it might be informative to run MIGRATE on the Bristol Bay populations, where the dispersal rate is predicted to be high, as a check on the program. In addition D. Pike made the point that the satellite tagging data could be presented and analyzed in greater detail to provide a backup for the genetic results.

S. Carr also wanted to know how the dispersal rates populations have been estimated outside of the genetic data which were used to determine them in this study. S. Carr emphasized that the MIGRATE program bases its emphasis solely on genetic data, the same as those used by the other methods, and is not an independent estimate of "demographic separation." The authors responded that movement data from tagged seals are difficult to obtain and are not currently available for any Alaska harbor seal populations for more than one season and that the genetic data is probably a better representation of dispersal because it reflects which seals are successfully reproducing following migration.

M. Kretzmann felt that mention should be made of microsatellite data alluded to in Westlake and O'Corry-Crowe (2002), as this would help distinguish reproductive independence from demographic independence.

Clarification was asked for by all on-site reviewers regarding the section in the AIBS Form and Guide concerning resource management. The section reads "Please comment on the relevance of the report for resource management. Does the report present appropriate, usable guidelines for resource management?" After considerable discussion it was determined that the reviewers should focus on the question of whether the report presents the best science to inform the decision makers. The relevance of the report was felt to be significant, but management decisions are to be made in another forum.

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EXECUTIVE SUMMARY (verbatim from report LJ-03-08)

Background

In the 1995 Alaska Marine Mammal Stock Assessment Reports, the National Marine Fisheries Service (NMFS) defined three stocks of harbor seal (*Phoca vitulina*) 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. This report details the findings from these studies, which conclude that current evidence supports a minimum of 12 stocks of harbor seals in Alaska.

Harbor seals (*Phoca vitulina*) occupy a near-continuous distribution in the coastal and continental shelf waters of Alaska from Dixon Entrance in the southeast, west throughout the Gulf of Alaska and the Aleutian Archipelago to Kuskokwim Bay in the Bering Sea. This important marine predator occupies a diverse range of habitats, hauls out at thousands of discrete coastal sites and represents a significant marine resource to a range of users. Harbor seals have declined dramatically in some parts of their Alaska range over the past few decades while in other parts their numbers have increased or remained stable over similar time periods. These declines and differences in trend suggest areas with independent population dynamics, and therefore, highlight the need for the definition of biologically meaningful management units, also known as stocks. The spatial scale of these stocks is important for interpreting direct and indirect human-caused mortality in relation to abundance, population trend and other aspects of harbor seal biology.

Management Objectives

The stated management goals of the Marine Mammal Protection Act (MMPA) are that 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 [that] they should not be permitted to diminish below their optimum sustainable population." The former objective has been interpreted as maintaining the species range, while the latter as not allowing stocks to decline below 50% of their historical population size "keeping in mind the carrying capacity of the habitat". Reduction of the range and local depletion are also undesirable because they could violate the goal of sustained use by Alaska Natives. Range contraction or fragmentation may mean that hunters that traditionally hunted in one area may have to travel farther and spend more time to obtain the same number of seals.

To attain the objective of maintaining the species range, the definition of stocks should be based on semi-isolated groups or sub-populations of seals that seldom exchange individuals. If that is not done, there is a risk of local depletion or extirpation. Therefore, an understanding of population structure and patterns of dispersal is central to identifying biologically meaningful stocks.

Genetic analysis of population structure and dispersal patterns

Genetic analysis is a long-established method of analyzing population structure and dispersal patterns and of defining units of conservation. Because of its maternal mode of inheritance and rapid rate of evolution, mitochondrial DNA (mtDNA) is an ideal marker in the investigation of the demographic relationships among groups of animals such as harbor seals. The analysis of variation in this marker can be used to identify management units where the primary objectives are to preserve the species

range as well as maintain healthy populations. In this report we present our findings from an extensive genetic study of population subdivision and dispersal patterns of harbor seals in Alaska using mtDNA.

Investigating population structure and dispersal in harbor seals in Alaska presented a number of challenges, including how to assess population subdivision in a continuously distributed species. To meet these challenges:

We conducted extensive sampling across the species' range. In an earlier study we sampled individuals from the major centers of harbor seal abundance. While that study revealed much about the evolutionary history of the species and documented broad-scale patterns of population structure, it could not fully uncover the scale and form of population partitioning because of major gaps in sample coverage. In the present study, we analyzed a total of 881 seals sampled at 180 separate locations throughout the range. These samples were provided by Alaska Native hunters, scientists and from tissue archives.

We used information on the distribution, abundance and movement behavior of harbor seals in Alaska to define a set of 31 initial units (equal groupings of sampling sites) for comparison in the analysis of population structure (Figure ES-2). These initial units were defined so as to be small enough in area to minimize the risk of missing structure and yet large enough to minimize the effects of low sample size. However, sample size in some of these units was determined to be too low to be representative of the underlying genetic composition and so these units were excluded from further analysis.

We employed a variety of methods to analyze the genetic data for evidence of population structure, including the geographically-constrained clustering method, Boundary Rank, classic distance-based cluster and phylogeny reconstruction analyses and statistical hypothesis-testing. We estimated rates of annual dispersal of seals among areas using a maximum-likelihood approach based on coalescent theory. We compared our genetic results to recent findings from studies on harbor seal movement and foraging behavior, trends in abundance, and diet in order to better resolve the spatial pattern of population structure of this species in Alaska.

The analysis of mtDNA variation in 881 harbor seals sampled from 180 sites throughout Alaska revealed: Substantial population subdivision across the State. The pattern of genetic differentiation was correlated with geographic distance indicating that female dispersal distances are a fraction of the species range and that when harbor seals do disperse, it is primarily to neighboring areas. The form of genetic differentiation, in conjunction with the non-uniform abundance of seals across their range, indicates that Alaskan harbor seals are subdivided on spatial scales of 150-540km, depending on region, into a series of partially isolated sub-populations. Using a variety of clustering methods, we identified 12 clusters of sampling sites that differed from each other at $p < 0.1$, using a chi-square permutation test. We estimated that dispersal among neighboring pairs of these 12 areas occurs at demographically low levels (~4.25 females/year). Thus, despite their near-continuous distribution along the Alaska coast, harbor seals in Alaska consist of a series of discrete sub-populations that seldom exchange individuals.

These findings are consistent with other information relating to harbor seal dispersal patterns and population structure, including movement patterns, trends in abundance and foraging ecology.

Conclusions

1. 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.

2. 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.

3. 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.

4. 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.

KRETZMANN CRITIQUE

Following our interaction with the authors at the on site review, most of my questions were addressed and I have no reservations about endorsing this report as the best available science to inform management decisions regarding harbor seals in Alaska. In particular, the very narrow mandate under which the authors were operating became clear; i.e., required assessment of stock structure under the Marine Mammal Protection Act (MMPA), and the narrow definition of demographic independence among populations (internal dynamics more important than immigration). My original review is below, with all comments added after the panel discussion in **bold**. In addition, at the end of this document, I have commented on where I think the anonymous reviewers may have misinterpreted some of the information presented in the report (because they did not have access to the many clarifications provided by the authors on site).

GENETIC SAMPLES AND DATA

A very large number of samples (881) was collected from most of the harbor seal's Alaskan range (180 locations), although sampling was heavily concentrated in South East Alaska, Prince William Sound and Kodiak areas. Limited samples from the Aleutians, the Pribilof Islands, and the Bering Sea may have affected the ability to clearly define distinct populations in these regions, but this limitation is acknowledged by the authors (points 3 and 4 in conclusions to the Executive Summary). In addition, the study is limited to a single genetic locus (mitochondrial DNA [mtDNA] control region), which reflects only female movements. The need for analysis of multiple nuclear DNA markers (e.g., microsatellites) is not acknowledged here, but is apparently underway, based on the final sentence in Westlake and O'Corry-Crowe (2002).

ANALYTICAL METHODS

The mtDNA control region is a rapidly evolving piece of DNA, well suited for distinguishing genetic differences among populations of a single species. The authors explain in Appendix 1 that genetic differences will be more difficult to detect with nuclear markers due to likely male-biased dispersal. However, very rapidly evolving nuclear microsatellite DNA may be more reflective of current levels of gene flow than is mtDNA. If enough males are moving among the putative populations defined here, they may not need to be managed separately. **At the review, the authors mentioned their ongoing microsatellite analysis, which to date shows the same large-scale general patterns of differentiation as the mtDNA data presented in Westlake and O'Corry-Crowe (2002). This information should be included in the report, as it strengthens the authors' contention that these groups of seals are genetically distinct from one another, with an independent technique using nuclear DNA (and, therefore, including the effects of male as well as female movements). However, it became clear that given the narrow definition of demographic independence among populations mandated by the Marine Mammal Protection Act, the mtDNA control region analysis undertaken by the authors is, indeed, the most appropriate measure. Although mtDNA reflects only female movements, this is all that matters to the ability of harbor seals to repopulate an area following a decline in numbers.**

The primary analytical method is a new unpublished statistical approach, Boundary Rank (BR), developed by one of the authors. The method is clearly described and appears to be validated in large

part by comparison with more traditional methods for assessing genetic differences among groups. The primary difference between the BR approach and other measures of differentiation is that the BR cluster analysis is constrained by geographic proximity.

The new method does not remove the need for defining initial population units, which is a very difficult to do in this continuously distributed coastal species. Because harbor seals typically do not move more than 50 km from their haulout sites, all sampling sites within 50 km of one another were grouped together, unless this made sample sizes too low, in which case more distant groups (up to 100 km apart) were combined. It would be better to apply a consistent 50 km rule, because it has some basis in what is known about the species' life history. That might mean eliminating a few more groups from the analysis when sample sizes are too low, as was done for 14 of the 180 sites. Geographical features were also used where present to help define initial units; this also may not be valid without information on how those features affect harbor seal movements. **Following discussion with the authors, I agree that while an objective application of initial units separated by 50 km would be ideal, it is not practical given the highly uneven sampling regime.**

The original reference of Martien et al., which applied the BR method to data for bottlenose dolphins in the Gulf of Mexico, shows that detailed expert information is important in defining initial units. For example, a small group of animals that appeared to be genetically distinct from other nearby groups was missed if not defined as an initial unit by experts with extensive knowledge of local dolphin movements. In addition, the expert analysis allowed links between populations that were not geographically adjacent (e.g., dolphins found in inland waterways as opposed to coastal regions), which would not be allowed under the strict stepping-stone model used here for harbor seals (p. 18). **Reasonable linkages were allowed among several initial units in South East Alaska and Prince William Sound. The units in Prince William Sound clustered together, while those in South East Alaska did not. In addition, the fact that the other clustering methods without geographic constraint agreed with the results of boundary rank (with the exception of some deep nodes in the trees) suggests that the assumptions of boundary rank were reasonable ones.**

The adjusted sample size omits all individuals with unique haplotypes at each site, because these individuals do not contribute anything to frequency-based comparisons (Appendix 3). **The authors clarified that these samples are not eliminated from the analysis, but are omitted only to calculate the adjusted sample size in order to decide whether to include or exclude a sample.** However, the presence of individuals with rare haplotypes contains valuable information (they would not exist if the population had been small and isolated for a significant time). **The authors explained that, given the very large number of haplotypes detected in these harbor seals, sharing of rare haplotypes could be a result of random small samples in each area, and may not contain any information about relatedness among groups. The kind of isolation that leads to the loss of rare haplotypes is not the focus of this study, as migration rates may be above 1% per year, enough to prevent significant differentiation on an evolutionary timescale.**

The elimination of all sampling sites for which adjusted sample size was less than 4 (p. 17) seems rather arbitrary (Kamishak and the Pribilofs are included, with N_a values of 5 and 6, respectively). A small sample will not capture representative genotypes of the region, and this would bias estimates of differences among populations downward. However, this seems like a costly loss of information. All of the Aleutian Island samples were eliminated by this criterion. **There was substantial discussion of the arbitrariness of this cutoff, and one of the authors provided an objective rationale for eliminating**

the Kamishak sample (N_a = 5) but keeping the Pribilof sample (N_a = 6), and this is the approach I would now recommend. The idea is to eliminate a known source of bias, i.e., when a very genetically diverse group is insufficiently sampled. In the case of Kamishak, a small sample was obtained from a large population in which haplotypic diversity was very high, and therefore the sample is unlikely to be representative. In contrast, the Pribilof sample represents a significant fraction of the total animals at that site, and haplotypic diversity is lower, so that a representative sample is likely obtained. This seems a much stronger and scientifically defensible rationale for inclusion/exclusion of samples than the current one of keeping everything with an $N_a \geq 4$.

