SC/13/WWG/O2

PROCEEDINGS OF A WORKSHOP ON THE POTENTIAL APPLICATION OF MARK-RECAPTURE METHODS TO ESTIMATE THE SIZE AND TREND OF THE PACIFIC WALRUS POPULATION

Anchorage, Alaska
March 12-13, 2002

U.S. Fish and Wildlife Service
Marine Mammals Management
1011 East Tudor Road
Anchorage, Alaska 99503

USFWS Technical Report MMM 03-1

February 2003

ACKNOWLEDGMENTS

The mark-recapture workshop was hosted and funded by the US Fish and Wildlife Service and the US Geological Survey. This report is based on the oral and written contributions of the workshop participants (Appendix I). Chad Jay, Brendan Kelly, Bryan Manly, Gennady Smirnov, and Sandra Talbot guided workshop discussions. Svetlana Potten provided English/Russian translation during the workshop. Mary Cody provided logistical support to workshop participants. The workshop proceedings were compiled and edited by Joel Garlich-Miller.

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EXECUTIVE SUMMARY

Between 1975 and 1990, the United States and Russia collaborated on a series of aerial surveys to estimate the size of the Pacific walrus population. These collaborative surveys were suspended after 1990 due to unresolved problems with aerial survey methods which failed to produce reasonable estimates of population size or trend (Gilbert 1999). In 2000, a panel of experts recommended exploring the potential application of mark-recapture methods as an alternative approach to population estimation (Garlich-Miller and Jay 2000). In addition to an estimate of population size, a mark-recapture study could also provide information on animal movement patterns, survivorship, and population structuring. This information is also important for effective population management.

Sample size requirements for a mark-recapture estimate of the Pacific walrus population were investigated through simulation (Manly 2000). Initial sampling simulations from a modeled walrus population indicated that even with a large number of animals sampled (15-30,000 annually) the precision of the resulting population estimates was quite poor. Incorporating age into the mark-recapture analysis dramatically increased the efficiency and precision of the estimate. When the age of sampled animals was incorporated into the mark-recapture analysis, simulated sampling rates as low as 1,500 animals annually produced population estimates with a coefficient of variation of approximately 20%. The age of walruses can be estimated in the field based on their skull and tusk morphology (Kelly *et al.* 2000).

Mark-recapture methods require the ability to identify individual animals. The use of genetic markers to identify individuals has several potential advantages over traditional methods of identification such as brands, marks, or tags. Genetic markers are permanent, they are present in all individuals, and they can be collected through relatively non-invasive sampling techniques. Considerations for the development and use of genetic markers for a mark-recapture study include: avoiding cross contamination of samples; identifying sufficient number of reliable and appropriate markers; the assumption that there is a sufficient level of genetic variation in the population to identify individuals, and; potential complications relating to population sub-structure. Genetic material can be extracted from skin samples collected with biopsy darts propelled by crossbow or rifle.

The successful application of mark-recapture techniques to estimate the size and trend of the Pacific walrus population would require the ability to determine age, gender, and identify individual animals with negligible error rates. Field and laboratory trials are required to evaluate these assumptions and to refine techniques. Pacific walruses range across a large, remote and dynamic habitat.

Collecting representative samples from the population is expected to present significant logistical challenges. Field trials are necessary to refine sampling strategies.

INTRODUCTION

Background

The Pacific walrus population is an ecologically important component of the Bering Sea ecosystem. While foraging for clams and other invertebrates, walruses exert a strong influence on the structure of the benthic community and play an important role in nutrient cycling. Walruses also serve as an important food source for top level predators, including man. Pacific walruses have been harvested by subsistence hunters for thousands of years. Today, walrus hunting remains an important component of the economy and culture of Native communities along the Bering and Chukchi Sea coasts.

