# ALASKA
# HARBOR SEAL RESEARCH PLAN

**2003**

**National Marine Fisheries Service**
Alaska Region, Protected Resources Division
Alaska Fisheries Science Center, National Marine Mammal Laboratory
Southwest Fisheries Science Center

**Alaska Department of Fish and Game**
Division of Wildlife Conservation

**Alaska Sealife Center**

**Alaska Native Harbor Seal Commission**

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## Alaska Harbor Seal Research Plan

## Introduction

Harbor seals, *Phoca vitulina,* are one of the most widely distributed pinnipeds in coastal Alaska, ranging from the southern part of the state at Dixon Entrance, west to the Aleutian Islands and into the southern Bering Sea. Harbor seals represent a significant marine resource to a range of users: subsistence harvesters, visitors, local Alaska residents, and others. Once considered abundant throughout their range, counts of harbor seals have declined in some areas of the state over the last 20 years.

The decline in numbers is most apparent in the Kodiak Archipelago of the Gulf of Alaska, as well as in Prince William Sound (PWS). Tugidak Island, in the Kodiak Archipelago, has historically been the focus of much of the research directed at harbor seals, and has yielded a large amount of information on this pinniped in Alaska. The number of harbor seals at Tugidak Island declined by approximately 90% from the mid-1970s through the early 1990s (Pitcher 1990, Small *et al*. 1998). Counts of harbor seals in Prince William Sound declined by 63% between 1984 and 1997 (Frost et al. 1998). Other pinniped species in Alaska, notably Steller sea lions and fur seals, have also experienced a decline in numbers over roughly the same time period. Concern for the status of harbor seals on a local and statewide basis highlights the need for continued and expanded research on this species in Alaska.

Research on Alaska harbor seals that is funded through the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (NMFS) is carried out principally by the following groups: NMFS, Alaska Fisheries Science Center's National Marine Mammal Laboratory (NMML); Alaska Department of Fish and Game, Division of Wildlife Conservation (ADF&G); NMFS, Southwest Fisheries Science Center (SWFSC); the Alaska Sealife Center; and the Alaska Native Harbor Seal Commission. The Alaska Native Harbor Seal Commission contracts subsistence harvest assessment to the Alaska Department of Fish and Game, Subsistence Division. Research efforts by the above-mentioned institutions support management needs directed at conserving healthy harbor seal populations in Alaska. Within NMFS, the management responsibility for harbor seals in Alaska lies with the Alaska Region, Protected Resources Division.

The combined research efforts by the above-listed groups focus on statewide harbor seal population abundance estimation, stock identification, trends in abundance, general biology and life history, and human interactions. Additional research on Alaska harbor seals is also being conducted by independent researchers, University researchers, and by the National Park Service in Glacier Bay National Park and Preserve.

Native Alaskans traditionally hunt harbor seals for subsistence food and handicrafts and have an accumulation of traditional knowledge associated with this species. The Alaska Native Harbor Seal Commission (ANHSC) represents Native interests on matters associated with harbor seals. On April 29, 1999, NMFS and the ANHSC signed an agreement as partners in the co- management of the subsistence uses of harbor seals in Alaska (NMFS/ANHSC 1999).

**Management Needs**
As mandated by the Marine Mammal Protection Act of 1972 as Amended (MMPA), NMFS is required to maintain the health and stability of marine ecosystems. Consistent with this major goal, three explicit objectives of the MMPA are to 1) maintain stocks at their optimum sustainable population (OSP) levels and as functioning elements of their ecosystems, 2) restore depleted stocks to OSP levels and 3) reduce incidental mortality and serious injury (from commercial fisheries) to "insignificant levels approaching a zero mortality and serious injury rate" (MMPA 1995, Barlow et. al 1995).

Therefore, the fundamental objective of management is to prevent "depletion" of a species or population stock or to restore a species or population stock to its OSP. A species or population is said to be depleted when the Secretary of Commerce "determines that a species or population stock is below its optimum sustainable population [OSP]." OSP is "the number of animals which will result in the maximum productivity of the population or the species, keeping in mind the carrying capacity of the habitat and the health of the ecosystem of which they form a constituent element" (MMPA 1995).

