

EVOSTC ANNUAL PROJECT REPORT

Recipients of funds from the *Exxon Valdez* Oil Spill Trustee Council must submit an annual project report in the following format by **Sept. 1 of each fiscal year** for which project funding is received (with the exception of the final funding year in which a final report must be submitted). **Please help ensure that continued support for your project will not be delayed by submitting your report by Sept. 1. Timely receipt of your report allows more time for court notice and transfer, report review and timely release of the following year's funds.**

Satisfactory review of the annual report is necessary for continuation of multi-year projects. Failure to submit an annual report by **Sept. 1** of each year, or unsatisfactory review of an annual report, will result in withholding of additional project funds and may result in cancellation of the project or denial of funding for future projects. **PLEASE NOTE:** Significant changes in a project's objectives, methods, schedule, or budget require submittal of a new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.

Project Number:..... **070853**

Project Title:..... **Pigeon Guillemot Restoration Research in Prince William Sound**

PI Name: **David Irons and Dan Roby**

Time period covered:..... **September 2008-September 2009**

Date of Report:..... **September 1, 2009**

Report prepared by:..... **David Irons**

Project website (if applicable):

Work Performed: Summarize work performed during the reporting period, including any results available to date and their relationship to the original project objectives. Explain deviations from the original project objectives, procedural or statistical methods, study area or schedule. Also describe any known problems or unusual developments, and whether and how they have been or can be overcome. Include any other significant information pertinent to the project.

This was largely an analysis year, although we resurveyed the Naked Island group for birds to confirm the extremely low numbers that we had found the two previous years. The numbers of Pigeon Guillemots, Tufted Puffins, and Parakeet Auklets remained low.

We obtained a preliminary report on the genetic origin of the mink on Naked Island and other parts of the sound. It is not a black and white result as is often seen on TV with genetic analyses. I have copied the Report below. In a sentence, the ancestors of the mink on Naked Island appear to be comprised of a mixture of introduced mink from Montague Island and other mink in PWS that were not introduced. They are still working on the time of arrival to Naked Island, but it may have been about the time of the oil spill.

Copy of Mink Report

Probable origins of Naked Island mink - based on mtDNA control region sequences, 10 nuclear microsatellite allele frequencies, and bottleneck simulations.

Brief review and update of data from previous report of October 2008.

In our previous report, we discussed how 105 mtDNA control region sequences allowed us to 1) distinguish mink from different parts of North America, 2) confirm that fur farm mink have ancestors from both east and west of the Rocky Mountains, and 3) show that mink samples from Knight, Montague and Naked Island in PWS include individuals with an eastern haplotype also found in fur farm mink, suggesting fur farm ancestry. We also discussed how PWS populations were currently structured based on data from seven microsatellite loci and how this structure further allows us to distinguish mink from Cordova, Northwestern PWS (mainland and Esther I.) and Montague, from those from Western mainland, Evans I. Knight I. and Naked I.

Since October we have obtained an additional 27 control region sequences, mostly from PWS, and microsatellite genotypes for 10 loci for 209 individuals that are 95% complete (less than 5% missing data). The data set now includes 24 Naked Island mink, up from 8. We also expanded our coverage of genotype diversity elsewhere in AK. By rerunning a handful of SE Alaska samples from our previous work, we reconciled our past data set of eight loci for SE AK with the PWS data set, as we had previously for interior AK. Subsequently, we ran the two new markers used with the PWS samples with 16 SE AK samples and 16 interior AK samples (of the 35 included in our last PWS report).

The control region sequences added for PWS were similar or identical to previous sequences for the various localities and quite distinct from one another, reinforcing the apparent mtDNA structure in the sound (Table 1) – Cordova and NWPWS in particular being quite distinct from one another and from the western mainland and adjacent PWS islands sampled. Most importantly for the purposes of this report, all 8 Naked Island mink sequenced had an identical haplotype (#27) that was also found in eastern localities and among fur farm mink. This does not necessarily mean that the Naked Island population was introduced from a fur farm, however. That haplotype was also found on Knight Isl. (3 of 7 samples) and Montague (1 of 9 samples) Isl., so may reflect earlier fur farm ancestry on those islands (and others in the area, like Latouche) and subsequent migration to Naked.