If we want to err on the side of overprotection (p. 6), then this may be a reasonable approach. However, the authors have already biased their analysis in this direction by choosing a cutoff for statistical significance of ≥ 0.1, instead of the traditional 0.05 (p. 22). **During the authors' presentation, a strong case was made for using the 0.1 rather than the 0.05 value, due to the low statistical power associated with these highly diverse small samples. In addition, they presented how the results would differ if managers chose to interpret the data using the 0.05 cutoff for statistical significance. This would result in 9 demographically independent units (lumping Frederick Sound with Ketchikan, Kamishak with West Kodiak, and East Kenai with Prince William Sound). All of these are reasonable groupings, given what we know about harbor seal movement patterns in Alaska. However, if we believe the migration estimates provided by the program MIGRATE, the level of dispersal between each of these pairs is very low, and they all clearly meet the strict definition of demographic independence.**

RESOURCE MANAGEMENT

Figure 2 clearly illustrates possible outcomes of a 50% reduction in harbor seal numbers. Because the goal is to manage these populations to meet the objectives outlined in the MMPA, outcomes b (loss of range) and c (fragmentation) are not acceptable. I agree with the authors that only outcome d, in which losses are proportional to population size, is acceptable under this criterion. But the question is how those populations should be defined. I support the idea that management should err on the side of overprotection where possible, but I am also concerned that overprotection of an abundant species like the harbor seal could lessen our ability (by decreasing available resources and political will) to protect more threatened species. **Following discussion with the authors, I understand this to be a moot point. The National Marine Fisheries Service is required to evaluate stock structure for all marine mammal species, and the authors of this report are not directly advocating any particular management action.**

It would be useful to know where subsistence hunting is concentrated in relation to both 1) numbers of animals, to evaluate potential impact on depletion and recolonization probability, and 2) genetic distinctiveness among favored hunting grounds. There are 6 distinct areas identified in the agreement with Native Alaskans, with apparently highly variable numbers of harbor seals present (Cook Inlet ~2800, versus South East Alaska ~45,000; Table 2). The ability of harbor seals to re-colonize an area following depletion is not necessarily easily predicted from estimates of current movement patterns based on genetic (or other) data. Movement patterns might change if one population was depleted (e.g., to exploit decreased competition for resources). **The authors admitted that density-dependent migration would change their predictions about movement patterns based on the genetic data. But, there is no direct evidence of this, and in fact, at some sites where harbor seals have been**

substantially depleted (e.g., the islands off the south end of Kodiak), there has been no significant re-colonization of this area over the last few decades. I therefore conclude that the best currently available data support demographic independence among these groups.

DISCUSSION AND INTERPRETATION OF OTHER STUDIES

The evidence for population structure among harbor seals in Alaska presented in Westlake and O'Corry-Crowe (2002) is not as strong as this report makes it sound (p. 7-8). F_{ST} estimates among geographically adjacent Alaska sampling sites were generally very low (e.g., 0.006 for Prince William Sound and Kodiak), and F_{ST} values incorporating additional information about evolutionary relationships among haplotypes were not significant. This means these populations have not been diverging for long, although they may not be exchanging many female migrants currently. **I now understand this to be the only question of interest for the purposes of this report.** This is where information from rapidly evolving nuclear markers would be especially valuable. If microsatellite data are available (see Westlake and O'Corry-Crowe 2002), they should be mentioned here and compared to those obtained using mtDNA. **While important to establish whether these areas are reproductively independent, microsatellite data will not help to resolve demographic independence, and are, therefore, not directly relevant to this discussion. The authors should make their narrow mandate very clear in the introduction (distinguish clearly between reproductive and demographic independence among populations, explain what sorts of genetic data can be effectively used to examine each of these issues, and why).**

The Boundary Rank (BR) method and the more traditional clustering methods based on genetic distance alone yielded similar results with a few exceptions. Both the Unweighted Pair Group Method with Arithmetic mean (UPGMA) and neighbor-joining trees linked Glacier Bay with Kamishak, and west Kodiak with the Pribilof Islands. Given what we know about harbor seal movements (well-documented with many relevant references in the Discussion pp. 35-36), these unexpected results are most likely attributable to small sample size in these areas (p. 32). **If Kamishak were eliminated from the analysis due to inadequate sampling of a large and very diverse population, one of these results would disappear. The other one (linking West Kodiak with the Pribilofs) occurs very late in the cluster analysis, and may reflect an evolutionary signal about relationships among these seals, but may not be relevant to the discussion about current patterns of movement.**

CONCLUSIONS

This report convincingly demonstrates the inadequacy of the currently recognized three stocks of harbor seals in Alaska for meeting the goals laid out in the Marine Mammal Protection Act. Based on rapidly evolving mtDNA control region data, the authors have demonstrated that 12 populations are distinct enough from one another that they should be managed separately (with independent hunting quotas, and intervention required in case of major losses). A slightly more conservative interpretation of the data would define 10 major units for management. The addition of nuclear microsatellite data would help to clarify the most appropriate management units. **I believe that mtDNA is best suited to define demographic independence, and that the difficulties the authors have encountered in defining structure in certain regions are due to sampling problems, rather than lack of resolution provided by the genetic markers they used. With the addition of more samples from some of the**

areas that had to be excluded for this report, I would feel very confident about relying on this definition of demographically independent units for management.

OTHER COMMENTS

Comments on Anonymous Reviewer #1: This reviewer voiced concern over basing the identification of management units solely on the basis of genetic considerations. First, the report does not designate management units; but, it does strongly support the existence of 12 demographically independent units based on genetic data, which managers would presumably consider along with other sources of information and constraints. As it stands, the genetic data are in fact strongly supported by what we know about harbor seal movements in Alaska (e.g., more extensive in Bristol Bay, and those seals are always the first to cluster together using the mtDNA data). In the absence of long-term data on dispersal of individuals, this is the best available data set.

This reviewer also was uncomfortable with the (UPGMA and NJ) groupings on the basis of genetic distance linking areas that are not close geographically, and the authors' dismissal of this result on the basis of low sample size. As discussed above, one of these sites (Kamishak) should probably have been excluded from the analysis on the basis of poor sampling relative to group size and haplotypic diversity. The areas that group together early in the analysis are typically very well sampled (e.g., Prince William Sound).

I initially agreed with this reviewer that, "If it is difficult to detect population structure, perhaps there is less stock structure than proposed." However, after discussion with the authors, I was convinced that given several sources of systematic bias against detecting structure with these data, the fact that they have demonstrated fairly significant differences among areas should be taken as strong evidence of demographic isolation.

This reviewer suggested that other harbor seal studies have suggested longer movements than the 50 km used in defining initial units here (based mostly on Atlantic harbor seals, whose movement patterns may be very different). However, if the Alaska harbor seals regularly made longer movements, there should not have been detectable structure among areas linked by those frequent movements. The fact that pups in Alaska are the ones documented to make longer movements is not relevant to genetic structure if they do not remain in those areas for breeding. At least some pups making long movements have been documented to return to the area of tagging.

I agree with this reviewer that trend counts and differences in foraging ecology among harbor seals in different areas cannot be used to support the idea of separate stocks, but the authors do not make a strong case for this.

While there may be continued revisions to the picture of genetic mixing as more samples are collected and analyzed, I disagree that this report should not be used as a basis for management decisions. We often can not wait for the perfect data set in order to take action, and the recommendations of the report are clearly an improvement over the currently recognized 3 stocks of harbor seals in Alaska.

Comments on Anonymous Reviewer #2: This reviewer had many of the same questions that I did in my initial review. We both wanted to see some justification for the choice of which areas to include and exclude, rather than the arbitrary cutoff of adjusted sample size larger than 4 (see discussion above). The sensitivity analysis on the initial groupings was further explained at the on site review,

which would probably have addressed this concern, as well. Anonymous reviewer #2 is correct to suggest that details of this analysis should be included in the report. The stepping stone model for dispersal initially appeared to be problematic, because allowing for longer distance connections might be compatible with harbor seal natural history. However, because the 2 other unconstrained clustering methods reached essentially the same conclusions, this is not a significant drawback of the BR approach. The density dependence dispersal issue is another that I brought up during discussion (see above). Anonymous reviewer #2 also would like to see the 0.05 significance level discussed (as the authors did in their presentation), and (as suggested by the on site reviewers) that they include it as an alternative interpretation in the text and with an additional table.

SUGGESTED REVISIONS

It may be feasible to combine all samples from the Aleutian Islands to obtain a large enough sample to test for genetic distinctiveness in this region. **There is some evidence from movement data and differences in habitat to support much more extensive movements among harbor seals in Bristol Bay. The two initial areas in this region, although separated by considerable distance, were clearly not genetically differentiated, which supports the data from tracking seal movements. In addition, this area is impacted by seasonal ice, which likely explains the movements. There currently is no available data to address whether this rule of more extensive movements applies to the Aleutian chain, but if such information is obtained in the future, pooling of samples in the Aleutians might be justified.**

Some discussion of alternative management schemes (such as the clusters that would be defined if a more conservative ≥ 0.05 was used) is warranted. This would provide managers with a scientifically valid alternative plan, which might be favored under certain circumstances, depending on the economic and political climate. It is possible to figure out how the results would vary if information in Table 4 were combined with Figure 7. For example, the two units closest to coalescence in Figure 7 (see also p. 27) were Ketchikan and Frederick Sound, and West Kodiak and Kamishak. These two pair-wise comparisons are not significantly differentiated at $p \geq 0.05$. If those 2 pairs were collapsed, 10 significantly different units would be left for management. The Ketchikan and Frederick Sound pair seem particularly worthy of collapse into a single unit, considering the estimate of > 4 females exchanged per year (Table 3). **As discussed above, the collapse of these units (and East Kenai with Prince William Sound) is a scientifically defensible option. However, given the very low expected number of females exchanged between each of these pairs, the internal dynamics in each area are likely to swamp any effect of immigration. The authors should include the alternative scenario of 9 independent units, along with their reasons for favoring the 12-unit interpretation. In addition, they should add a table showing p values associated with hypothesis testing for the differentiation among all 12 independent units (in addition to Table 4, which shows the results for the 16 initial units included in their analysis). This would greatly assist the reader in drawing his/her own conclusions.**

PIKE CRITIQUE

GENETIC SAMPLES AND DATA

Sampling bias

Little detail of the sample collection program is given in the report, but it was apparently a combination of hunter-kill, tagging, biopsy, and other directed and opportunistically collected samples taken over several years (time period not specified). Consequently, the sampling scheme is biased in at least 3 ways that may have a bearing on the findings.

The spatial distribution of samples is not the same as the spatial distribution of animals. The authors acknowledge this and deal with it by adjusting sample size and excluding sites that are inadequately sampled. They also acknowledge that the number and location of stock divisions has been affected by inadequate sampling and that further work is needed in some areas.

Samples apparently were taken over at least 20 years (p. 9, paragraph 2), but no attempt was made to test if there was temporal heterogeneity within sampling sites. If there was, this would confound spatial comparisons. Temporal heterogeneity within sites appears to be unlikely for this species, but it has been observed in other marine mammals, e.g., beluga (Palsbøll et al. 2002). Given the long period of time over which samples were collected, it is quite conceivable that there may have been distributional shifts over the period. This should have been tested at those sites where sample numbers were adequate. If this was not possible due to inadequate sample sizes, as appears to be the case, the potential ramifications of this should have been discussed.

The authors acknowledged that the possibility of temporal change within sites had not been thoroughly addressed, but that this would not be possible due to insufficient samples at all but a few sites. They considered it likely that, if temporal change was occurring within sites, the combination of samples from several years would reduce the power of the analyses to discriminate between sites.

Animals were not sampled randomly from the population. Hunter-kill samples can be particularly problematic in this respect because hunting often occurs in discrete events when many animals from a specific location/time are taken. Depending on the social structure of the species, this can lead to a situation where all or nearly all the samples from a location were taken in one hunting event, and are from one group of closely related animals (i.e., pod, herd, extended family group) (Palsbøll et al. 2002). If a stock is made up of several such groups, this may lead to an overestimation of the number of stocks in the area. In this respect, it would have been helpful to have more detail on the following: a) information about the origin of the samples, including date and collection method; b) a more detailed description of the social structure of harbor seals in the area; and c) a description of the hunting methods used.

The authors considered that this was unlikely to be a problem in the present analyses. The samples come from several sources, but originate mainly from hunter-kills and biologists conducting tagging and other studies. The pattern of hunting is such that multiple kills in a single hunting event are rare. In some cases, multiple samples are obtained from tagging at a single site on a single day, but this is not common. The combination of samples taken over several years would tend to reduce the possibility of

such biased sampling influencing the results. The potential problem was also addressed by excluding sites with low effective samples sizes.

Samples were taken at various times of the year, but the analyses are not seasonally disaggregated. If seal distribution varies seasonally, this could lead to a situation where 2 or more stocks are sampled at a single site in a given year, confounding the resolution of the spatial distribution of stocks.

The authors conceded this possibility but noted that the satellite tagging information, though limited, did not indicate any pronounced seasonal migrations of seals in most areas. However, if such seasonal shifts in distribution were occurring, the most likely effect would be a reduction in the power of the analyses to resolve spatial stock structure, and the identification of too few groupings.

This reviewer concluded from these discussions that most of the potential sampling biases identified would lead to a reduction in the power of the analyses to resolve spatial stock structure. It would be useful for the authors to discuss more thoroughly in the report these potential biases and their likely effects.

ANALYTICAL METHODS

Laboratory analyses

I preface my comments by emphasizing that I have no expertise in this area.

Sample quality:

The authors do not report whether any tests were carried out to assess sample quality. Problems with sample quality have been noted for cetacean samples for microsatellite DNA (e.g., Givens et al. 2004). The samples were collected from several sources and different methods of preservation were used. However, it does not appear that any tests were performed to determine if preservation method had any effect on haplotypic variation, i.e., would the same sample preserved by 2 or more methods give the same result; nor was any literature cited as evidence that this was not a problem.

The authors explained that several protocols were in place, both at the laboratory and analytical phases, to ensure the quality and reliability of the genetic data. Both DNA strands were sequenced in all cases. Problematic samples were redone and discarded if results were ambiguous. A minimum of 10% of all samples was reanalyzed as a standard protocol, and disagreements were rare. It was also noted that the most likely effect of laboratory errors, if any occurred, would be the introduction of "noise" and the reduction of the discriminatory power of the analyses.