The current size and trend of the Pacific walrus population are unknown. Between 1975 and 1990, the United States and Russia collaborated on a series of aerial surveys to estimate the size of the Pacific walrus population. These collaborative surveys were suspended after 1990 due to unresolved problems with aerial survey methods that failed to produce reasonable estimates of population size or trend (Gilbert 1999). Future work to evaluate the size and trend of the Pacific walrus population is considered a high priority by Russian and American scientists.

In March 2000, the U.S. Fish and Wildlife Service (USFWS) and U.S. Geological Survey (USGS) hosted a workshop concerning walrus survey methods. Workshop participants included American and Russian experts in walrus biology and survey design, subsistence hunters and resource managers. The goal of the workshop was to identify and evaluate various survey techniques and approaches to estimate the size and trend of the Pacific walrus population (Garlich-Miller and Jay 2000). Workshop participants recommended exploring the potential application of genetic finger printing techniques in a mark-recapture study. This recommendation was based on the success of a genetic tagging study carried out on humpback whales in the North Atlantic (Smith *et. al*. 1999). In addition to information on population size, a mark-recapture study could also provide information on animal movement patterns, survivorship, and population structuring. This additional information is also important for effective population management.

Sample size requirements for a mark-recapture estimate of the Pacific walrus population were investigated through simulation (Appendix II: Manly 2000). Initial mark-recapture sampling simulations from a modeled walrus population of 300,000 animals indicated that even with a large number of animals sampled (15,000 to 30,000 samples annually), the accuracy of the resulting population estimates was quite poor. Additional simulations were conducted where the age-class of sampled animals was known. Incorporating age into the mark-recapture analysis dramatically increased the efficiency and precision of the estimate. Simulated sampling rates as low as 1,500 animals annually, produced population estimates with a coefficient of variation of approximately 20%. The age of free-ranging walruses can be estimated in the field based on their skull and tusk morphology (Appendix II: Kelly *et al.* 2000).

Workshop objectives

Based upon the encouraging results of the mark-recapture simulation trials, the USFWS and USGS convened an ad hoc workshop to discuss and evaluate the mark-recapture approach to estimating the size and trend of the Pacific walrus population.

The objectives of the workshop were to:

$ Review the results of mark-recapture simulation trials
$ Review information on field techniques for estimating the age of walruses
$ Review information on genetic fingerprinting techniques
$ Review information on biopsy sampling techniques
$ Review information on the seasonal distribution of walrus herds
$ Discuss potential sampling strategies
$ Develop a list of recommendations for future work

The following report is based on the oral and written contributions of workshop participants (Appendix I). Prior to the workshop, participants reviewed a package of background information on mark-recapture simulations, field identification of walrus age/sex classes, and genetic analyses of Pacific walrus (Appendix II).

WORKSHOP TOPICS

Mark-recapture simulations *(Bryan Manly)*

Sample size requirements for a mark-recapture estimate of the Pacific walrus population were investigated through simulation (Appendix II: Manly 2000). The exchange of walruses between six hypothetical sampling locations, and unsampled sites (e.g. the pack-ice of the Chukchi Sea) was modeled using site-transition probability matrices. The modeled population consisted of 150,000 male and 150,000 female walruses. Capture probabilities for male and females at each sample site were estimated based on the age/sex composition of the herds observed at the haulouts during an aerial survey in the fall of 1990, with the assumption that the animals using the haulouts spent 50% of their time away from the haulout foraging.

A conventional mark-recapture study was simulated without consideration of the age of sampled animals. The Manly and Parr (1968) method was used to estimate population size with separate estimates for males and females. Simulation trials indicated that even when a relatively large number of animals were sampled (5-10% of the population) the accuracy of estimates were quite poor.

Incorporating age into the mark-recapture analysis dramatically increased the efficiency of the estimate. For example, it can be assumed that all animals observed to be 1, 2 or 3+ years old during year $t + 1$ were alive in year t, therefore, they can be added to the group used to estimate the probability of capture in year t. Using the Manly and Parr method in this way sampling with probabilities of capture of 0.01 (approximately 1,500 animals per year) for three or four years, resulted in population estimates with a coefficient of variation of approximately 20%. Samples collected from harvested animals could potentially provide additional recapture data.