To meet the overall management objectives as mandated by the MMPA, specific information must be available to managers. Management must be able to identify and describe a given group of animals (i.e., a stock or population of harbor seals); describe the status of this unit, including minimum population estimates and the trend in numbers; estimate human-induced mortality levels, including those resulting from commercial fisheries and subsistence removals; as well as have knowledge of the species' biology and ecology to determine how the species may be affected and by what factors. The principal categories of research presented in this plan are designed to provide information to meet the management objectives described above. Using this information, management is tasked with designing appropriate measures that will mediate negative impacts to harbor seals.

In addition, NMFS has recently entered into a new management partnership with the ANHSC to co-manage the subsistence use of harbor seals in Alaska. The agreement between NMFS and the ANHSC includes, as a principal objective, to provide for the maintenance of harbor seal population levels that will allow for long-term sustainable harvests. Information collected by the various research projects will also contribute towards ensuring that this management objective can be met.

As part of the Co-management Agreement between NMFS and the ANHSC, an Action Plan was developed in January 2001 to serve as the guiding document for joint and separate management actions by the ANHSC and NMFS related to the conservation and management of subsistence uses of harbor seals. This Action Plan details the research to be conducted and information to be collected by each party that will serve to meet management needs. The Action Plan covers three broad areas: Population monitoring (abundance and trends, vessel disturbance, contaminants, food habits, stock identification, and fisheries impacts), Harvest management (harvest monitoring and biosampling) and Education. The research components of this Action Plan feed directly into the current Harbor Seal Research Plan.

**Goals of the Harbor Seal Research Plan**

Prior to the development of the first Alaska Harbor Seal Research Plan document in 2000, no consolidated research plan for all NMFS-funded harbor seal research, which is carried out by several institutions, existed. The document produced in 2000 detailed NMFS-administered research then being conducted by NMFS and the ADFG. Since the creation of the 2000 Plan, two other groups have also received NMFS pass-through funding for harbor seal research in Alaska: the Alaska Sealife Center and the Alaska Native Harbor Seal Commission. The current document is intended to consolidate all of these research efforts.

A consolidated plan will provide the opportunity for a more effective evaluation of existing Alaska harbor seal research. It will provide a platform for modifications to be made to this research based on priorities that satisfy management objectives. The timescale is intended to be a five-year outlook; although the document will be revised annually. A five-year timescale was chosen to fit with budgeting cycles within the agency and to accommodate the full cycle of aerial surveys for the complete range of harbor seals in Alaska.

The principal objectives of this Research Plan are the following:

1. Consolidate the research plans of currently-funded projects into a single coordinated effort;
2. Describe additional research projects that are currently unfunded, but for which funds are being sought;
3. Increase the dialogue, coordination, and collaboration among NMFS-funded harbor seal researchers and NMFS managers through the process of annually reviewing, evaluating and updating the Research Plan; and
4. Ensure that research on Alaska harbor seals satisfies management objectives.

The Research Plan will also serve a useful role in the co-management process by drawing together the current and proposed descriptions of harbor seal research projects funded by NMFS. In doing so, the plan should enhance communication between NMFS and the ANHSC by describing NMFS-funded research plans and priorities. Similarly, the plan is expected to provide helpful information to the Alaska Scientific Review Group about NMFS-funded harbor seal research.

The Research Plan has been organized according to broad research categories with individual projects outlined in greater detail. Each task includes a research category overview, project objectives, justification, methods, product, project status and project lead. The scope of research projects includes both short- and long-term tasks, depending on the nature of the investigation. This Research Plan will be evaluated and revised annually as research and management objectives are met, needs evolve and funding commitments change.