Analytical artifacts:

Bandelt et al. (2001) identify 5 major types of errors that can occur in the analysis of mitochondrial DNA, and identify measures to identify such errors. It was not clear initially if the authors checked for artifacts that may have been introduced during the analysis. This would seem to be particularly important because of the high proportion (60%) of haplotypes that were found in only one sample.

As explained above, the authors noted that several protocols were in place to reduce the possibility of analytical errors.

Statistical analyses:

Boundary Rank (BR):

The performance of BR in placing stock boundaries under the conditions of a spatial cline in stocks and uneven sampling over the cline was investigated in a simulation study by Butterworth and Brandão (2004). They found that non-uniform sampling led to a bias in the placement of the boundary, with the boundary being "attracted" towards the area with the most intense sampling. In this case, sampling intensity was very uneven across the initial groupings, ranging from n = 20 to n = 87. This may have lead to misplacement of stock boundaries in some cases, although other analyses have, in most cases, confirmed the boundary assignment by BR.

The authors noted that Butterworth and Brandão (2004) had used a different version of the Boundary Rank algorithm that did not use the same distance measure used in the present study. They also noted that the conditions of the simulation used by Butterworth and Brandão (2004) were highly unrealistic. Extensive simulation testing of the BR algorithm used here did not reveal the biases shown by Butterworth and Brandão (2004).

Initial boundary assignment:

BR requires an initial assignment of boundaries that is partly subjective. The authors report that they investigated the effect of changing the initial boundaries used in the BR analysis on the outcome, and reported little or no effect in most areas. However, these investigations are not reported in detail. In one case (East Kodiak), an initial grouping was dropped from the analysis because of uncertainties in placing the initial boundaries, but again, the reasoning behind this is not well explained. More detail about this would have been useful, particularly considering that 9 of the 16 groupings used in the analysis were identified as potential management stocks. It was not clear if it was possible to investigate the effect of initial unit size on the outcome, i.e., using fewer, larger initial units than those used here. It is also not clear if smaller units could have been used in some areas. Initial boundary placement is to a large extent dictated by the availability of samples and topography. As more samples become available in the future, it may be useful to use smaller and smaller initial groupings. This should allow for a more precise assignment of stock boundaries.

The authors noted that sensitivity tests on initial boundary placement had been conducted, but agreed that these had not been extensively reported in the document. In the case of East Kodiak, boundary placement to a large extent dictated the outcome because sampling was highly concentrated at 2 adjacent sites, and this was the main reason the site had been left out. The authors noted that there was at present no completely objective way to place initial boundaries, but that they were established with consideration of the known biology of the species, so in this sense, it was not unexpected that some initial units were retained as final groupings.

Dispersal rates:

Dispersal rates are estimated from genetic data. Taylor et al. (2000) found that such estimations can be highly biased, and Schweder (2003) noted that estimation is particularly problematic in cases where the 2 stocks for which dispersal is being estimated differ greatly in abundance and/or sampling intensity. Thus, it may not be appropriate to take the realized dispersal rates at face value. An alternative might have been to calculate dispersal rates for all possible pairs and assess them proportionally.

The authors agreed that the dispersal rates calculated using the MIGRATE program could not be taken at face value because the assumptions used in the program were not all supported for these data. However, they indicated that these potential violations of assumptions would likely lead to an overestimation, rather than an underestimation, of dispersal. Therefore, the dispersal rates calculated are likely positively biased and therefore support their view that the 12 units identified are demographically isolated. The possibility of calculating dispersal for all possible pairs was considered, but it is not feasible with the software available today.

Dispersal rates estimated from genetic data reflect the mating history of the animals over hundreds or even thousands of years. In this sense, they may not be relevant to current management. This may be particularly important, as the ecosystem in this area has apparently changed greatly due to regime shifts, climate change, fisheries, and the near elimination of several species of cetaceans. Some of these changes have occurred quite recently (in the past 20 years). It would, therefore, not be surprising if current stock structures and dispersal rates do not reflect the historical pattern. The authors could address this by discussing what effect extreme changes in dispersal rates in the past 20 years would have had on the genetic signal from samples taken today. For example, it would be interesting to know if population declines causing a cessation of dispersal between areas that previously had high dispersal would be detectable. It would also be useful to confirm the genetic dispersal estimates by other methods, most obviously tagging studies.

The authors considered that any significant level of dispersal would probably wipe out the genetic signal within a very short time, probably only 2 to 3 generations. However, they offered no compelling evidence for this assertion. The converse situation, where dispersal has recently ceased, might be impossible to detect, because some time would be needed for genetic drift and mutation to cause changes in haplotype frequencies. Therefore, the authors concluded that genetic methods were very unlikely to detect structure where none existed.

The case of the continuum:

Anonymous Reviewer 2 raises the issue of a single population distributed along a coast with a continuous gradient in genetic structure, and considers that the methods used would falsely identify 2 or more stocks under such conditions. While I concur that this is true, it would appear that under these conditions, some sort of management regime that distributes harvest along the gradient would be necessary to preserve the full range and prevent range fragmentation, as required under the guidelines to the Marine Mammal Protection Act (MMPA) (Wade and Angliss 1997). At any point or block along the continuum, dispersal would not be sufficient to prevent local depletion if the entire allowable harvest was taken there. The identification of “management stocks” along the coast might, therefore, be a useful management device to prevent local depletion. Therefore, I do not concur with Anonymous Reviewer 2 that management stocks are not required under such a condition, even if the boundaries themselves might be meaningless, in the sense that they would not separate true biological stocks. In addition, the authors noted that even though harbor seals were distributed nearly continuously in some areas, there were, nevertheless, great variations in abundance and near gaps in distribution that were indicative of potential stock boundaries.

RESOURCE MANAGEMENT

The report is, of course, highly relevant for the management of harbor seals in Alaska. However, the authors do not go into any detail about the implications of their findings to management, as it was apparently not the purpose of the report to do so; management recommendations are presumably made in another forum in this case.

Management stocks:

The report identifies 12 units that could be used as management stocks, and it is likely that several more will be identified when sufficient samples become available from other areas. This means that each unit should be managed separately, i.e., in terms of setting quotas and assessing the impact of other activities, such as by-catch.

Given the known biology of this species, as reviewed in the report, it seems likely that they are for the most part non-migratory and have a tendency to form rather small biological stocks with little dispersal between areas. Therefore, even in the absence of other information, a precautionary approach to management would suggest that the area be managed as several units in order to prevent local depletion and range fragmentation. The 12 groupings suggested by this study offer a good first approach to stock delineation for management, and would appear to be the best scientific basis available at this time. However, given the uncertainties identified, particularly with reference to the relevance of the genetic signal to present patterns of distribution and dispersal in a changing environment, these findings should be confirmed by other means.

In general, the potential for satellite and other forms of tagging as a method of stock delineation and/or confirming the results of the genetic analyses is underestimated. The authors state that limits on the duration of the tracking period preclude the use of tagging in estimating dispersal. I assume the maximum lifespan of such a tag is approximately 1 year, as they would be shed during molting. If a relatively large number of tags can be deployed, and they can be applied over a broad area, it is possible to use the data to delineate stock boundaries and estimate dispersal between areas, as has been done for polar bears in Nunavut (Bethke et al. 1996). This has an advantage over genetic methods in that the data are relevant to the present, whereas genetic changes have accumulated over many generations and may be irrelevant to the situation prevailing at present. A relatively large number of tags have already been applied, so it may be possible to undertake such an analysis with the data available. If not, then further tagging should be undertaken in a designed study. The potential for analyzing the genetic and tagging data in a common framework, as suggested by Anonymous Reviewer 2, should also be investigated.

DISCUSSION AND INTERPRETATION OF OTHER STUDIES

The interpretation of other, non-genetic evidence relevant to harbor seal population structure was logical and appropriate. However, as discussed above, the potential to use data from satellite tag applications as a method of stock delineation and for estimating dispersal rates between areas is underestimated.

CONCLUSIONS

In one important sense, the 12 population units defined are not consistent with definition of stocks provided in the MMPA. The MMPA defines a stock as "a group of marine mammals of the same species or smaller taxa in a common spatial arrangement, that interbreed when mature." As the differentiation of the 12 population units is based on maternally inherited mitochondrial DNA, it does not provide explicit evidence that interbreeding among the units does not occur. The authors note that dispersal is often greater for males, and this is often the case for seals, particularly for immature males. Although this is not assessed in this report, it is highly probable that bi-parentally inherited DNA, such as microsatellites, would not show the degree of differentiation seen for mitochondrial DNA, because higher rates of dispersal among males would prevent differences from developing. A classic example of this among cetaceans is the case of the North Atlantic humpback whale, where a single breeding stock forms 5 feeding stocks, which can be discriminated from one another using mitochondrial DNA analysis, but not using nuclear DNA analysis (International Whaling Commission, 2002).

Wade and Angliss (1997) conclude that demographic isolation is the most important criterion in defining stocks for management, and I agree with this conclusion. Therefore, I agree that the delineation of stocks through maternally inherited DNA is an acceptable method, as it does indicate demographic isolation. However, one can at least conceive of cases where this might not be the case. If, for example, the hunt was heavily biased towards male seals, the establishment of small management units based on mitochondrial DNA might not be necessary. Dispersal of males from neighboring subgroups might be enough to maintain the hunted subgroup, even if the hunting pressure was concentrated in one area. However, in this case, the authors report that the harbor seal hunt is not heavily selective for males.

As stated previously, the genetic results are evidence that the 12 populations have been largely demographically isolated in the recent past. It is not proof that they are so at present. Alternatively, populations that have become isolated recently would not be detected by genetic means. Given the seemingly unstable state of the marine ecosystem in this area, it is not safe to assume that past patterns are being maintained. Therefore, as recommended earlier, a tagging program should be conducted to confirm the genetic results.

OTHER COMMENTS

No other comments are noted.

SUGGESTED REVISIONS

The report would benefit from the following:

1) A more complete description of present and past seal harvesting, including harvests by area and year, sex and age composition, and hunting methods. Any other sources of human-induced mortality, such as by-catch, should also be described.

2) A more complete discussion of the management implications of these findings.

3) A more complete discussion of the need for additional research, especially the need for tagging studies, the requirement for more samples, and the need to change the survey design to provide individual abundance estimates for the identified units.

4) A more complete discussion of identified biases and their probable direction. In most cases discussed above, the probable biases would lead to a lack of power in identifying stock divisions. If this is so, the conservation ramifications of this should be discussed.

5) A more complete description of the sensitivity tests carried out, particularly with reference to the effect of changing the boundaries of the initial groupings on the results from Boundary Rank and the other clustering methods. In this regard, a better explanation of rationale for the exclusion of East Kodiak grouping is required.

6) A discussion of the relevance of the genetic signal to present day stock boundaries and rates of dispersal. Specifically, it would be interesting to know if the genetic signal would be preserved if the situation has altered radically in the past 20 years.

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4) Givens GH, Bickham JW, Matson CW, and Ozaksoy I. Examination of Bering-Chukchi-Beaufort Seas bowhead whale stock structure hypotheses using microsatellite data. Document SC/56/BRG18 for the IWC Scientific Committee, 2004.

5) International Whaling Commission (IWC). Report of the Scientific Committee, Annex H. Report of the Sub-Committee on the Comprehensive Assessment of North Atlantic Humpback Whales. *J. Cetacean Res. Mange.* 2002;4:230-260.

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7) Schweder T. Genetic dispersal in eastern Atlantic minke whales. Report of the Scientific Committee, Annex D, Adjunct 3. *J. Cetacean Res. Manage.*2003; 6*(Suppl.)*:182.

8) Taylor B L, Chivers SJ, Sexton S, and Dizon AE. Evaluating dispersal estimates using mtDNA data: Comparing analytical and simulation approaches. *Conservation Biol.* 2000;14:1287-1297.

9) Taylor B. A new method to increase statistical power by removing highly variable sites from mtDNA sequences thereby reducing rare haplotypes. 2004; Document SC/56/SD2 for the IWC Scientific Committee.

CARR CRITIQUE

GENETIC SAMPLES AND DATA

The methods of selecting, collecting, and handling samples were adequate relative to the conclusions drawn, and limitations of the sampling scheme and data were adequately acknowledged and considered. As in most such studies, sampling does not include all parts of the range, is not evenly distributed over the range, and some individual samples series are too small. Still, absence of samples from the Gulf of Alaska makes it difficult to evaluate the alternative hypothesis of clinal variation between the Gulf and Southeast Alaska stocks. This is adequately acknowledged.

ANALYTICAL METHODS

The authors are established, well-published experts in this area; conventional and established laboratory methods have been appropriately applied.

Haplotype DNA sequence data in a user-friendly format, e.g., MEGA or NEXUS files were not made available to the reviewers until the meeting. A dendrogram of these data should be presented and commented upon as well.

Table 4 (p. 41 of the report) presents the statistical significance of observed F_{ST} values, but not the differentiation indices themselves. It is suspected from the text that these values are quite small. Demonstration of statistically significant differences in the positions of multivariate centroids is not equivalent to demonstration of biologically significant subdivision of management units from which population samples are drawn. See Carr and Crutcher (1998) for a discussion of this in a management context. Throughout the manuscript, there is a segue from “statistically significant” to “biologically distinct,” which may be an over-interpretation.