The results of microsatellite analyses run previously also did not change significantly with the addition of more samples and loci and will thus be reviewed only briefly at this time (data not shown). The microsatellite data were error checked for a final time and tests of Hardy-Weinberg equilibrium, linkage disequilibrium and an exact test of population differentiation were performed using GENEPOP. All loci were in Hardy-Weinberg equilibrium (HWE; no allelic dropout causing an excess of homozygotes) and unlinked. All PWS populations were also in Hardy-Weinberg equilibrium individually and all populations were differentiated from one another except for Copper R. and SW AK (as previously). Another measure of genetic differentiation,

Table 1: Distribution of control region haplotypes in PWS (in color and bold) compared to other localities (black). MtDNA clades were determined from cyt b and control region sequences for non-PWS haplotypes (from a previous study) and are included to show evolutionary relationships. The “?” indicates the uncertainty about the relationship of three CR haplotypes to those in the previous study. Haplotypes from eastern localities, a western fur farm and Ireland, where they are introduced (from Genbank), are also in bold to highlight haplotypes of eastern origin that may have been introduced to the west (#27) and vice versa (#19) via fur farms.

haplotypes	?			Western clade									North Pacific Coast clade									Eastern clade						
localities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	*28
Int. BC/MT					1	2	3		4																			
Interior AK												4				1		1										
SW AK														1	1			1										
Copper R.							1									1		1										
Cordova							4	1																				
NW PWS																		1	4									
W PWS			2															5										
Evans I																	1	4										
Knight I			3						1																		3	
Naked I																											8	
Montague I																		8									1	
East																			1								3	13
Fur Farm												2			3			3			1						1	
Feral Ireland																			1				1		1	1		
SE AK	2	1			1				5	2	1					1		6	1	2			1					
Vancouver I				7															7									

*28 includes 12 different eastern haplotypes from 13 mink, combined to save space.

pairwise Fsts, was again calculated using ARLEQUIN and all but Copper River and SW AK were significantly different (again). STRUCTURE was run with SE AK as an additional putative population and it was identified as an 8th cluster - the rest of the clusters were largely unchanged. Naked Isl. did not form a distinct cluster independent from WPWS, Evans and Knight. The distinctiveness of the Montague Isl. sample from the rest of PWS, however, was shown to be due to similarities with both PWS and SE AK (the location of the fur farm that provided the mink for the 1951 introduction).

The remainder of this report will focus on the new analyses we were able to perform with the increased number of samples from Naked Island (24) and the increased number of microsatellite loci (10). These include tests of hypotheses concerning the origin of Naked Island mink, specifically 1) that the population was recently founded, 2) the probable number of founders, 3) the probably source population(s) and 4) how long ago.

New Analyses: Identification of population bottlenecks

The software BOTTLENECK (Cornuet & Luikart 1996) was used to determine whether the microsatellite data could detect the presumed small population of mink that colonized and/or was introduced to Naked Isl. and Montague Isl. compared to other PWS and Alaska populations where mink have long been resident. Recently bottlenecked populations show a greater loss of allele numbers relative to heterozygosity compared to large populations in mutation-drift equilibrium (HWE). BOTTLENECK utilizes several simple statistics to evaluate the significance of deviations from HWE under different models of microsatellite evolution. The two used here are the Sign test and Wilcoxon signed rank test, both valid for tests of 10 or more loci. The former is valid only for larger sample sizes of 20-30, while the later is valid for samples as small as 4.

BOTTLENECK results support the occurrence of population bottlenecks in mink on Montague Isl. and Naked Isl., consistent with the recent colonization of these islands. The Montague Isl. sample had reduced gene diversity relative to heterozygosity as indicated by both the significant sign test ($p < 0.01682$ under the infinite allele model, IAM) and the Wilcoxon test ($p < 0.00342$ under the IAM). The same effect was observed in the Naked Island sample with the sign test and IAM ($p < 0.02627$) but not the Wilcoxon ($p = 0.09766$). The lack of significance for the latter, reputedly more sensitive, test can be attributed to the Naked Island sample having two monomorphic loci. With no heterozygosity, there could be no possible change in heterozygosity for these two loci, thus the sample size for the latter test was reduced by two, reducing the test's power.