## RESEARCH CATEGORIES

## A. ABUNDANCE AND TREND ESTIMATION

**Overview**
This research category comprises tasks that relate to estimation of abundance and trends in population size of Alaska harbor seals. Because the interdependence of the tasks is important to the justification for the research, a brief overview seems appropriate. Three of the tasks, numbered A.1, A.2, and A.4, describe surveys to count harbor seals on their haul-out sites. Task A.1 describes surveys and analysis to estimate statewide abundance of harbor seals on about a 5-year cycle. Because this 5-year cycle is too long to provide timely estimates of population trends, task A.4 describes surveys and analysis required to estimate trends in selected regions of the state. These tasks differ in their objectives and geographic scope, but are subject to the same factors that introduce variability in the proportion of the seal population that is ashore to be observed and counted during the surveys. Therefore, all three survey tasks are subject to the same constraints and difficulties of designing the surveys to either minimize variability from factors (covariates) such as tides, weather conditions, time of day, and date (in the seals' seasonal life history), or to adjust for the state of those covariates during the surveys. Even when counts are adjusted for covariates, estimating the total population size requires estimating the proportion of the population that was not included in the survey because some seals were in the water (unavailable to be counted). Task A.3 is concerned with estimating a correction factor for the proportion missed. With the development of techniques for covariate adjustment, special care must be taken to ensure that the correction factors can be estimated in a way that is compatible with covariate-adjusted counts. Task A.2 is a special effort to address practical difficulties of surveying harbor seals that use glacial ice for hauling out.

### A.1. Surveys to estimate population abundance

**Objective:** Estimate statewide distribution and abundance of harbor seals in Alaska.

**Justification:** The 1994 amendments to the Marine Mammal Protection Act (Section 117) require that NMFS produce a Stock Assessment Report (SAR) on the status of each species under its jurisdiction. Certain key population parameters are required to describe the status of the stock, including population size. Minimum population estimates are also needed for the calculation of a Potential Biological Removal (PBR) level, also required in the SARs. Since 1991 the NMFS National Marine Mammal Laboratory, in cooperation with ADF&G, has conducted yearly censuses of harbor seals in Alaska to provide baseline population information and for purposes of calculating a minimum population estimate for the SARs.

**Methods:** For purposes of conducting abundance surveys, the state of Alaska has been divided into 5 survey regions: (1) north side of the Alaska Peninsula and Bristol Bay; (2) Aleutian Islands; (3) Gulf of Alaska; (4) northern Southeast Alaska; and (5) southern Southeast Alaska. Each subdivision is surveyed on a rotating schedule. Logistics and resources preclude the completion of a more comprehensive survey on a more frequent basis. Two complete abundance estimates are, therefore, produced every decade. It should be noted that when zones already surveyed as part of the aerial survey trend routes (task A.4.1) are present in the survey area, these zones are not double-counted (i.e., the counts from the trend routes are incorporated in the abundance estimates).

Fixed-wing aircraft are used to photograph harbor seals hauled out during the molt season in August. Aerial surveys are flown between 100 and 300m altitude at 90 knots, within 2 hours on either side of low tide. The entire coastline is surveyed several times initially to identify haul-out sites, then observers fly from site to site including all previously known haul-outs. Four to six repetitive counts on separate days are planned for each major haul-out site within each study area over the 2 week survey period. For small groups of seals (<10), counts are obtained by observers during the surveys. For larger groups, the seals are photographed with digital or 35 mm cameras equipped with a 70-210 mm lens. Seals are later counted on digital displays or on projected transparencies.

Covariate analyses consistently show that date, time relative to mid-day, and time of day, and tide height influence counts. The survey counts are therefore adjusted for the covariate conditions prior to estimating total population abundance (task A.3).

**Product:** The total abundance of harbor seals statewide and for each of the regions will be estimated on a 5-year cycle. These estimates will comprise minimum population sizes in the form of the actual numbers of seals counted on aerial surveys, counts adjusted for covariates, and estimates of abundance obtained by correcting for the proportion of the population unavailable to be counted during the aerial surveys (task A.3).

**Five-year project status:** Area-specific abundance estimates will be produced according to the following schedule (the rotation schedule will be repeated):

| | |
|---|---|
| 2003 | Southern Southeast Alaska |
| 2004 | Aleutian Islands |
| 2005 | North side of the Alaska Peninsula and Bristol Bay |
| 2006 | Gulf of Alaska |
| 2007 | Northern Southeast Alaska |

Funded FY03.

**Project lead:** NMML

### A.2. Glacial survey methodology

**Objective:** Develop and apply methods specific to estimation of harbor seal population abundance at glacial haul-out sites.