Table 4 is used to identify significantly differentiated groups. Choice of a non-standard critical $p < 0.1$ appears to have been done *a posteriori*, and more seriously, no Bonferroni correction is made for multiple comparisons. Thus the procedure “over identifies.” However, at the meeting, the authors addressed this question. They maintained that since these are multiple independent tests, no Bonferroni correction is required.

The Boundary Rank (BR) method seems to mandate contiguous units, and thus is predisposed to draw boundaries. This may be inappropriate if the data actually suggest clinal variation, allowing for absence of key intermediate samples. The algorithm should be presented. At the meeting, the Boundary Rank method became clearer. This method can be used with any criterion for linking data. It would be good to know if the essentially linear stepping stone arrangement of populations can be evaluated by a suitable multiple range test.

Resolution is limited by the relatively modest length of mtDNA sequence used. An alternative whole-genome approach may provide the necessary resolution to answer the questions asked. This approach, based on human, Atlantic Cod, and Harp Seal data (Carr et al. in prep.) can be seen at http://www.mun.ca/biology/scarr/AESN_Biodiversity_Genomics.html.

RESOURCE MANAGEMENT

The report supplies extremely valuable information for resource management. Correctly interpreted, these data and their analysis can contribute to the development of a sound, biologically based protection strategy.

DISCUSSION AND INTERPRETATION OF OTHER STUDIES

The authors have briefly reviewed non-genetic evidence (population trends), citing conclusions without including primary data. The actual estimates of dispersal and movement seem weak. Were this a Committee on the Status of Endangered Wildlife in Canada (COSEWIC) report, tables and graphs demonstrating population trends and declines should be provided, which would go towards establishing threat levels for the 12 proposed units.

CONCLUSIONS

Contrary to assertion, Boundary Rank and Neighbor Joining (NJ) results seem to contrast rather sharply. The BR method seems to agree with the current division of the range into the three current non-overlapping management units. In contrast, the NJ results suggest populations within the present Gulf stock have affinities inconsistent with simple isolation by distance in a stepping-stone structure. Much of the discussion of the evident anomalies seems to be pleading for a cleaner model. It appear there is a notion that contiguous, non-overlapping stocks are required *a priori* for ease of management, and the analytical approach should attempt to identify defensible boundaries between such units. A concern remains that choices of which populations to include and exclude seem guided by *a posteriori* results; thus, I would advise against excluding any further populations at this point, which would be specifically *a posteriori*.

The “twelve” units are not defined in the Executive Summary, and the bulk of the manuscript analyzes 16 population samples. They have to be extracted from Figure 10.

A plausible case can be made from the same data for nine units, with allowance for clinal variation. Please see the figures in Appendix I.

OTHER COMMENTS

None are noted.

SUGGESTED REVISIONS

1. Include primary haplotype data, and one or more dendrograms of these.
2. Include data in upper triangle of Table 4.
3. Include a clear map and list of the 12 units. Consider alternative models explicitly.
4. Review all uses of the word “distinct” for precision of meaning.
5. Include tables of demographic data.
6. Include dendrograms of BR, NJ, and Unweighted Pair

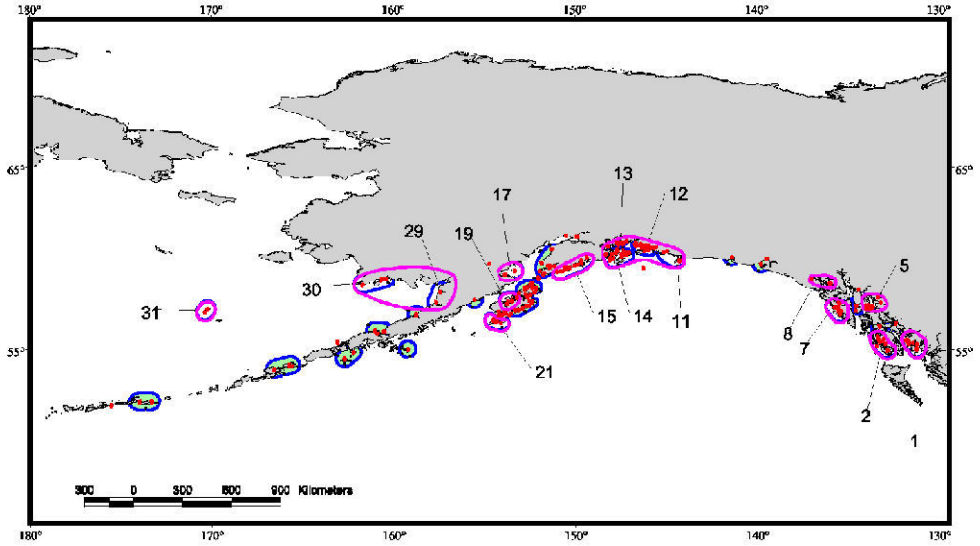
Group Method with Arithmetic Mean (UPGMA).

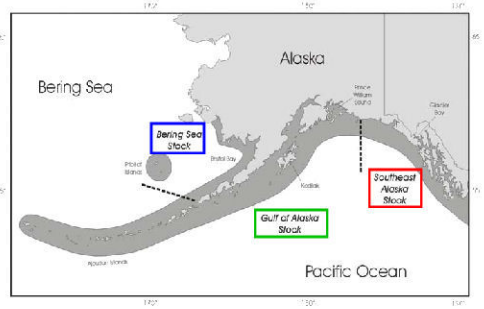
7. Include a number of figures from the PowerPoint presentation. Refer to the PowerPoint presentation handout supplied by the authors at the meeting and see Appendix II for the actual figures.

a. Section VI, page 3, slide 3. Include gloss in the table within the table and use boldface for the samples that are kept, rather than those excluded.
b. Section VI, page 4, slide 3. Include gloss in the table to show why exclusion of 15 samples leaves 16 units of the original 31.
c. Section VI, page 6, slide 2. Include counts of numbers of genotypes and sample size N after exclusions.
d. Section VI, page 7, slide 1.
e. Section VI, page 7, slide 2.
f. Section VII, page 1, slide 2.
g. Section VII, page 1, slide 3.
h. Section VII, page 2, slide 1.
i. Section VII, page 2, slide 2.
j. Section VII, page 2, slide 3.
k. Section VII, page 3, slide 1.
l. Section VII, page 3, slide 2.
m. Section VII, page 3, slide 3.
n. Section VII, page 7, slide 2. Include Kodiak Island series to show ambiguity of boundary.
o. Section VII, page 7, slide 3.
p. Section VII, page 8, slide 2. Be clear that MIGRATE is based on genetic data and is not an independent measure of demographic dispersal.
q. Section VII, page 8, slide 3.
r. Section VII, page 9, slide 2. It is critical to include dispersal estimates based on MIGRATE for Bering Sea populations to establish whether the algorithm can give intermediate or high dispersal levels.
s. Section VII page 10, slide 3.
t. Section VIII, page 1, slide 3. No formal correlation test was done; it is suggested to use the Mantel test. The methods do not give similar results; discrepancies should not be minimized.

Appendix I

Figures Illustrating S. Carr's Nine Unit Hypothesis



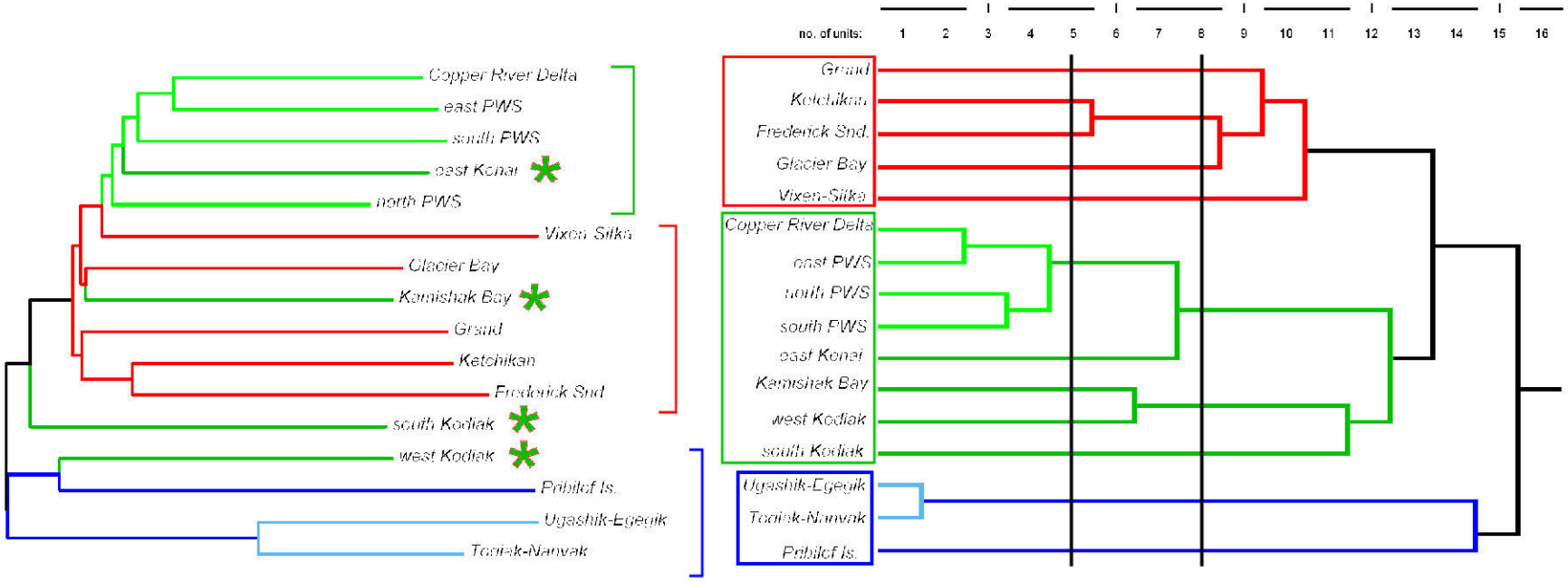


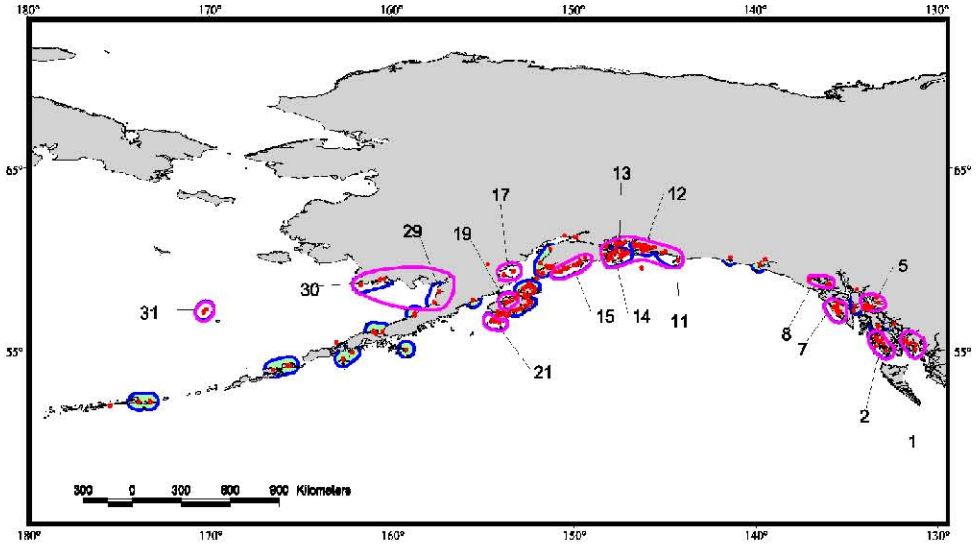
29	Ugashik-Egegik
30	Togiak-Nanvak
31	Pribilof Islands

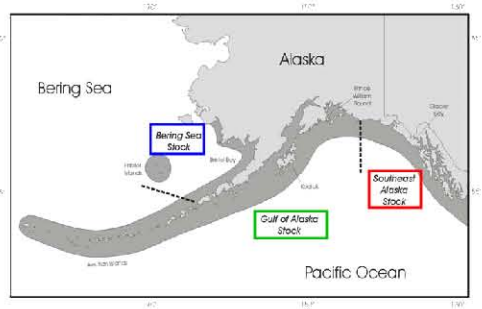
11	Copper River Delta
12	East Prince William S$^{nd.}$
13	North Prince William S$^{nd.}$
14	South Prince William S$^{nd.}$
15	East Kenai
17	Kamishak
19	West Kodiak
21	South Kodiak

1	Ketchikan
2	Grand Island
5	Frederick S$^{nd.}$
7	Vixen-Sitka
8	Glacier Bay

Figures 5 + 10. Twelve management units recommend by report (in magenta).
[group 11, 12, 13, & 14, and 29 & 30]
16 numbered samples with sufficient sample size (in blue).
15 samples of original 31 excluded from analysis (shaded green).