The effects of a recent population bottleneck were not detected in any other sample. Despite the Western PWS sample having the lowest allelic diversity, its proportionately low heterozygosity suggests that the population has had sufficient time to recover from a (more ancient) bottleneck or that the population reduction causing a loss of alleles has been more gradual.

New Analyses: Bottleneck simulations

GENELOSS

We used the program GENELOSS (England & Osler 2001) to test scenarios for the origin of the Naked Isl. mink population. The program uses Monte Carlo sampling to simulate the effects of a population bottleneck on allelic diversity over multiple generations using allele frequency information from potential source populations, putative bottleneck sizes (in numbers of breeding pairs), and bottleneck durations (in generations). However, it ignores the potential effects of demographic processes after the bottleneck, such as population growth rate, on genetic diversity. Instead, it provides idealized estimate that may be most accurate for populations that rapidly regain sufficient size to avoid additional loss of diversity due to genetic drift. Mink populations on new islands probably grow fast enough, but may not get large enough to avoid post-bottleneck allele loss due to genetic drift.

At the outset, we predicted that the number of founders was likely to be rather small in the case of a natural colonization, given that Naked Island's 6 km distance from the next nearest island. In contrast, an intentional introduction from elsewhere in PWS or from a fur farm would probably involve more founders (16 females and 8

males were introduced to Montague in 1951). Thus we used founding population sizes ranging of 1, 5 and 10 breeding pairs in our simulations.

Assuming that the habitat for mink on Naked Island is similar to that on other mink inhabited islands in PWS, we also expect that the bottleneck duration would be brief once mink colonized the island. Mink populations invading new habitats in Eurasia and South America expand rapidly and the island environments of the North Pacific coast support the highest densities of mink recorded (2 or more per km of shoreline). Thus we simulated bottleneck sizes of 1, 3 and 5 generations without population growth, which correspond to durations of 1, 3 and 5 years because mink breed at one year old.

We used the above parameters and allele frequencies from three different populations: Knight Island (n=25), Montague Island (n=25) and a typical west coast fur farm (n=24). Knight was chosen due to its proximity to Naked and the results of the STRUCTURE analysis that placed the two samples in the same cluster. Montague was chosen because of its history as an introduced population that may have inspired further introductions from Montague to other islands. The Fur farm sample was chosen to test the hypothesis that an introduction of fur farm mink independent from that on Montague was responsible for the mink population on Naked. Simulations for PWS "populations" NWPWS, Cordova and WPWS were run for a subset of these variables, namely 1-5 founders for a one generation bottleneck, to identify other potential sources of immigrants/introductions. One thousand iterations of each scenario were run.

GENELOSS provides mean expected heterozygosity (H_e) and mean number of alleles retained (N_A) over iterations, as well as the proportion of simulations in which each allele in the source population is retained (allelic retention rate). Hanson & Taylor (2008) and Taylor & Cooper (1999), in their studies of known and suspected introduced populations of possums and wallabies, used the allelic retention rate to estimate a probability that allele loss patterns simulated for various putative source populations (e.g., Knight, Montague, fur farm) would deviate in the manner observed in the population of interest (in our case, Naked Isl.). We followed their method, multiplying the proportion of simulation replicates lacking each allele common in the putative source population (frequency > 5%) but absent on Naked Isl. Because of the non-independence of alleles in a genotype (particularly relevant in the case of small founder populations over short bottleneck durations), this method is not strictly correct, but it does provide a way to ranking the probabilities of different source and bottleneck scenarios.