**Justification:** Glacial ice represents a significant haul-out substrate for harbor seals in Alaska, and thus must be included in census survey efforts conducted for statewide abundance estimates. Additionally, modified or new trend routes may include glacial ice haul-outs in the future. Difficulties arise in the application of conventional aerial survey techniques to glacial ice floe substrate. Seals are conventionally photographed from fixed wing aircraft that circle over haul-out locations. In terrestrial haul-out areas the substrate provides a point of reference for the observers, allowing the observers to ensure that all areas of the specific haul-out have been photographed, but not duplicated. In glacial ice areas the uniformity and expansive size of the substrate is problematic for observers photographing large numbers of seals. Without specific reference points it is difficult to ensure that complete coverage is obtained and duplication is prevented. Rocks and dirt commonly found in glacial ice pose additional challenges for identification and counting of seals from hand-held 35mm photographs such as those used in conventional surveys of terrestrial sites. For glacial ice areas with relatively few animals, the conventional aerial photography may suffice. However, under circumstances where large numbers of seals are spread out over large areas, determining the areas of overlap in the resulting photographs is very difficult, and hence estimates are less reliable. In these cases, the conventional methodology produces abundance estimates, which take considerable time to generate and are of unacceptably high variation (Small 1998). Alternative methods for surveying ice substrate types are required.

**Methods:** In 1999-2002, several alternative survey methods were tested by ADF&G and NMML. These included medium format photography, infrared imagery, and large format photography. Large format (9") color negative photography proved suitable for surveys of large numbers of seals distributed over large expanses of floating ice. In this method, the negatives are obtained from a commercial photogrammetry vendor directed to obtain complete (overlapping) coverage of the fields of ice emanating from tidewater glaciers. The negatives are scanned digitally at high resolution and then viewed with image analysis software to assist in orientation and counting seals on the images.

During development of the methods, the glacial haul-out sites in Alaska were surveyed partially in 2001 and completely in 2002. To provide regular updates that will complement the 5-year cycle for estimating statewide abundance of harbor seals, photogrammetric surveys will be conducted at the same time as the conventional aerial surveys of the Gulf of Alaska, the northern part of southeast Alaska, and the southern part of southeast Alaska. In the two alternate years, the funding for this project will be used to continue the timely processing of the images, which is labor intensive.

**Product:** Population surveys of glacial ice haul-out sites, where a substantial fraction of the harbor seals in Alaska are found.

**Five-year project status:** Unfunded in FY03 and beyond.

**Project lead:** NMML

**A.3. Correction factor study**

**Objective:** Estimate the proportion of seals not counted during aerial abundance surveys to calculate a "correction factor" that is applied to the counts, providing an estimate of total abundance.

**Justification:** Harbor seals are censused from aircraft by photographing the seals hauled out on land or ice during the molt period (August). The abundance of seals cannot be estimated from these surveys alone because an unknown proportion of the population is missed (there will always be some seals in the water, unavailable to be counted). The proportion missed or, equivalently, a correction factor, can be estimated by independent studies using radio telemetry to monitor the haul-out patterns of marked individual seals. With the development of techniques for covariate adjustment of survey counts, special care must be taken to ensure that the correction factor can be estimated in a way that is compatible with covariate-adjusted counts. Simpkins et al. (2003) demonstrated a technique for estimating a correction factor that takes into account the relevant covariates. They found that haul-out proportions, after adjusting to covariate values that were (locally) the best for seals hauling out, were not significantly different between Grand Island (0.813) and Nanvak Bay (0.857), even though those two sites were 970 miles apart and surveyed 6 yr apart. The similar estimates for both sites were consistent with a hypothesis that harbor seals in different regions behave similarly under locally ideal conditions, at least during the molt season. The two sites compose a very small sample, however, and further validation of the apparent constancy of ideal haul-out proportions will require estimating haul-out proportions for other regions and years.