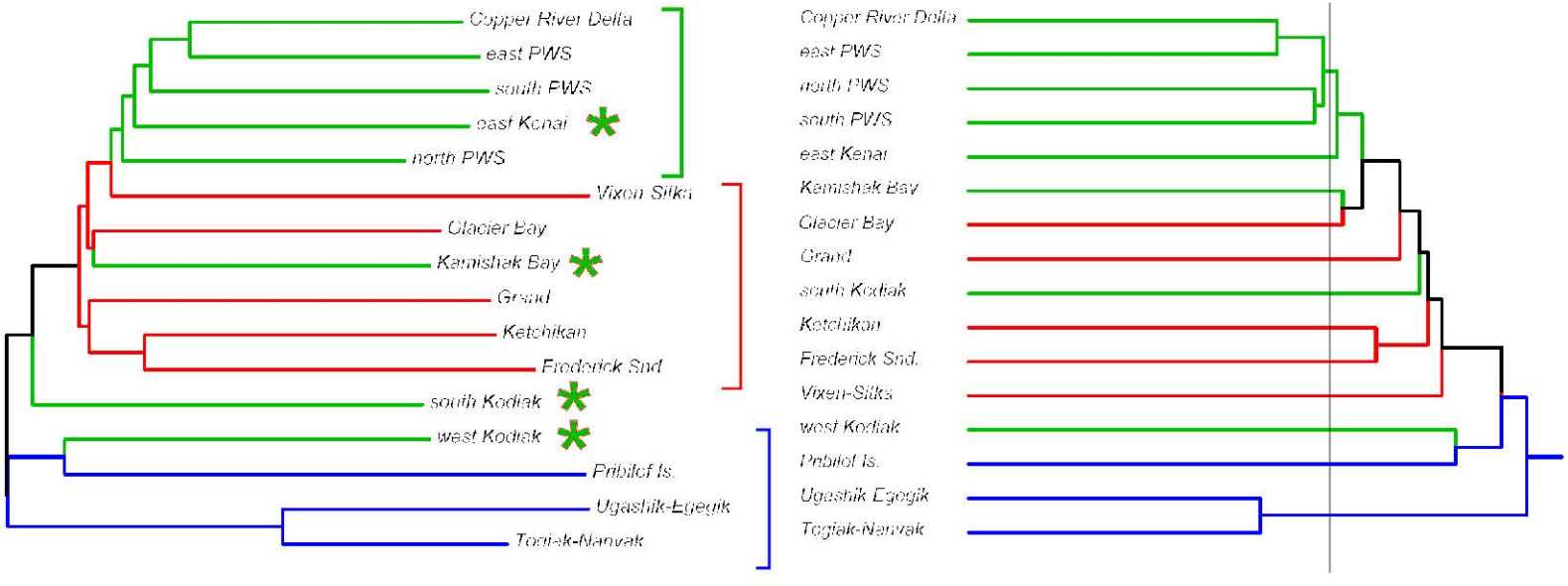


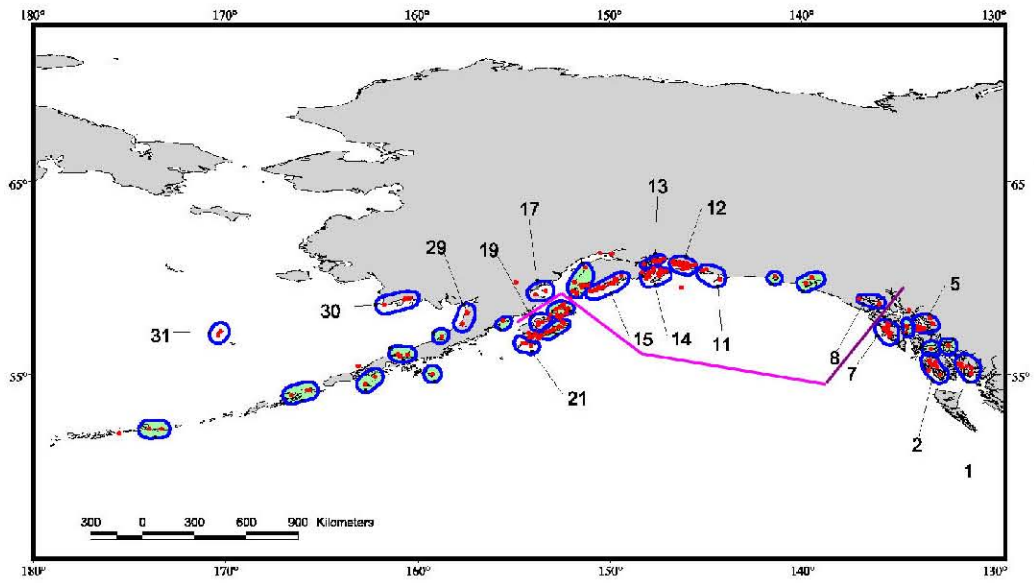
29	Ugashik-Egegik
30	Togiak-Nanvak
31	Pribilof Islands

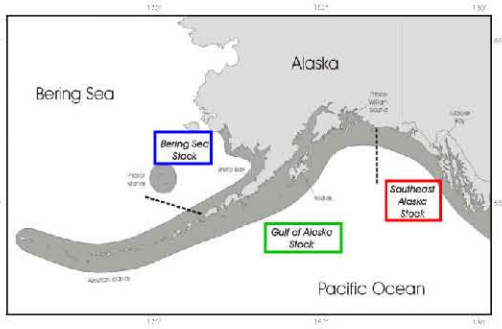
11	Copper River Delta
12	East Prince William S$^{nd.}$
13	North Prince William S$^{nd.}$
14	South Prince William S$^{nd.}$
15	East Kenai
17	Kamishak
19	West Kodiak
21	South Kodiak

1	Ketchikan
2	Grand Island
5	Frederick S$^{nd.}$
7	Vixen-Sitka
8	Glacier Bay

Figures 5 + 10. Twelve management units recommend by report (in magenta).
[group 11, 12, 13, & 14, and 29 & 30]
16 numbered samples with sufficient sample size (in blue).
15 samples of original 31 excluded from analysis (shaded green).







1 Ketchikan
2 Grand Island
5 Frederick Snd.
7 Vixen-Sitka

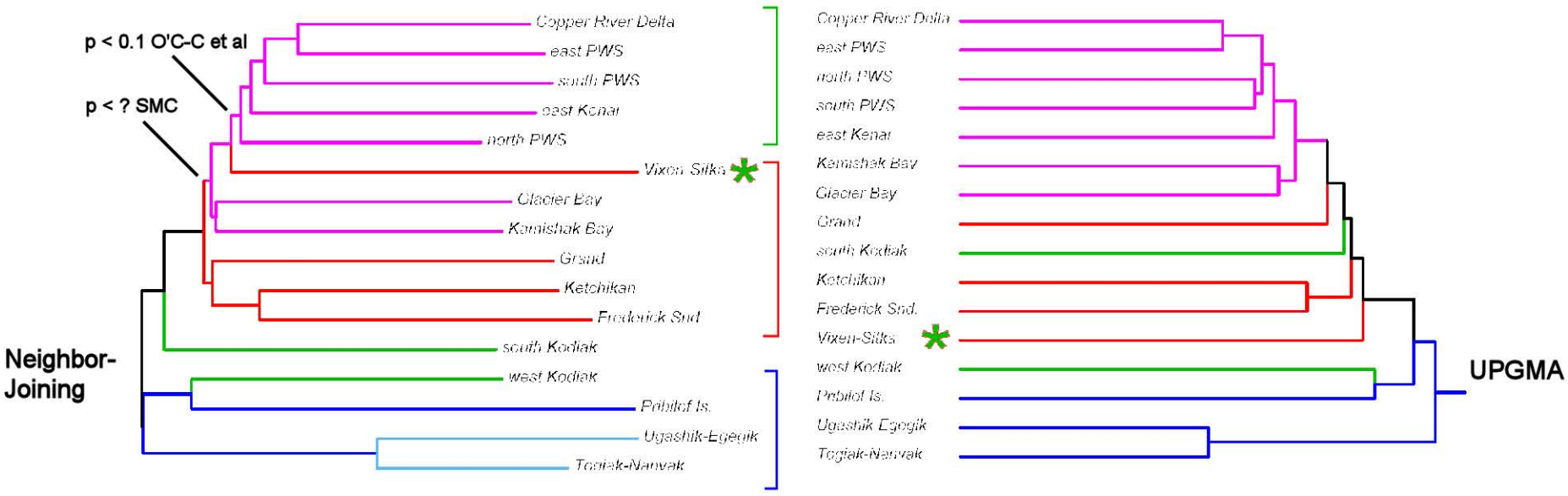
8 Glacier Bay
11 Copper River Delta
12 E Prince William Snd.
13 N Prince William Snd.
14 S Prince William Snd.
15 East Kenai
17 Kamishak

19 West Kodiak
21 South Kodiak

29 Ugashik-Egegik
30 Togiak-Nanvak

31 Pribilof Islands

SMC's Nine Distinguishable Units: include #8 (Glacier Bay) & #17 (Kamishak) in Gulf of Alaska Stock
clinal variation of #7 ➡ #8 ➡ #11 ?



	Ketchikan	Grand Is.	Frederick Snd.	Vixen-Sitka	Glacier Bay	Copper River D.	east PWS	north PWS	south PWS	east Kenai	Kamishak Bay	west Kodiak	south Kodiak	Ugashik-Egegik	Togiak-Nanvak	Pribilof Is.
sample size (n):	42	34	44	50	27	22	79	30	87	47	20	37	51	35	32	16
Ketchikan	0															
Grand Island	0.029	0														
Frederick Snd.	0.063	0.003	0													
Vixen-Sitka	0	0.001	0.002	0												
Glacier Bay	0.019	0.088	0.014	0.017	0											
Copper River Delta	0.042	0.048	0.025	0.051	0.439	0										
east PWS	0	0.018	0.002	0.011	0.144	0.780	0									
north PWS	0.050	0.246	0.05	0.089	0.383	0.483	0.713	0								
south PWS	0	0.007	0.003	0.005	0.145	0.338	0.670	0.602	0							
east Kenai	0	0.016	0.035	0.002	0.092	0.338	0.272	0.570	0.341	0						
Kamishak Bay	0.035	0.109	0.019	0.027	0.317	0.092	0.392	0.354	0.273	0.111	0					
west Kodiak	0	0	0.001	0	0.002	0.039	0	0.002	0	0.01	0.085	0				
south Kodiak	0	0	0	0	0.020	0.028	0	0.027	0.003	0.018	0.03	0	0			
Ugashik-Egegik	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Togiak-Nanvak	0	0	0	0	0	0	0	0	0	0	0.001	0	0	0.924	0	
Pribilof Is.	0.001	0	0	0	0	0	0	0.001	0	0	0.018	0.016	0.001	0	0.001	0

Appendix 2

Figures from O'Corry-Crowe, Martien, and Taylor: PowerPoint presentation October 12-14, 2004, Juneau, Alaska

Sample size, genetic diversity and power

We examined the sample size (n) relative to genetic diversity (H) within each of the 31 initial units to determine whether the sample set sufficiently represented the underlying haplotype frequencies for the unit to be included In the analysis of population differentiation

$n_a = n - h$

Used a threshold value

A further 27 samples from 14 sites were not placed in any of the initial strata because of very low sample size. East Kodiak, despite having and n_a=9 was excluded from the analysis because of uncertainty in defining the limits of this unit

site no.	Unit name	no. of sampling sites	sample size n	proportion unique	haplotypic diversity H	adjusted sample size n_a
1	Ketchikan	8	42	0.781	0.981	10
2	Grand Island	8	34	0.609	0.977	11
3	Red Bay	1	8	1.000	1.000	0
4	Wrangell	1	10	0.714	0.911	3
5	Frederick Sd.	6	44	0.667	0.948	20
6	Angoon	3	9	1.000	1.000	0
7	Viten-Sitka	13	50	0.520	0.959	25
8	Glacier Bay	2	27	0.824	0.923	10
9	Yakutat Bay	3	21	0.882	0.967	4
10	Icy Bay	1	20	0.944	0.984	2
11	Copper River Delta	3	22	0.643	0.939	8
12	East Prince William Sd	15	79	0.625	0.970	39
13	North Prince William Sd	10	30	0.727	0.975	8
14	South Prince William Sd	15	87	0.682	0.964	43
15	East Kenai	13	47	0.593	0.964	20
16	West Kenai	10	23	0.895	0.957	4
17	Kamishak	2	20	0.800	0.958	5
18	North Kodiak	12	27	0.960	0.922	2
19	West Kodiak	4	37	0.636	0.958	15
† 20	East Kodiak	10	29	0.650	0.973	9
21	South Kodiak	5	51	0.759	0.954	22
22	Pasle Bay	1	8	1.000	1.000	0
23	Shumagin	1	5	0.750	0.900	1
24	Sanak	2	11	0.900	0.982	1
25	Aleutns-UnAlaska	3	8	1.000	1.000	0
26	Atka	2	6	0.500	0.867	2
27	Nelson-Prt. Moller	3	8	0.714	0.972	1
28	Prt. Heiden	1	8	0.667	0.929	2
29	Ugashik-Egegik	3	35	0.647	0.901	18
30	Togiak-Nunvak	3	32	0.750	0.942	12
31	Pribilof Islands	2	16	0.800	0.892	6
		166	854		0.975	

a. Section VI, page 3, slide 3: Include gloss on the table within the table and use boldface for the samples that are kept, rather than those excluded.