The computer programs CMRSIM and CMRSIM1 are available to simulate mark-recapture estimation with or without age information (Appendix II: Manly 2000). These programs can be used to explore different sampling scenarios such as sampling with a constant probability of capture; sampling with a relatively high probability of capture at the start and end of the sampling period; sampling with varying effort at different sampling sites, or, sampling every other year. The effects of grouping various age-classes (for example, 0 and 1-year-old animals) on sample size requirements could also be examined. In general, the more precisely each age-class can be defined, the more efficient sampling will be. The models can be adjusted for different capture probabilities for males and females, but assume that all age-classes (per gender) have an equal capture probability. Additional modeling effort would be required to examine the effects of heterogeneous capture probability across age-classes, or the effects of population sub-structuring on sample size requirements.

Field identification of age/sex classes *(Brendan Kelly)*

Manly (2000) demonstrated through simulation that incorporating age into mark-recapture analysis can dramatically increase the efficiency and precision of a population estimate. The age and gender of free ranging walruses can be estimated according to their facial (skull and tusk) morphology (Appendix II: Kelly *et. al.* 2000). The distinguishing morphological characteristics (ratio of tusk length to snout width) were derived from a series of harvested specimens for which age was estimated through counts of growth layers in the lower canine teeth.

Field identification techniques were tested and refined during a series of research cruises in the 1980s and 1990s to estimate rates of productivity, survivorship and recruitment (Appendix II: Kelly *et. al.* 2000). Ice-reinforced ships and coastguard icebreakers were used as operational bases for sampling free-ranging walrus herds in the pack ice. In general, smaller ships were better at maneuvering through the ice and approaching groups of walruses without disturbance. Scale drawings were used in the field as standards to help classify walruses as calves of the year, yearlings, 2-year-olds, 3-year- olds, 4-5-year-olds, 6-9-year-olds, 10-15-year-olds, and animals >15 years-old. Gender was estimated for all mature animals (more than 6 years-old) which have sufficient dimorphism.

Consistent criterion for assigning animals to different age-classes is essential for accuracy. A comprehensive training program is required to standardize methods of observation and data collection. Observers occasionally have difficulty distinguishing the gender of mature animals. In the case of a genetic study, the gender of sampled animals could potentially be determined through DNA analysis. Inexperienced observers also had some difficulties distinguishing between calves of the year and yearling animals (calves have no visible tusks, yearlings have tiny stubs that are not always visible below their mustachial pads). While this distinction is essential for estimating productivity rates and survivorship between age-classes, it may be less important for a mark-recapture population estimate.

The age classification method requires a full frontal facial view to assess the ratio of tusk length to snout width. This sampling requirement may not be fully compatible with the need for collecting multiple biopsy samples rapidly from a group of animals. Field trials are recommended to determine potential sampling rates across various age-classes.

Using genetic markers in mark-recapture studies *(Sandra Talbot)*

Mark-recapture methods require the ability to identify individual animals in a population. The use of genetic markers to identify individual animals offers several potential advantages over the more traditional methods of identification such as brands, marks, or tags. Genetic markers are permanent, they are present in all individuals, and can be collected through relatively non-invasive sampling procedures.

The use of genetic markers in a mark-recapture study requires that there is a sufficient level of genetic variation in the selected markers to identify individuals in the population. The number of co-dominant markers necessary to identify individuals is dependent upon the level of relatedness in the population.

Other considerations include the need to develop a reliable method for collecting genetic samples and the careful handling of samples to avoid contamination in the field or lab. Laboratory investigations need to be carried out to identify potential sources of error and quantify levels of allelic drop out. Selected micro-satellite markers should show strong amplification without co-amplification of non-target DNA.