**Methods:** Several weeks prior to annual assessment surveys (August), up to 50 seals will be captured and outfitted with flipper (rear) mounted VHF transmitters. All haul-out sites in the vicinity of the capture site(s) will be monitored with data-logging VHF receivers to record the presence/absence of all radio-tagged seals ashore. The presence/absence data will be used in a regression model to estimate the proportion of seals hauled out under a standardized set of covariate conditions (e.g., date, time of day, tide height, weather), as in Simpkins et al. (2003). This proportion and its variance form the basis of a correction factor for converting covariate-adjusted survey counts to estimates of absolute abundance.

**Product:** This study will provide factors to correct harbor seal counts for seals missed during aerial surveys.

**Five-year project status:** Current plans call for conducting this project annually using the same schedule as the aerial assessment surveys for abundance. Unfunded in FY03 and beyond

**Project lead:** NMML

**A.4. Surveys to estimate population trends**

**A.4.1 Aerial surveys of trend routes**

**Objective:** Estimate population trends for harbor seals in the areas of Ketchikan, Sitka, Prince

William Sound (PWS), the Kodiak Archipelago, and Bristol Bay. We will explore the feasibility of conducting trend routes in the Aleutian islands, and south Kenai Peninsula to monitor population trends in those areas should sufficient funding and personnel become available.

**Justification:** Changes in harbor seal numbers over time have been monitored through aerial trend surveys that estimate the number of seals (i.e., counts) at designated sites within selected survey routes distributed throughout the state (Frost *et al.* 1999, Small *et al.* 1999). Trend surveys are aimed at determining whether harbor seal numbers are increasing, stable, or decreasing in different parts of their range in Alaska. The locations of trend routes currently monitored were selected to be generally representative of harbor seals throughout the state. This trend information is fundamental to an understanding of population status (Gerber *et al.* 1999) and to the implementation of the PBR approach to management under the MMPA. Continued monitoring of these routes will allow researchers and managers to follow population trends and make adjustments to research objectives, as well as to implement management measures that would assist in recovery of depressed populations and to monitor the effectiveness of management measures.

**Methods:** Standardized aerial survey techniques will be used to count animals at all haul-out sites that comprise each route. Overflights of these sites will be conducted from fixed wing planes, over a four hour period, two hours on either side of mean low tide. Surveys will be conducted during the peak molt period in August. Observers will first obtain a visual count, then use a 35mm camera, with 400 ASA color slide film and an 80-200mm zoom lens, to photograph all seals hauled out. Seals are later counted from projected slide images on a white surface. Small groups of seals (10-15) may not be photographed, as the visual count is sufficient. Environmental conditions such as time, and tide height (i.e., 'covariates') will be recorded when each site is surveyed. Four or more replicate counts will be obtained to achieve sufficient precision in the trend estimate. A separate model for each combination of covariates and trend trajectories are determined, with the final trend estimate and standard error obtained as a weighted average of trend estimates from the individual models (Small et al. 1999).

**Product:** Harbor seal population trend estimates for each of the trend route areas; i.e., Ketchikan, Sitka, Prince William Sound (PWS), the Kodiak Archipelago and Bristol Bay. Trend estimates can be calculated for the entire time period for which counts are available, as well as shorter periods, e.g., the most recent 5-year period. A manuscript was published in Marine Mammal Science (Small et al. 2003) presenting trend data from 1983-2000 in Ketchikan, Sitka, and Kodiak areas. A synthesis of trend data in PWS from 1990 through 2002 is currently in progress with a manuscript expected to be drafted and submitted for publication in 2003-04.

**Five-year project status:** Aerial survey routes with representative haul-out sites for trend analyses have been established for Ketchikan (1983), Sitka (1983), the Kodiak Archipelago (1993), PWS (1984), and Bristol Bay (1998). These trend routes are surveyed annually until the weighted standard error associated with the trend estimates is <1.0, at which time a biennial survey schedule will be considered. The Ketchikan route was shifted to a biennial schedule following the 1996 survey; the next survey is planned for 2003. Trend estimates will be calculated annually once counts are obtained. Due to a reduction in funding in FY02 we will not be flying all trend routes in August

2003. Those areas that have shown a consistent trend and are not areas of concern in terms of the direction of that trend will be bypassed this year. The current plan for 2003 trend surveys is to survey PWS, where populations continue to decline and Bristol Bay where we do not have enough years of data to establish a consistent trend. ADF&G will also conduct trend surveys in the Ketchikan area in conjunction with the NMML abundance surveys in that area. Because of consistent positive trends in the Kodiak and Sitka areas and reduced funding, those survey routes will be bypassed this year. Funded at reduced level FY02, unfunded FY03 and beyond.