To minimize the possibility of the analysis of genetic differentiation being biased by low sample size relative to haplotypic diversity, we excluded from the final analyses all initial units with $n_a \leq 4$

15 of the original 31 units were excluded

site no.	Unit name	no. of sampling sites	sample size n	proportion unique	haplotypic diversity H	adjusted sample size n_a
1	Ketchikan	8	42	0.781	0.981	10
2	Grand Island	8	34	0.609	0.977	11
3	Red Bay	1	8	1.000	1.000	0
4	Wrangell	1	10	0.714	0.911	3
5	Frederick Sd.	6	44	0.667	0.948	20
6	Angoon	3	9	1.000	1.000	0
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† 20	East Kodiak	10	29	0.650	0.973	9
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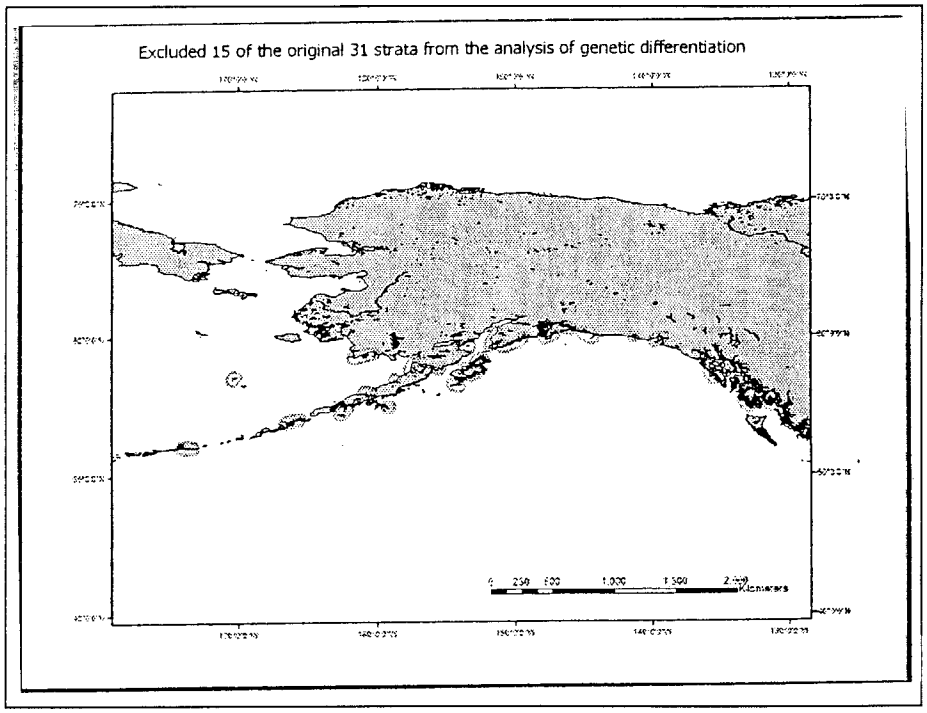
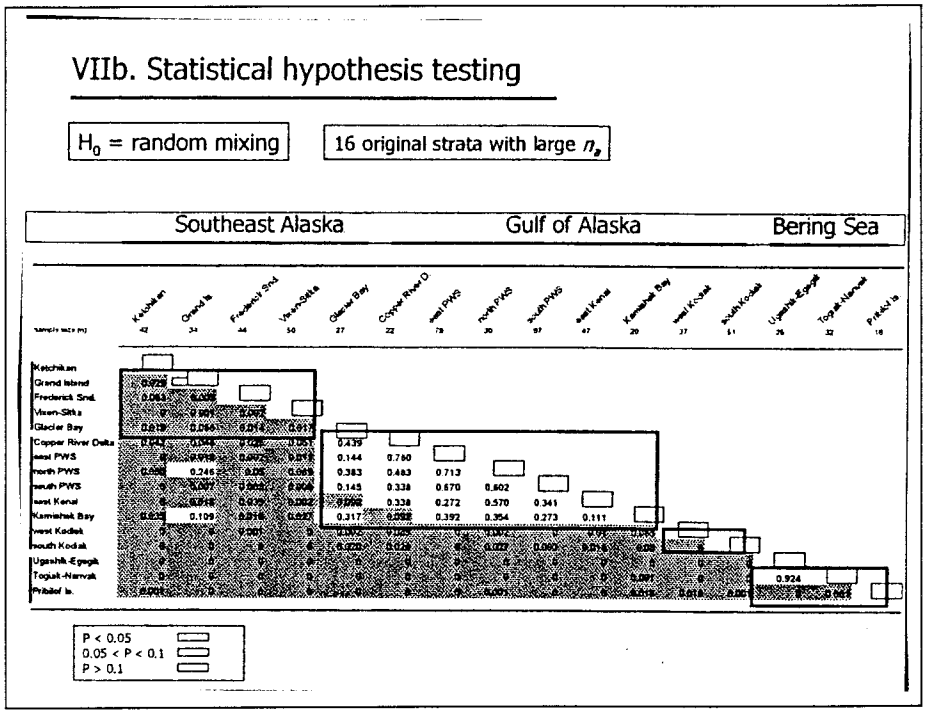
30	Togiak-Nunvak	3	32	0.750	0.942	12
31	Pribilof Islands	2	16	0.800	0.892	6
		166	854		0.975	**303**

b. Section VI, page 4, slide 3: Include gloss in the table to show why exclusion of 15 samples leaves 16 units of the original 31.



c. Section VI, page 6, slide 2: Include counts of numbers of genotypes and sample size N after exclusions.



d. Section VI, page 7, slide 1

VIIb. Statistical hypothesis testing

Substantial levels of population subdivision detected among most of the original 16 units and were found to be significant at α=0.05 under a H_o of random mixing

A general isolation by distance model of population structure was evident

In many cases, significant differentiation was found among neighboring strata that indicated population structure over spatial scales of 153-541km

Comparison	*p* value	swim distance (km)
Ketchikan v. Grand-Klawock	0.003	153
Vixen-Sitka v. Frederick Snd.	0.002	174
Pribilof Isl.s v. E. Bristol Bay	0.001	541

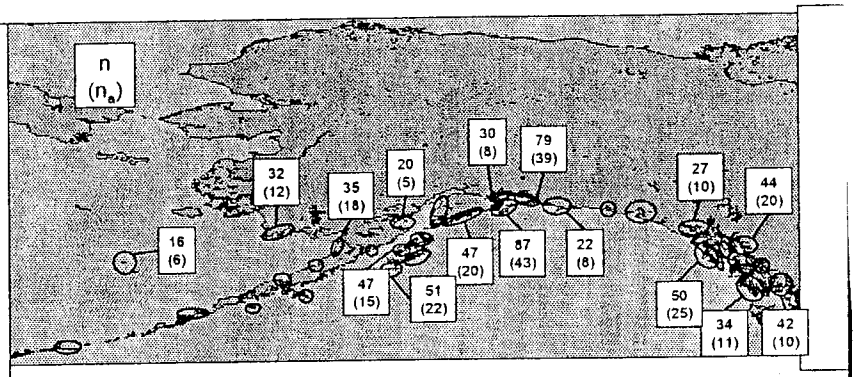
By contrast, a number of adjacent units were not found to be significantly differentiated.

East PWS v. South PWS	0.670	75
North PWS v. East PWS	0.713	70
Ugashik-Egegik v. Togiak-Nan	0.924	226-270

20

e. Section VI, page 7, slide 2

Initial Units



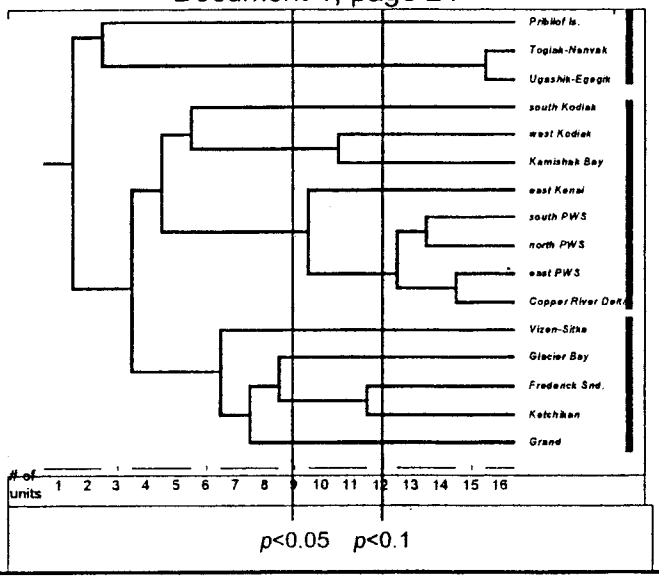
f. Section VII, page 1, slide 2

Boundary Rank
Geographic Constraint

g. Section VII, page 1, slide 3

Boundary Rank Results

Document 1, page 24



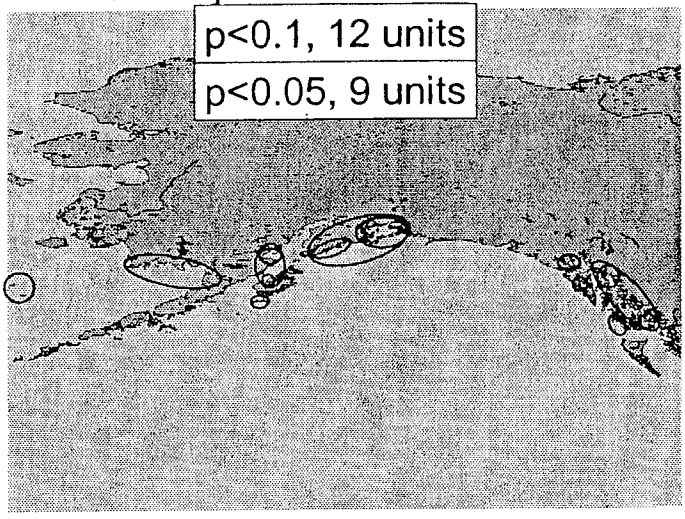
h. Section VII, page 2, slide 1

BR Results

All adjacent units differ at

p<0.1, 12 units

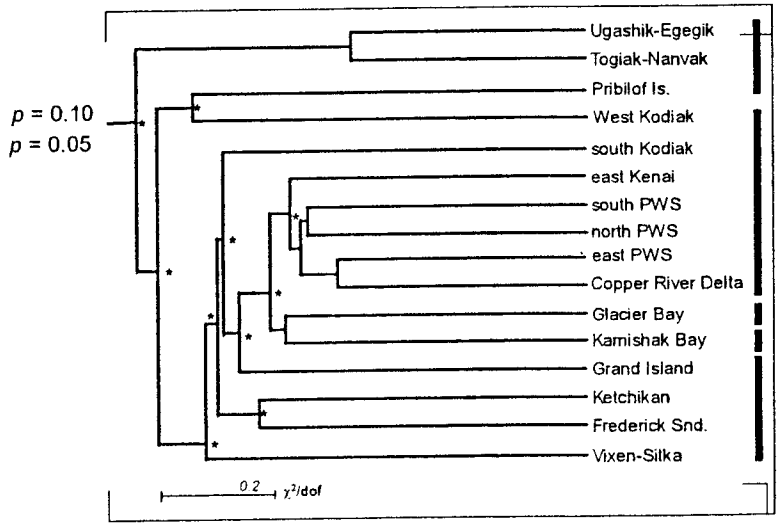
p<0.05, 9 units



i. Section VII, page 2, slide 2

UPGMA Results

Document 1, page 25



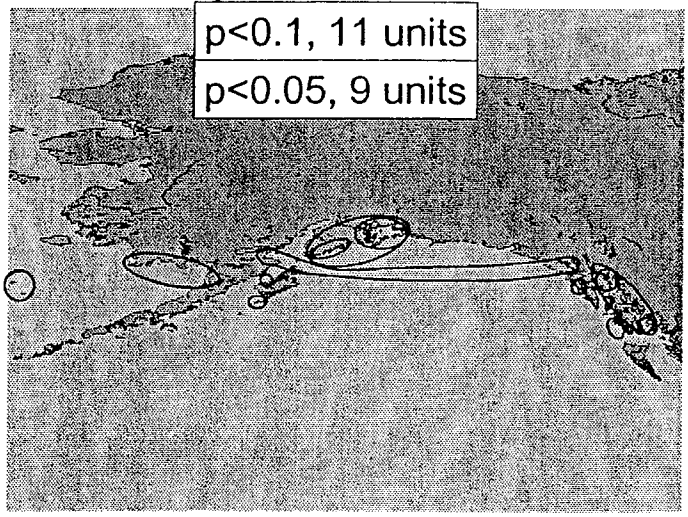
j. Section VII, page 2, slide 3

UPGMA Results

All adjacent units differ at

p<0.1, 11 units

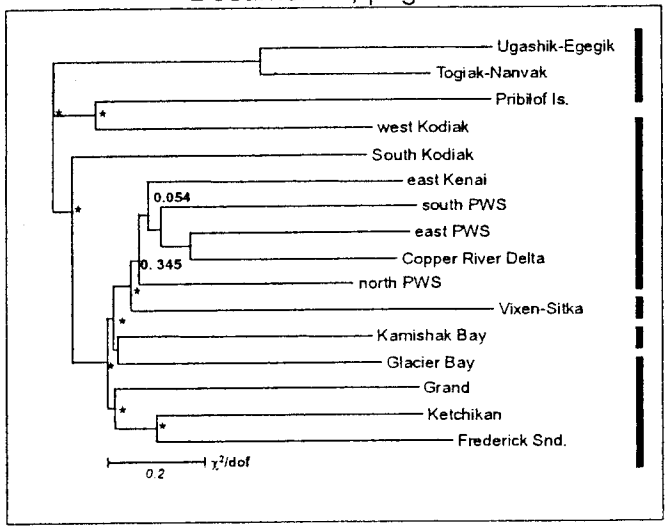
p<0.05, 9 units



k. Section VII, page 3, slide 1

Neighbor Joining Results

Document 1, page 26



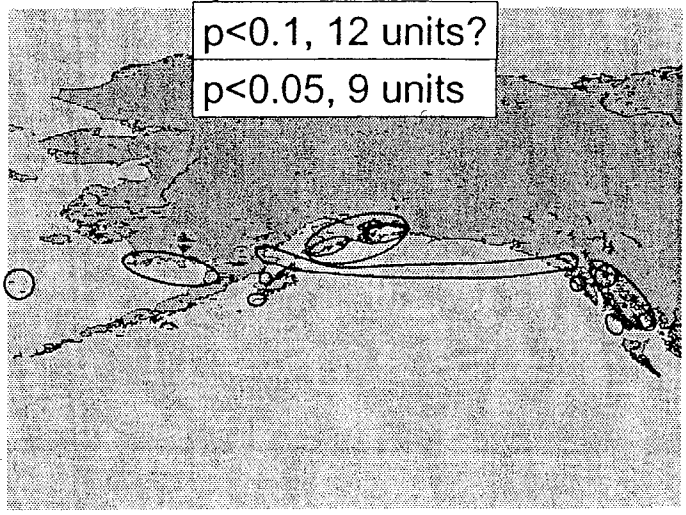
l. Section VII, page 3, slide 2

Neighbor Joining Results

All adjacent units differ at

p<0.1, 12 units?