The USGS Molecular Ecology Lab (MEL) has been investigating the feasability of using genetic markers to identify individual walruses. Preliminary results suggest adequate variation and number of identified markers to identify individuals, however follow-up studies are recommended to establish confidence bounds about individual exclusion probability estimates, and to investigate the level of potential sub-structuring within the population (Appendix II: Talbot *et al.* 2002).

The MEL is primarily set up for phylogenetic and population level research, and not for conducting large-scale projects such as the proposed walrus mark-recapture study. Once laboratory methods have been established, other laboratories, better equipped to process large numbers of samples, could be contracted to carry out the genetic analysis. For example, the USFWS laboratory in Anchorage is well equipped to process large numbers of genetic samples. The advantage of having the laboratory analysis done in-house is that it will be easier to resolve any sampling questions.

Biopsy sampling techniques *(Chad Jay)*

Genetic material can be extracted from skin samples collected with biopsy darts propelled by crossbow or rifle. One advantage of crossbows is that they are quiet, reducing the potential of disturbing other animals in a group. Biopsy darts typically have a cutting head that removes a small sample (approximately 1 cm^3) of skin. Biopsy cutting heads come in various shapes and sizes. The ones that have been used to sample walruses have three internal barbs that retain the tissue sample when the dart is pulled away. Biopsy tips can be modified to penetrate to different depths.

The effective range of most biopsy systems is approximately 20 yards. Field experience suggests that walruses hauled out on land or ice can be approached closely if efforts are taken not to disturb them. Male walruses sampled at Bristol Bay haulouts exhibited little or no reaction to biopsy darts. Biopsy darts are normally retrieved using a line and reel. Floatable darts can also be used to sample animals in the water without lines attached. Several darts, prepared in advance, can be used to sample multiple animals from a single group. Care must be taken not to sample the same animal in a group more than once to eliminate lab costs associated with unnecessary replication.

There do not appear to be any technical hurdles to overcome in collecting biopsy samples from walruses, however field trials are required to develop effective sampling procedures and sampling rates for animals in different habitats.

Seasonal distribution patterns of the Pacific walrus population *(Gennady Smirnov)*

The entire Pacific walrus population over-winters in the pack ice of the Bering Sea. During the winter months, large concentrations of walruses are found in polynas south of St. Lawrence Island, in the Gulf of Anadyr, and outer Bristol Bay. It is likely that the St Lawrence Island and Gulf of Anadyr groups intermingle, however this has not been verified.

In the spring, most of the population migrates north through the Bering Strait into the Chukchi Sea, however some animals, mostly adult males, moves south to Bristol Bay and the Kamchatka Peninsula.

The ice is often slow to move out of the Gulf of Anadyr in the spring. This may explain why mixed (age/sex classes) herds persist in this region. Once the sea ice melts, these animals begin to use land haulouts at Rudder and Meechkin Spits. The mixed haulouts in the Gulf of Anadyr often persist into June and July. Telemetry data supports the theory that the haulouts in the Gulf of Anadyr are an inter-linked complex (animals move between haulouts). In 2001, the composition of walrus herds at the coastal haulouts in the Gulf of Anadyr was examined. Approximately 4.5% of the animals using the haulouts were calves of the year, 3.5% were yearlings, and 4% were two-year olds. The gender of adults was approximately equal. By the end of July, most of the females and dependent calves have migrated north into the Chukchi Sea. In recent years, there has been an increase in the number of females and young using the coastal haulouts in the Gulf of Anadyr. It has been suggested that the shift in summering areas is related to changing ice conditions.

By August and September, most of the Pacific walrus population has moved into the pack ice of the Chukchi Sea. Large concentrations of walruses are normally found in the vicinity of Wrangel Island (Russia) and along the northwestern coast of Alaska. In minimal ice years, large mixed haulouts develop on Wrangel Island, however these haulouts do not form every year. In late September or October, the walruses that summered in the Chukchi Sea begin to move south. At the same time, at least some of the male walruses that summered along the Kamchatka coastline and in Bristol Bay move north towards the Bering Strait.