**Project lead:** ADF&G

## A.4.2 Trends at land-based counting sites

### A.4.2.1 Nanvak Bay land-based site

Objective: Collect land-based counts of harbor seals at Nanvak during the pupping and molting periods to continue long-term population trend monitoring for this index site.

**Justification:** Nanvak Bay is the only site in the Bering Sea where harbor seals have been consistently monitored across years on a daily basis during either the pupping and/or molting periods. Specifically, land-based counts at this site, the largest haulout in northern Bristol Bay, were conducted during the pupping and molting periods from 1990-2001. Counts from this site represent the longest time-series available for the Bering Sea stock. In additional, Nanvak Bay is near the geographic boundary for harbor seals in western Alaska, a geographic area likely to be affected by global climate change, and thus offers the opportunity to examine harbor seal population status in an area that may provide insights on how this species can adapt to dynamic environmental conditions.

**Methods:** Spotting scopes and binoculars are used to conduct daily counts of seals from mid May through September or October, with additional counts collected during 15 days centered around both the peak pupping and molting periods.

**Product:** A population trend analysis, including the effect of covariates, of all counts collected. Additionally, evaluate population trend estimates based on land-based counts compared to trend estimates derived from aerial surveys.

**Five-year project status:** Surveys will be completed annually (as funding and staffing allows) during the pupping period in late May through June and the molting period from late July through early September. Population trend estimates from 1990-2000 for Nanavak Bay were presented in a manuscript submitted for publication in fall 2001 currently back from review awaiting revision to combine with manuscript of Tugidak Island data. Due to cutback in funding and staff, no counts were obtained during pupping or molting in 2002 or 2003 at Nanvak Bay.

**Project lead:** ADF&G

## A.4.2.2 Aialik Bay Population Monitoring

**Objective:** Collect counts of harbor seals on ice associated with Aialik and Pederson Glaciers in Aialik Bay from May through September to resume long-term population monitoring initiated in 1979.

**Background:** Aialik Bay, located in the Kenai Fjords National Park, is a unique location for studying seals that use glacier ice. It also is the oldest field-monitoring site on the outer Kenai Peninsula and the only glacial ice haulout monitored in southcentral Alaska. Aialik Bay is a particularly useful area to study glacial ice inhabiting seals as Squab Island provides a convenient central location for observing seals drifting on ice around the island. Harbor seals in Aialik Bay were first investigated in 1964 when seals were being commercially harvested (Bishop 1967). With the creation of the Kenai Fjords National Park and the expected increase in tourism traffic, the National Park Service funded studies of the status of harbor seals in Aialik Bay (Murphy and Hoover 1983, Hoover 1982, 1983). The studies were supplemented by additional counts in 1989, associated with evaluating the effect of the *Exxon Valdez* oil spill (Hoover-Miller 1989). Long-term monitoring was continued in spring 2002 with the placement of remote-controlled video cameras on Squab Island and at Pederson Lake in Aialik Bay. Cameras are operated during the summer, from June through September.

**Justification:** Numbers of harbor seals in Aialik Bay have decreased from counts of more than 1,600 seals in 1980 to about 230 seals in 2002. The decline in numbers corresponds to a major harbor seal population decline in the Gulf of Alaska, but may be augmented by increasing vessel traffic associated with tourism and decreasing ice availability from Aialik Glacier. Since 1994, numbers of seals in the Kodiak archipelago have stabilized and begun to recover. Population data for upper Aialik Bay are being reevaluated relative to environmental conditions, and sampling effort.

**Methods:** Remote video monitoring is being used to count seals and document haulout activity and ice availability in upper Aialik Bay. Four remotely controlled video cameras equipped with a 300x (25x optical) lens have been installed for summer operations. Two cameras are located on Squab Island and two are mounted near Pederson Lake. Images from one camera at a time are transmitted to the Alaska SeaLife Center for processing and analysis.