p<0.05, 9 units



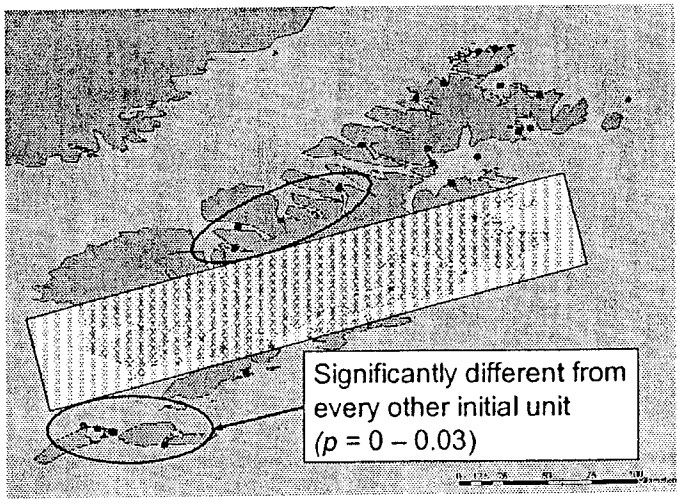
m. Section VII, page 3, slide 3

Kodiak Initial Units

- Alternate initial unit definitions on Kodiak Archipelago all result in a boundary between the northwestern and southeastern portions of the archipelago
- Exact location of that boundary on the eastern side of Kodiak Island varies depending on initial units

n. Section VII, page 7, slide 2: Include Kodiak Island series to show ambiguity of boundary.

Kodiak Initial Units



o. Section VII, page 7, slide 3

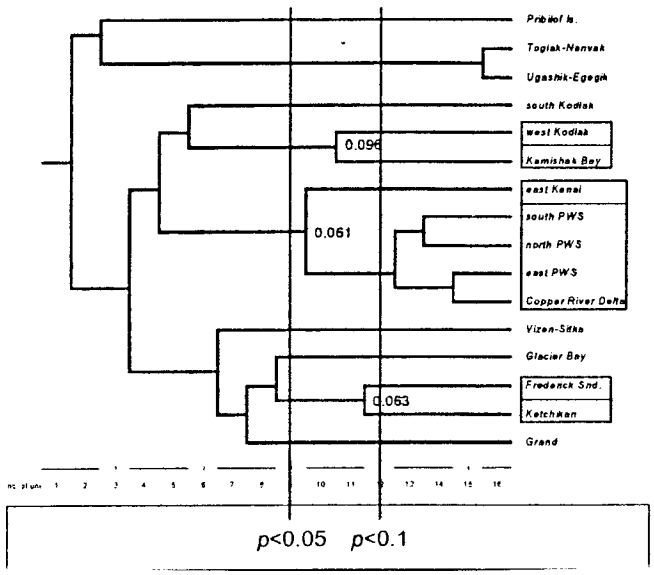
Migrate

(Beerli and Felsenstein, 1999, 2001)

- Maximum likelihood method
- Estimates $N_e dT$ and $N_e \mu$, with confidence intervals
- Need an estimate of effective abundance in order to convert the estimates from *Migrate* into an annual dispersal rate

p. Section VII, page 8, Slide 2: Be clear that MIGRATE is based on genetic data and is not an independent measure of demographic dispersal.

Estimating Dispersal



q. Section VII, page 8, slide 3

Dispersal Rate Estimates

Dispersal rate from:	Annual # female dispersers	d	Percent Dispersal
Frederick Sound to Ketchikan	4.25 (2.2 – 6.2)	0.0003	0.03%
Ketchikan to Frederick Sound	0.8 (0.7 – 1.0)	0.0002	0.02%
West Kodiak to Kamishak Bay	0.9 (0.5 – 1.1)	0.0002	0.02%
Kamishak Bay to West Kodiak	1.6 (0.6 – 1.8)	0.0018	0.18%
Prince William Sound to Kenai Peninsula	1.1 (0.9 – 1.2)	0.002	0.2%
Kenai Peninsula to Prince William Sound	1.1 (0.9 – 1.2)	0.0003	0.03%

d = Annual # dispersers / (current abundance / 3)

r. Section VII, page 9, slide 2: It is critical to include dispersal estimates based on MIGRATE for Bering Sea populations to establish whether the algorithm can give intermediate or high dispersal levels.

Dispersal Rate Estimates

Dispersal rate from:	Annual # female dispersers	d	Percent Dispersal
Kenai to PWS w/o N. PWS	0.75 (0.64-1.18)	~0.00018	~0.018%
PWS w/o N. PWS to Kenai	2.41 (2.10-2.82)	0.004	0.4%
Kenai to Kamishak Bay	0.31 (0.21-0.67)	0.00007	0.007%
Kamishak Bay to Kenai	1.05 (0.72-1.64)	0.0014	0.14%

d = Annual # dispersers / (current abundance / 3)

s. Section VII, page 10, slide 3

A. Current stocks are too broadly defined to meet the management objectives of the MMPA

Substantial levels of genetic differentiation, indicative of demographically low levels of dispersal, were found on spatial scales smaller than those of current stocks

1. Recent study of macro-geographic structure concluded that the current stocks are too broadly defined

   Westlake and O'Corry-Crowe (2002)

2. The current study found: genetic differentiation on the scale of 150-540km

   genetic differentiation was correlated with geographic distance

   12 clusters of sampling sites differ from each other at $p<0.1$

   dispersal among neighboring pairs of these 12 areas occurs at < 4.45 females/yr

   all methods used gave similar results

3. Recent study of nDNA variation revealed macro-geographic structure

t. Section VIII, page 1, slide 3: No formal correlation test was done; it is suggested to use the Mantel test. The methods do not give similar results; discrepancies should not be minimized.

ANONYMOUS REVIEWER #1 CRITIQUE

GENETIC SAMPLES AND DATA

I have two general comments/concerns regarding the genetics data.

1) Genetic samples were taken from a subset of the available sites where seals are found. In the Kodiak area, this does not seem to be a problem, but in Southeast Alaska, it may lead to spurious conclusions. In Figure 6 B, it appears that there is a continuous distribution of locations where harbor seals have been observed hauled out. I counted about 500 harbor seal haulout sites in Figure 6 B, and genetics samples have been taken from around 43 of them. Paetkau (1999) notes that, "Perhaps the most important analytical issue currently limiting extrapolation from genetic data to movement patterns is that it must be possible to distinguish between continuous and subdivided populations and to identify the subdivisions that exist: only when this has been done should the issue of assess the relationships between those subdivisions arise. This issue is often ignored by geneticists who treat the study areas forming the basis of their sampling as cohesive natural groups and move straight to the task of quantifying the relationships between those sampled areas. Such an approach is adequate for learning about the general partitioning of genetic diversity across a species distribution, but not for identifying management units (MUs)." Taylor and Dizon (1999) stress that management unit criteria cannot be based solely on genetic parameters. Conclusions based on the present set of samples must be linked to movements and dispersal tendencies of harbor seals. As evaluated below, the movements data are open to question and alternate interpretation, and therefore the definition of management units is likely premature.

2) The times when genetics samples were collected are not distributed evenly throughout the year. I received a data set from Westlake and O'Corry-Crowe (2002) that I am presuming are part of the data used in LJ-03-08. In this set, there were 530 records of samples taken from Alaska where the date was known. Of those, 202 were taken in May and June, 165 were taken between July and October, 34 were taken between November and January, and 128 between February and April. If there was seasonal movement into and out of the areas where collections were made, the genetics data would not be representative. Below I argue that this could be the case.

ANALYTICAL METHODS

This is not my area of expertise. However, a clarification is needed regarding a statement on page 32. If areas grouped solely on the basis of genetic distance link areas that are not close geographically and are dismissed as being an artifact of low sample size, it also seems possible that the grouping of nearby areas could be an artifact of low sample size.

RESOURCE MANAGEMENT

Harbor seal subsistence take occurs at all times of year, including the winter months (Wolfe 2001). If stock definitions are based on primarily summer genetic samples, and movements are based primarily on summer samples (see below), then the stock identification does not consider the potential for winter movement overlap of stocks, or that a village may be impacting different stocks at different times of the year.

On page 22, the authors state, "We believe it is appropriate to use the precautionary value of 0.1 because a large abundance of harbor seals coupled with their continuous distribution makes it very difficult to detect population structure using genetics." If it is difficult to detect population structure, perhaps there is less stock structure than proposed.

DISCUSSION AND INTERPRETATION OF OTHER STUDIES

The authors state that numerous studies have examined harbor seal movement patterns (p. 14), and cite several papers to state that, "the vast majority of seals stayed within 50 km (31 miles) of the original capture area." Bonner and Witthames (1974) actually emphasize how much common (harbor) seals move, stating, "the records presented here show that Common seals area capable of wide dispersal and the population in the Wash cannot be considered self-contained." Leopold et al. (1996) offer evidence of winter dispersal from the Wash in England. Pitcher and McAllister (1981) collected data only during the summer, and noted both considerable fidelity to haulout sites during this time, but also movements of 194 and 74 km. Harkönen and Harding (2001) used branding to mark their animals, but did not note the distribution of re-sighting effort.

Lowry et al. (2001) attached satellite-linked transmitters to harbor seals in Prince William Sound. A close reading of the paper leads to an interpretation of the results slightly different from those in LJ-03-08. What I read is that harbor seal movements are quite variable, with some being residential, some moving out to sea over 50 km offshore, and some moved to Copper River and other locations. Juveniles moved more than adults. Also, the data from Lowry et al. (2001) are primarily from summer months, with late winter months having few samples.

Small and Verhoef (2001) develop the 50 km rule applied in LJ-03-08, where they state less than 1% of the satellite locations of foraging adult and subadult harbors seals were greater than 50 km from the haulout site. However, they do not say whether the haulout site changes over time. In a companion report (Rehberg and Small, 2001), they note that first summer pups are quite variable in distances moved, and some moved several hundred kilometers. (Lowry et al. (2001) also noted that a few seals did move outside the study area before the tags stopped functioning).

The authors ignore the movements of harbor seals in the Western Atlantic Ocean, where considerable seasonal movements have been noted for many years. Payne and Seltzer (1989) note winter occurrence of harbor seals in Southern New England and Waring et al. (2003) note that in winter, harbor seals occur regularly as far south as New Jersey, but generally do not pup south of Maine. Williams (1999) notes that harbor seals were regularly bi-caught in gill-nets out into the middle of the Gulf of Maine, greater than 100 km from shore. Lowry et al. (2001) noted that harbor seals were nearly always found in waters less than 200 m deep, and this is consistent with observations in the Gulf of Maine.

Recent information from a study of the effect of an offshore windmill park at Horns Reef has refuted the impression that the distribution of harbor seals in the North Sea is predominantly coastal (Tourgaard et al. 2003). The foraging area of the Dutch Wadden harbor seals extends from the northern German Bight and most of the Danish North Sea territory into the central North Sea and the Norwegian North Sea sector (see http://www.hornsrev.dk/Miljoeforhold/miljoerapporter/Hornsreef%20Seals%202002.pdf).

The point of this discussion is to note that the species has significant plasticity in behavior and movement, and that observations taken at certain seal densities that indicate little movement may not

be applicable at higher or lower densities. (The Fretwell-Lucas theory comes to mind here.) Even in the studies in Alaska, a certain fraction of the population does move.

The trends in abundance information (4.4.2, p. 37) is based on a series of efforts going back to Pitcher and McAllister (1981) that correct a set of trend counts for covariates and more recently correct the adjusted count for the fraction not out of the water under ideal conditions (Boveng et. al. 2003). However, the trend counts assume there will be no shift in distribution or that no new sites will be occupied. Simpkins et al. (2003) note any movements can confound trend evaluations.

The discussion of foraging ecology on page 38 offers no support for separate stocks. Harbor seals feed on a variety of fish species, and the fact that different food habit studies have found different results in different subregions is not substantive. I would also expect one would find different food habits in the same region at different times of the year.