The simulation that best fit the Naked Island data involved five founding pairs from Knight Isl. that experienced a three generation bottleneck (Figure 1a & 1b). The greatest overlap between the number of alleles (N_A) and heterozygosity (H_e) per locus for the Naked Island data versus the simulated data is observed when Knight is the source population over the entire range of scenarios (Fig. 1a and 1b). By themselves, simulated N_A and H_e estimates cannot distinguish source populations because similar N_A and H_e estimates can be generated with entirely different alleles. However, allele loss patterns also support Knight as the source population (Figure 1a and 1b; $P = 9 \times 10^{-4}$). Other scenarios with allele loss patterns more similar to that observed on Naked Isl. (e.g., Fur farm, Fig 1e and 1f; one founding pair and a five generation bottleneck, $P = 2 \times 10^{-3}$) did not fit the Naked Island data as well overall because their corresponding N_A and H_e values were too low (fewer alleles found on Naked were retained).

In some cases single founder scenarios using allele frequencies from Montague (Fig 1c and 1d) and fur farm samples generated allele loss probabilities lower or nearly as low as those from Knight, while scenarios with five founding pairs from these populations inevitably resulted in the fixation of an allele that was absent on Naked (and allele loss probabilities equaling zero). To make sure that there wasn't a founder number between one and five pairs that was more likely to generate an allele loss pattern similar to Naked Island's than that found using five pairs from Knight, additional scenarios with two founding pairs were run. In most subsequent scenarios, simulations based on Montague and fur farm mink had one or more alleles become fixed that were lost on Naked. The one exception was the simulation with fur farm mink bottlenecked for five generations, but fur farm mink still remained less likely than mink from Knight to produce the allele loss pattern observed for Naked Island ($P = 1.5 \times 10^{-5}$, $N_A = 1.94 \pm 0.1237$, $H_e = 0.3091 \pm 0.393$).

The allele distribution of the Naked sample is also not simply a subset of any one source population and the alleles on Naked Island that are missing in various source populations should also be considered (Table 2) in determining the most likely source population. The Naked sample shared the greatest majority of its alleles (21 of 22) with Knight and the additional allele was found in only a single individual. It is not unusual to find additional alleles relative to the source population in populations that have experienced known bottlenecks. There are three possible

explanations: 1) microsatellites mutate very rapidly and a new allele may have arisen post-bottleneck, 2) sample sizes of close to 30 are preferred in microsatellite studies because they are likely to include all alleles present in the population at frequencies greater than 5%, but it is possible that a rarer allele not sampled in the source population could be sampled in the smaller bottlenecked population, and 3) post-bottleneck immigration may introduce additional alleles.). The “extra” allele in the Naked Isl. sample was present at low frequencies on Montague and in eastern mink. Thus, the second explanation, that the allele is present at low frequency on Knight seems most likely. Knight and Montague share other alleles and one CR haplotype that are unusual in PWS but found in eastern or fur farm mink, indicative of migration between the two and/or fur farm ancestry on both.

Table 2: Sample size (n), total number of alleles (N_A) at all 10 loci for 7 PWS sampling localities, number also found among the 22 total alleles in the Naked Island sample (n=24), and the proportion shared between Naked Island and other “populations”.

	n	Total N_A	N_A shared w/Naked	Proportion of Naked N_A shared
Knight	26	31	21	0.95
Montague	26	37	19	0.86
Fur farm	24	52	18	0.82
NW PWS	11	30	17	0.77
Cordova	15	28	16	0.73
W PWS	15	22	18	0.82
Evans	11	22	15	0.68

BOTTLESIM (in first person because I haven't even mentioned the following to Joe yet)

Another bottleneck simulation program, BOTTLENECK (Kuo & Janzen 2003) assumes the source of migrants is known, but can then take into account the demographic variables that GENELOSS does not to estimate when a bottlenecked population was established. However, doing so requires specifying not only founding population size and bottleneck duration, but also how post-bottleneck population size has varied over time. Assuming that a newly founded population in a favorable environment is going to grow rapidly, a logistic growth curve seemed reasonable to describe mink population growth on Naked Island. To generate this curve, however, we need a population growth rate, r , and the carrying capacity of the environment, K . I have made some educated guesses about these values, and have run several simulations, but I would prefer to investigate further to be sure the values chosen are meaningful in the context of Naked Island mink before I go too far in interpreting them.