**Product:** This study contrasts harbor seal attendance and current conditions with those in 1979-1981. Video records of the number and distribution of seals and ice quality and distribution throughout the day are kept using time-lapse video recordings. These images depict the distribution of seals and ice, and record concurrent environmental conditions. Data maps from 1979-1981 and 1989 are being digitized and contrasted with current conditions. A report will identify changes in harbor seal attendance over time, evaluate the current population status, and assess the potential role changes in ice availability may have on recovery.

**Five-Year Project Status:** 2002 was the first year of study. Surveys will be conducted annually from June through September each year. Longevity of the project is dependent on funding. Funded FY02 and FY03.

**Project Lead:** ASLC
**Project Partners:** NPS (Oceans Alaska Science and Learning Center), Port Graham Corporation, USFWS

### A.4.2.3 Trends on the eastern coast of the Gulf of Alaska

**Objective:** Estimate population trend for harbor seals along the coast of the eastern Gulf of Alaska.

**Justification:** The vast majority of harbor seals along the eastern Gulf coast haul out on floating ice calved from glaciers in Icy and Disenchantment Bays. These areas are important for subsistence hunting of harbor seals. Because glacial ice as a haul-out substrate poses difficulties for the techniques used in conventional aerial surveys of trend routes, the trend in eastern Gulf coast populations can be better assessed by the photogrammetry methods described above in task A.2.

**Methods:** One of the major glacial haul-out areas in the eastern Gulf coast region (i.e., Icy Bay or Disenchantment Bay) will be selected as the trend indicator site. The photogrammetry and counting methods described in task A.2 will be applied on 3-4 replicate days per year during the peak harbor seal molt period. Environmental conditions and other covariates such as date, time, and weather statistics will be recorded when each site is surveyed. After sufficient annual replications, the population trend and its standard error will be estimated from a regression model that adjusts for the covariate conditions.

**Product:** A harbor seal population trend estimate for the eastern coast of the Gulf of Alaska region.

**Five-year project status:** Counts from both candidate trend sites were obtained in 2001 and 2002. Photogrammetry surveys not planned for 2003. Continuation of surveys in 2004 and beyond will provide trend estimates after 2005. Unfunded in FY04.

**Project lead:** NMML

### A.4.2.4 Tugidak land-based site

**Objective:** Collect land-based counts of harbor seals on the southwest and middle beach haulout sites on Tugidak Island during the pupping and molting periods to continue long-term population trend monitoring for this index site.

**Justification:** Tugidak Island, southwest of the Kodiak Archipelago, represents a unique study site for harbor seal research in Alaska. Several beaches on the island are haulout sites for some of the largest concentrations of harbor seals in Alaska. Studies of these haulout sites were initiated in the mid 1970s by the ADF&G. Since 1994, the population of harbor seals on Tugidak Island has been consistently and intensively studied. The uniqueness of this site with regard to the number of animals that haul out on the island's beaches, the excellent view of the seals afforded by the bluffs, and the long term historical record of land-based counts make it a focus of continued investigations on population dynamics and harbor seal biology.

**Methods:** Counts of harbor seals will be conducted from 30m bluffs overlooking the haulouts on the southern and western shores of Tugidak Island. Seals hauled out on the beach will be counted using a spotting scope and binoculars. Surveys will be conducted on a daily basis within 2 hours on either side of low tide during the pupping period in May and June and the molting period in later July through early September**.** Total counts will be made as well as counts by sex and year class (pups, yearlings, subadults and adults). Yearly counts (maximal and mean estimates) will be included in a time series of counts for this area for analyses of population trend of harbor seals in the Gulf of Alaska.

**Product:** A population trend analysis, including the effect of covariates, of all counts collected through 1999 has been completed and will be presented in a draft manuscript scheduled for completion in June 2000. The manuscript will also include an evaluation of land-based counts for documenting population trend, with a comparison to trend estimates derived from aerial surveys.

**Five-year project status:** Surveys will be completed annually during the pupping period in late May through June and the molting period from late July through early September**.** Funded FY03.