In October, large herds of walruses begin to come ashore along the Chukotka coastline. It is difficult to predict when and where these haulouts will form. In the U.S., walruses are frequently observed at haulouts on Big Diomede Island, King Island, St. Lawrence Island, and the Punuk Islands. With the continuing development of ice, most walruses move south of St. Lawrence Island and the Chukotka Peninsula by early to mid-December. In October and November, ice forms early in the Gulf of Anadyr due to the influx of freshwater from rivers. Groups of walruses groups begin to haulout onto the ice as soon as it is thick enough.

Sampling considerations for a mark-recapture population estimate

The successful application of mark-recapture techniques to estimate the size and trend of the Pacific walrus population will require the ability to determine age, gender, and identify individual animals with negligible error rates. Field and laboratory trials are required to evaluate these assumptions and to refine techniques.

A fundamental assumption of the mark-recapture approach to population size estimation is that all animals in the sampled population have some probability of capture. Collecting representative samples from the Pacific walrus population is expected to present considerable logistical and sampling challenges. Although many walruses use terrestrial haulouts during the summer months, for most of the year walruses are associated with pack ice. Large seasonal and inter-annual differences in sea ice cover make it difficult to predict when and where to locate aggregations of walruses. Sampling walruses offshore in the pack ice will require an ice-reinforced ship, which will add significantly to the expense of sample collection. There may also be age-specific differences in capture probability. For example, while it may be relatively easy to sample mature animals (particularly males) at haulouts, it is expected that it will be far more difficult to sample calves because their mothers tend to be wary and highly protective. Walruses also tend to haulout in large groups. Collecting a random sample from a large group of animals will be difficult because only those animals on the edge of the herd can be easily sampled. Genetic samples could be collected from animals swimming in the water, however it will be difficult to estimate age or gender without a full frontal view of their snout or tusks.

Sampling strategies

The modeling effort used to estimate sample size requirements was a theoretical exercise. The modeling exercise considered six hypothetical sampling locations representing complexes of land-based haulouts. Walruses in the offshore pack ice were not considered in this hypothetical sampling strategy; they were assumed to exchange with the sampled locations over time. While this hypothetical sampling approach was reasonable for simulations to estimate sample size requirements, the sampling strategy used in an actual mark-recapture study would likely have to be more complex. Additional information regarding site fidelity and animal movement patterns is necessary to refine a sampling strategy. This information could be collected through telemetry studies or by initiating a mark-recapture initiative and adjusting the sampling strategy based on the information obtained.

From a logistical perspective, the late summer or early fall may be the easiest time of the year to locate and sample large numbers of walruses because many animals are expected to be using land-based haulouts at this time. Unfortunately, the fidelity of walruses to the coastal haulout sites is poorly understood and seasonal movement patterns are difficult to predict. Depending on the ice conditions, walruses may use land-based haulouts one year and remain in the pack ice the next. Furthermore, the location of offshore aggregations of walruses (those animals that are not associated with land-based haulouts) will shift from year to year depending on the extent of the sea ice. A summer/fall sampling strategy would have to be flexible enough to adapt to annual changes in walrus distributions. A broad, flexible sampling effort might best be accomplished from a small ice-reinforced ship equipped with skiffs to carry out sampling. The ship could travel north along the Chukotka coastline, sampling walruses at the coastal haulouts along the way. Once the pack ice is encountered to the north, the ship could travel east along the ice-edge to collect samples from offshore aggregations of walruses [1]. Aerial reconnaissance would be very useful for finding offshore walrus aggregations. Walruses summering at terrestrial haulouts in the Bering Sea could be sampled from shore.

[1] During previous fall surveys, walruses were generally found within 150 kilometers of the southern edge of the pack ice (Gilbert 1999).