CONCLUSIONS

I agree that the current stocks of harbor seals in Alaska are too broadly defined to meet management objectives of the Marine Mammal Protection Act (MMPA). The findings do not provide an adequate framework from which to evaluate the need for future interpretations and data, but they do provide a start for future investigations. The genetic study is limited by sample coverage, and interpretation and conclusions should wait for this data. I disagree that the multiple small units that are defined as separate stocks will not change, and National Oceanic and Atmospheric Administration (NOAA) fisheries should be flexible and plan to change those stock definitions as information becomes available.

OTHER COMMENTS

This is a significant attempt to provide a basis for protection of individual harbor seal stocks. However, the data fall short of that necessary to make an informed assessment. Investigations in Alaska should adopt multiple working hypotheses (Chamberlin 1897) and not focus on collecting information supporting one hypothesis at the expense of another. Particularly, investigations should consider the possibility of longer and more frequent movements of harbor seals between haulout sites and perhaps greater genetic interchange with adjacent areas. A meta-population model may well be worth investigating, especially in areas where the seals are continuously distributed, as in Southeast Alaska.

An aside: The last sentence in appendix II on page 52 notes that some areas may be too small to be able to confirm the existence of stocks, including Puget Sound, the Gulf of Maine, or Cook Inlet. Perhaps Southeast Alaska is also in this category, since it is arguably the same size as the Gulf of Maine (see attached map).

SUGGESTED REVISIONS

Since I believe the definition of stocks requires more data and alternative interpretation models, I would not continue to use this report as a basis for management decisions.

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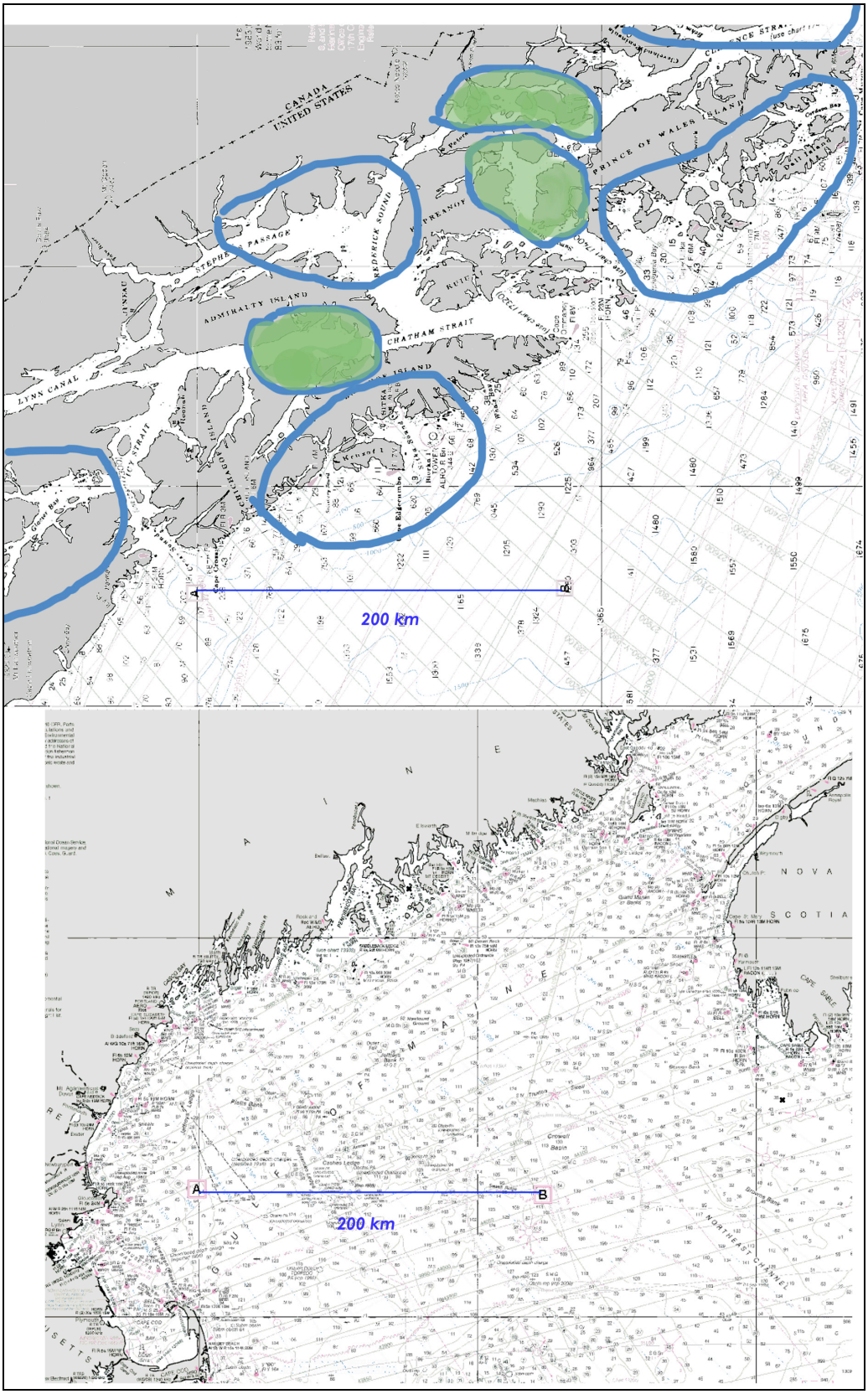
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ANONYMOUS REVIEWER #2 CRITIQUE

GENETIC SAMPLES AND DATA

The authors have carefully highlighted and attempted to deal with sampling limitations throughout the study. They suggest that a purpose-built, balanced sampling scheme leading to more data locally would be preferable, but that the cost is currently prohibitive. They address the imbalances in their data by using a sample size adjustment (discussed in Appendix 3, adjustments shown in page 11) and the limitations in local sample size partly by some initial clustering (page 14), and partly by censoring (page 17). The work deserves credit for identifying and dealing with these limitations and the general approaches used are appropriate. Following are some remarks concerning the details.

The motivation behind the adjusted sample size is understandable, but the definition (bottom of page 53) seems arbitrary. There are many functions that potentially can be used for adjusting sample size. Of these, the one used by the authors is admittedly the simplest. However, the objective here is not parsimony, but the elimination of bias. There is no evidence, presented or referenced, that the particular adjustment eliminates bias. Quite the contrary, there is some indirect evidence in the results of this study (remarked upon at the bottom of page 32) that the adjustment either does not fully correct or that it overcorrects.

Related to this problem is the choice of the critical value of adjusted sample size (N_a) used for censoring (page 17). It is not clear why the value of 4 was chosen.

The initial grouping of the sampling locations is also quite *ad hoc*. In this case, the authors quite rightly choose to carry out a sensitivity analysis, but their description of this appears incomplete. The relevant passages (pages 14 and 27) only amount to a statement that "boundaries between the initial units were altered but this caused no change to the essential conclusions of the study." Ideally, it would be good to know how (and how much) the boundaries were changed.

The methods of collecting and handling data, as well as the laboratory techniques, are outside this reviewer's field of expertise.

ANALYTICAL METHODS

It was enjoyable to read about Boundary Rank (BR), a purpose-built clustering method developed by two of the authors. It would be a matter of interest to know if the authors have considered formulating this as a Bayesian probability model with prior probabilities of clustering informed by distance. Also, it would be interesting to develop more informative ways of comparing the three methods of clustering used here. It was rather difficult to juxtapose their results simply using the dendrograms.

Still on the topic of BR, the presentation in the middle of page 18 raises two questions. First, the definition of "adjacent" was not clear. Even after examining Fig. 6 and its blow-ups, the criteria for linking two groups were still opaque. Second, it is not clear what measure of distance was used for BR. If Euclidean, given that seals do not swim on land, it is also not clear if this is a good approximation, given the shape of the coastline and degree of fragmentation of land.

The evidence for and necessity of a stepping-stone model (page 18) for dispersal is not entirely convincing. The fact that the probability of a particular jump decays with distance does not uniquely

lead to a stepping-stone model. It could just as well imply that longer jumps are rarer. Typically, the model is used for dispersers that are given a once-in-a-lifetime chance to jump and are only allowed to jump to the closest site. It is not clear if this is the way the model has been implemented here. If so, then it is not an appropriate dispersal model for seals. Either way, this needs some clarification.

The report contains other assumptions about dispersal that are equally unclear. For example, the biological significance of a transition matrix that is symmetric around its leading diagonal (page 22) remains unstated. Also, the authors initially mention "behavioral and ecological factors" (top of page 8), but then ignore the possibility of density dependence in dispersal (page 22).

RESOURCE MANAGEMENT

The report tackles an urgent and thorny problem of management on a particularly difficult species (due to its continuous distribution), using incomplete data. It is a worthy effort and a huge improvement on previous management plans. Here are two distinct comments that should be read more as discussion points and less as criticism of the current work.

First, this reviewer has a philosophical difficulty with the central premise of the work; that discrete subpopulations must exist. For simplicity, consider a continuous population arranged along a linear coast that contains no discrete subpopulations, but is nevertheless heterogeneous in the sense of a continuous gradient. Subdividing the range of the population by means of few arbitrary boundaries and sampling from within those will provide groups of animals that are statistically different. However, despite this statistical result, the position of the boundaries is completely meaningless because the animals immediately on either side of the boundary are almost identical. If, as is the case here, the determination of boundaries were done after sampling, then one would expect it to be completely at the mercy of the choice of sampling locations. It is not clear whether the results of this study are immune to this sort of problem. One of the authors' listed references that looked particularly relevant (reference 6) did not seem to address this potential problem.

Second, the arguments discussing the use of 0.05 for hypothesis testing (second paragraph on page 22) are not very convincing and could be removed. The following paragraph, describing the explicit examination of population dynamics, offers considerable improvement. It is refreshing that a genetics-oriented study should explicitly address questions of population dynamics and the authors should be encouraged to develop this sort of investigation further in the future. As it stands, it is more akin to an illustration of how population dynamics could critically affect management decisions, rather than a comprehensive investigation. Crucially, here the effect of population dynamics on relative isolation is affected by generation time because generation time differs at different population densities. This is obtained by averaging between generation time at carrying capacity (T_c) and at zero population size (T_0). This seems incorrect. Surely, the average should be weighted by the amount of time the population spends at each state. Even this would be a crude approximation, because the correct answer is the integral of generation time over the actual population time series. Also, this approach ignores the spatial heterogeneity in population dynamics. Taking this investigation forward could follow two routes: the population model used in the simulations could be expanded, or the simulations could be run on real population data. The latter is considerably better, if the data exist.

DISCUSSION AND INTERPRETATION OF OTHER STUDIES

The interpretation of other non-genetic evidence relevant to harbor seal populations was logical and entirely appropriate. This was one of the strong points of the report. In the spirit of BR (the combination of spatial and genetic distance between groups of animals), it was surprising to see that a discussion about how all these strands can be pulled together on a single analytical framework was omitted from the authors' list of future directions.

CONCLUSIONS

The introduction of the report walks the tightrope between conservation requirements and scientific completeness. As is often the case, management decisions need to be made before the data or analysis methods are complete and complementary. The authors have made a good stab at laying down the definitions of the quantities that are relevant and measurable. Indeed, they go to great lengths to ensure that their results are consistent with the definition of stocks, as provided in the Marine Mammal Protection Act (MMPA), and as implemented by the National Marine Fisheries Service (NMFS).

One complaint with regard to the first paragraph of the discussion on page 31 is that the authors state that their genetic results demonstrate the existence of relatively isolated subpopulations. This is quite different from assuming isolation *a priori* (as the authors have done) and delineating the subpopulations using clustering techniques. Consequently, the argument made here is unnecessarily circular. To illustrate this point, when distance was used in the clustering, (in BR) the pattern of isolation by distance was (unsurprisingly) adhered to. In contrast, when distance was omitted, the pattern was not nearly as straightforward.

On page 32, the authors suggest that such discrepancies between clustering methods might be an artifact of sampling bias (which should have been eliminated by their sample size adjustment, see above). They say (top of page 33) that BR is "less susceptible to being misled by poor sampling than the other clustering methods." Conversely, it could be argued that BR is more likely to conceal the telltale signs of poor sampling.

OTHER COMMENTS

Some minor editorial comments follow:

1) Page 17: It is not clear in what sense this is "parameter estimation." It is not clear which parameters are being estimated.

2) Page 22 (Top): Replace "…demographically independent…" by "… approximately demographically independent…."

SUGGESTED REVISIONS

Based on the above comments, here is a concise list of suggested revisions:

1) Include or refer to evidence, theoretical or empirical, that the proposed adjustment in sample size eliminates bias. Theoretical evidence may arise from asymptotic arguments such as those used to prove that an estimator is unbiased. Empirical evidence may be obtained by simulation. If such evidence is

unavailable, then this limitation should be clearly stated in Appendix 3 and the Discussion. Justify the choice of N_a and expand on the sensitivity of the results on the initial clustering.

2) State and justify the measure of distance used in BR and explain the definition of adjacency.

3) The particular model used for dispersal (stepping-stone, symmetric, density independent) needs to be defended. Alternatively, its limitations must be acknowledged in the Discussion.

4) State the limitations of the population model used here and outline how it can be improved.

5) Suggest ways in which the different strands of genetics, behavior, space, population dynamics, and telemetry can eventually be pulled together on a single clustering framework.

6) Clarify the apparent circularity of the argument in the first paragraph in the discussion.

BACKGROUND REFERENCES SUPPLIED TO THE REVIEWERS

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