For example, I've taken my initial estimates of K from two estimates of coastal mink population density – one from feral mink on islands in Europe (0.66 per km of coastline) and the other from coastal mink on island off Vancouver Island (2.0 per km of coastline) – and an estimate of the length of coastline in the Naked Archipelago derived from measurements made using GoogleEarth (80 km). This gives K values of 54 and 160 for the archipelago (reasonable or not?). I would appreciate being directed to any information any of you may have regarding the length of coastline in the archipelago! As for population growth rate, I haven't had the time to research this well but have tried $r = .30$, $.50$ and $.70$ and considered the lowest is least likely to approximate reality.

I've used the bottleneck population size and duration from the most likely scenario in GeneLoss (Knight Isl., ~5 founding pairs, bottlenecked for 3 generations) and looked at simulated allelic diversity measures for three time frames: 20 years (since the EVOS), 50 years (around the time mink were introduced to Montague) and 80 years (when mink farming first started becoming popular in AK).

So far, all of the values for N_a and H_e obtained over the three time frames overlap those observed on Naked Island when $K=160$. When $K=54$, simulations of the two lower growth rates and 80 year time frame result in allele numbers and heterozygosities far lower than observed on Naked Island (genetic drift). In contrast, in the $K=54$ simulation with $r = 0.70$, allele numbers are too high for only 20 years since founding. As I said, I don't want to over-interpret this based on my current justification for my r and K estimates, but I am encouraged that the simulation results are in the right ballpark.

Conclusions and further analyses

Allelic diversity measures from the Naked Island sample are consistent with the mink population there having recently undergone a bottleneck, presumably due to founder effects. Simulations suggest that a small number of founders from a population similar to our Knight Island sample and expanding within 2 or 3 years of their arrival, is more likely to produce allelic frequency distributions, numbers of alleles and heterozygosities similar to those observed in the Naked Isl. sample than other populations we sampled. We cannot yet tell how long these mink may have been on Naked Island, but Dave's hypothesis that they arrived shortly after the Exxon Valdes oil spill strikes me as quite likely.

The microsatellite data show the 24 Naked Island samples clearly clustering with samples from Knight/Evans/Western PWS (mainland) suggesting recent common ancestry and/or continuing gene flow among them. On the other hand, eight mink sequenced from Naked Island all had the same mtDNA haplotype found at a moderate frequency on Knight Isl and in Ontario (both ~43%) and at low frequency on Montague Isl. and in our fur farm sample (15%) (both 11%). The distribution of this geographic haplotype suggests that it was introduced to PWS by fur farms, via Montague, Latouche or possibly other western PWS localities that had fur farms. The combined information from nuclear and mitochondrial genetics supports an ancestry for Naked Island mink that includes 'hybrids' between fur farm and native mink.