Another sampling strategy might be to sample animals during the late winter or early spring in the pack ice of the Bering Sea. At this time of the year walruses are concentrated in areas of broken pack ice or persistent leads. All segments of the population may be aggregated together at this time. Sampling would require a dedicated icebreaker and some form of aerial reconnaissance to locate winter aggregations. Cold temperatures, lack of daylight, and the high costs associated with a polar-class icebreaker (necessary to withstand the heavy ice conditions) would present considerable sampling challenges during the winter months.

Another sampling strategy to be considered would take advantage of the large-scale migrations occurring through the Bering Strait in the spring. Subsistence walrus hunters from Bering Strait villages intercept large numbers of walruses each spring as they migrate through the Strait. The skills and resources of these hunters could be utilized to help collect biopsy samples during the spring migration before the animals become widely and unpredictably distributed throughout the Chukchi Sea. Animals that don=t migrate through the Bering Strait in the spring could presumably be sampled from shore at coastal haulouts in the Bering Sea. One challenge of sampling from small, shore-based boats is that weather and ice conditions often make it difficult to access herds of walruses as they migrate through the Strait.

Walruses also migrate through the Bering Strait in the fall. This typically occurs in October and November before the sea ice is thick enough to form ice haulouts. As a result, large mixed herds frequently come to shore to rest along the Chukotka coastline. During the fall migration animals are frequently exhausted from swimming when they come to shore and are therefore easy to approach. One challenge associated with this strategy is that the time and location of the fall haulouts along the Chukotka coastline are difficult to predict.

RECOMMENDATIONS

While there does not appear to be any insurmountable obstacles to carrying out a mark-recapture study with respect to sample collection, genetic identification or statistical estimation, field and laboratory trials are necessary to verify assumptions and refine techniques. Representative sample collection is likely to be the greatest challenge to a successful study.

Modeling

Relatively simple methods of estimation were used for the sample simulation trials. If the mark-recapture approach to estimating population size is considered further, alternative analysis methods should also be explored. Because of the complex nature of the data that would be collected (incorporating the age of sampled animals, movement between sites, heterogeneity in capture probability for different age/sex classes, harvest information etc.), it will probably be necessary to modify or customize an existing method of analysis. Although a preliminary study of analysis methods could be made at any time, the patterns of movement in the real population will be an important consideration for data analysis. The final decision regarding how to analyze the data will depend on the nature of the data actually obtained. Consequently, data would have to be collected for at least two years before modeling issues can be properly assessed. Otherwise, a lot of effort could be devoted to modeling that turns out not to be appropriate or necessary.

Genetics

A collection of samples from different geographical regions is necessary to evaluate the level of population sub-structuring. The null hypothesis is that the population is panmictic. If this is the case, it may require a relatively large number of alleles to identify individuals. In general, the more alleles required, the more complicated, expensive and error prone the laboratory analysis methods will be.

Because of the relatively large number of samples necessary for this proposed mark- recapture study, it would be desirable to determine the minimum number of loci necessary to identify individuals with reasonable certainty. Efficiencies are likely to evolve as more samples are collected. Reducing the number of loci necessary to identify individuals will result in a cheaper, more accurate, laboratory technique.

Field sampling

Population estimates from mark-recapture data will be biased if some segment of the population is unavailable for sampling. Movement data collected from a mark-recapture study or through telemetry studies could help assess this problem.

Field trials are also necessary to perfect sampling techniques and investigate potential sampling rates. A pilot project could provide information on movement patterns so that methods of estimation could be investigated further, and genetic samples that would help refine laboratory techniques. Samples collected through a pilot project could be used for the start of a long-term study.

A mark-recapture estimate will require a minimum investment of two years of sampling. The first year of sampling (marking) should be as broad and intensive as possible. Unless a significant sampling effort is made it is unlikely that any recaptures will be obtained - this would represent a wasted effort. As long as there are some recaptures a population estimate can be calculated.

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APPENDIX I: LIST OF WORKSHOP PARTICIPANTS

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APPENDIX II: BACKGROUND INFORMATION