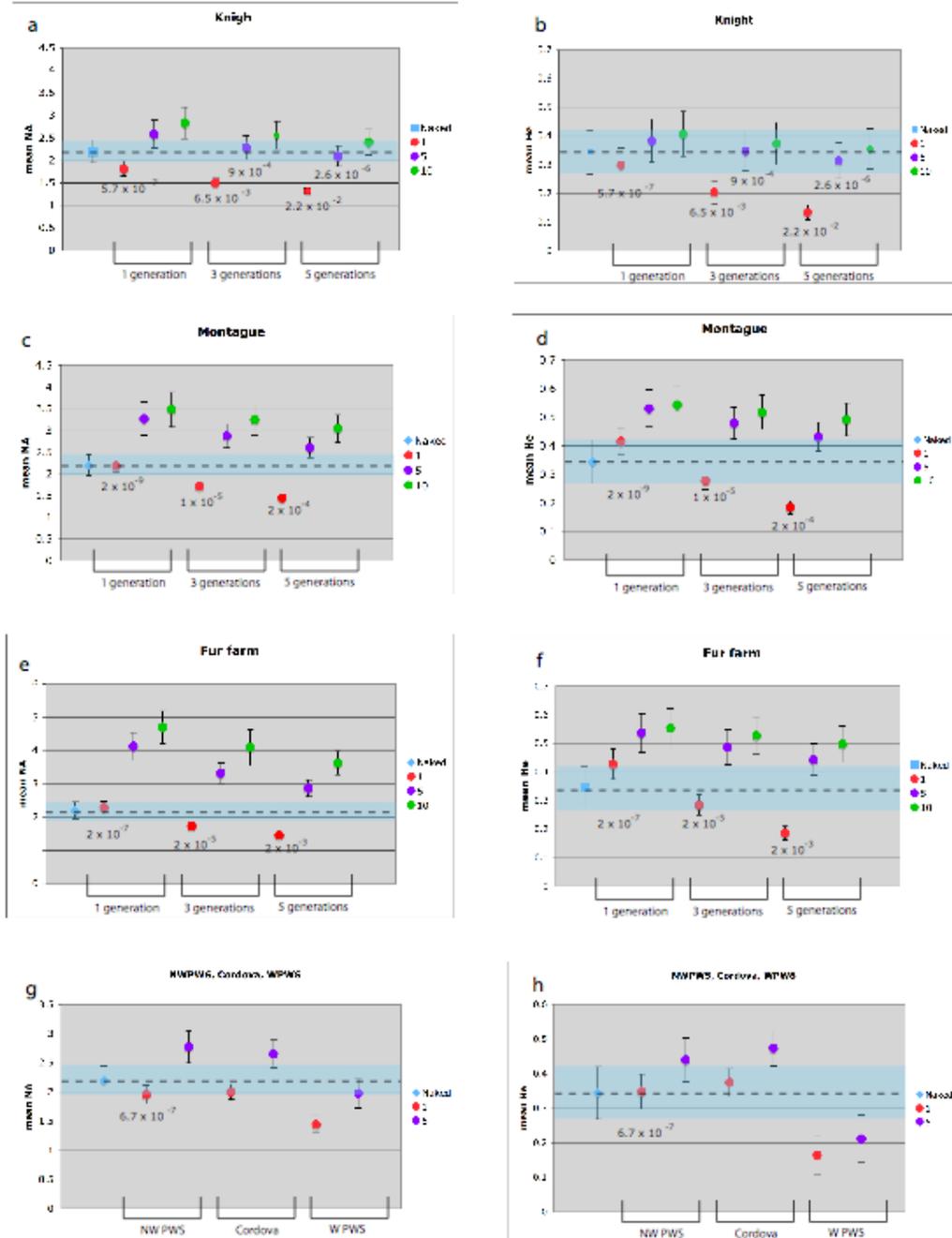
There are additional programs (MIGRATE, NeESTIMATOR, etc.) we can use to get estimates of population size and migration rates among localities in PWS (which should be helpful determining how quickly Naked Island might be recolonized if the current population were removed). I simply did not have time to undertake these in the 15 days since I've had all the data. I will start on these as soon as I get back from the American Society of Mammalogists meeting (June 24-29).

We realize that you are (understandably) eager for a final report and I will work hard to get it to you ASAP. Given a couple weeks of family commitments in July, I think it most likely that we will have a final report for you in the beginning (first week) of August (if not before) – fully updated and complete with citations and references. If it would help to have any information sooner (like MIGRATE results), I'd be happy to pass them on as they become available (within the next couple weeks). I just don't think I'll have time to finalize everything that quickly. If there is anything missing that you still want to know that you think genetics can inform you about, please ask! I haven't had time to sit down and think about these latest results as comprehensively as I would like (maybe on the plane on my way home after the meeting), so I would not be surprised if I am missing something obvious

Figure 1

Results of GENELOSS bottleneck simulations using Knight (a & b), Montague (c & d), Fur farm, (e & f) and NW PWS, Cordova and W PWS (g & h) as putative source populations. Mean (\pm standard error) of the simulation averages of allelic diversity (N_A ; left hand column) and expected heterozygosity (H_e ; right hand column) for 1000 iterations for 10 loci (y axis). Bottleneck lengths of 1, 3 and 5 generations and bottleneck sizes of 1, 5 and 10 pairs are indicated on the x-axis and by symbol colors of red, purple, and green, respectively. N_A and H_e for the Naked Island sample are provided first (light blue) and the mean \pm standard error for Naked Island is further shaded light blue across each figure. Note the change in scale for e (intentional) and g and h (accidental). Non-zero allele loss probabilities for a scenario are shown below the relevant symbol.

Figure 1



Kirsten Bixler, the graduate student who is working on this project has been analyzing data. She presented preliminary results at the AMSS in January.

She has found that the Pigeon Guillemot population declined roughly 90%, from about 1000 to about 100, since our last survey in 1998. Also the populations of about 500 Tufted Puffins and 700 Parakeet Auklets were decimated, only a handful of each remains. The EVOS funded marine bird surveys of PWS showed a sound wide decline of Pigeon Guillemots, excluding the Naked Island complex and during the same time period of about only 20%., so we know the declines on Naked, Storey and Peak do not reflect the sound-wide population trends. We expect that recently introduced mink are responsible for these declines.

We also contracted with Dr. Brown to conduct aerial surveys for forage fish around Naked Island to compare to surveys done in the mid 1990's. Preliminary results suggest that there as many or more forage fish around Naked Island now as in the 1990's. Beach seines were also done to sample abundance of forage fish to compare to the 1990's and results supported the results of the aerial surveys.

Future Work: Summarize work to be performed during the upcoming year, if different from the original proposal. Describe any proposed changes in objectives, procedural or statistical methods, study area or schedule. *NOTE: Significant changes in a project's objectives, methods, schedule or budget require submittal of a new proposal subject to the standard process of proposal submittal, technical review and Trustee Council approval.*

We plan to continue to analyze the data collected and write a report of the findings. Also the mink genetics data will be finalized and a final report will be written.

Coordination/Collaboration: Describe efforts undertaken during the reporting period to achieve the coordination and collaboration provisions of the proposal, if applicable.

This project has been to date a stand alone project.

Community Involvement/TEK & Resource Management Applications: Describe efforts undertaken during the reporting period to achieve the community involvement/TEK and resource management application provisions of the proposal, if applicable.

We contacted people in Chenega, Tatitlek, Cordova, Valdez and Whittier looking for trappers to hire to trap the mink in PWS for us. We found no one who wanted to do the trapping for us. We have also contacted several people in these villages to help determine the history of mink distribution in the sound. People were very helpful, we collected information that suggests that there were no mink on

Information Transfer: List (a) publications produced during the reporting period, (b) conference and workshop presentations and attendance during the reporting period, and (c) data and/or information products developed during the reporting period. *NOTE: Lack of compliance with the Trustee Council's data policy and/or the project's data management plan will result in*

withholding of additional project funds, cancellation of the project, or denial of funding for future projects.

Efforts to date include sample acquisition and management. Data management will compile with the Trustee Councils; data management policies.

Preliminary results were presented at the AMSS in January.

Budget: Explain any differences and/or problems between actual and budgeted expenditures, including any substantial changes in the allocation of funds among line items on the budget form. Also provide any new information regarding matching funds or funds from non-EVOS sources for the project.

NOTE: *Any request for an increased or supplemental budget must be submitted as a new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.*

We have stayed within our budget to date. We asked and were approved to do an additional survey of birds this year. We stayed in an inexpensive boat while surveying birds. We have asked for a no cost extension for one year to complete the project.

We can accept your annual report as a digital file (Microsoft Word or WordPerfect), with all figures and tables embedded. Acrobat Portable Document Format (PDF) files (version 4.x or later) are also acceptable; please do not lock PDF files or include digital signatures.

Please submit reports electronically in [ProjectView](#) or by email to mandy.migura@alaska.gov. Also, please be sure to post your annual report on your own website, if you have one.



*We appreciate your prompt submission of your annual report
and thank you for your participation.*