**Project lead:** ADF&G

**A.4.2.5 Aleutian Islands trend -aerial (ADFG)**

**Objective:** Estimate population trends for harbor seals along the Aleutian Islands.

**Justification:** Little is known about the status of harbor seals in the Aleutian chain, however, changes in behavior and declines in populations of other species (e.g., sea otters, sea lions, and killer whales) have been reported in the area. Changing ecosystem dynamics may result in population-level consequences for harbor seals that may occur on a shorter timeframe than can be effectively monitored during the abundance surveys conducted by NMML in that area every five years.

**Methods:** ADF&G has not established a trend route in the Aleutians. Given sufficient funding and adequate personnel, ADF&G will investigate the feasibility of conducting annual trend surveys in the area.

**Product:** Harbor seal population trend estimates for a trend route in the Aleutians, once a trend route is established.

**Five-year project status:** This objective was established as a research priority in a 2003 meeting of the investigators (ADF&G, NMML, NMFS, ASLC, and ANHSC) involved in harbor seal research in Alaska. Project lead was given to ADF&G but no action is planned at this time. Unfunded FY03 and beyond.

### A.4.2.6 South Kenai trend route

**Objective:** Estimate population trends for harbor seals in the southern Kenai area.

**Methods:** ADF&G has not established a trend route in the southern Kenai area. Given sufficient funding and adequate personnel, ADF&G will investigate the feasibility of conducting annual trend surveys in the area.

**Product:** Harbor seal population trend estimates for a trend route in the southern Kenai area, once a trend route is established.

**Five-year project status:** This objective was established as a research priority in a 2003 meeting of the investigators (ADF&G, NMML, NMFS, ASLC, and ANHSC) involved in harbor seal research in Alaska. Project lead was given to ADF&G but no action is planned at this time. Unfunded FY03 and beyond.

## A.5 Trend survey experimental design

**Objective:** Evaluate the current experimental design for conducting aerial surveys to assess population trends for harbor seals in Alaska. Determine factors that contribute to the greatest variability in counts and experiment with alternative survey designs that may be more representative of population trends for harbor seals in Alaska.

**Justification:** Assessment of population trend and abundance of harbor seals in Alaska is fundamental to understanding population status. Prior to the early 1980's, monitoring of harbor seal numbers occurred in only a few areas outside of Tugidak Island, and surveys were infrequent and not standardized. Beginning in 1983-84, standardized population trend surveys were established in PWS and Southeast Alaska, followed by the establishment of trend survey routes for the Kodiak Archipelago in 1993 and Bristol Bay in 1998.

Information from the trend and abundance estimates indicate population status varies across the state. Harbor seals declined by approximately 90% between 1976 and 1992 on Tugidak Island, south of Kodiak Island (Pitcher 1990, Lewis et al. 1996) and by 63% between 1984 and 1997 in PWS (Frost et al. 1998). These monitoring results have provided valuable information regarding the population status of harbor seals in the state. However, the original trend survey routes were determined primarily on logistical constraints, not on statistical sampling designs. Advances in harbor seal biology and life history as well as overall advances in marine mammal survey methodology raise new questions as to whether the current design for population monitoring is adequately and robustly estimating trends in abundance and overall statewide abundance (Small

1999). Relatively precise and accurate estimates of population trend and abundance are required to determine appropriate management strategies. In addition, there has been no comprehensive statistical review of the population monitoring methodology for harbor seals in Alaska. A review of the survey design for the *Exxon Valdez* spill area will be applicable to other parts of the state.

**Methods:** Current monitoring programs include aerial population trend and abundance surveys, and land-based counts at a key index site (Tugidak Island). Surveys occur over a variety of areas and haulout substrate types. Because of the continued decline in seal numbers in Prince William Sound, emphasis is focused on determining whether inclusion of other survey routes may be more representative of population trends for harbor seals in that area. Simulations using an operational model approach (Adkison et al. *in review*) to evaluate robust designs for trend surveys for harbor seals resulted in the conclusion that adjusting for the effects of covariates on the proportion of the population hauled out was essential and that inter-site variability in trends was a key factor in influencing the robustness and power of trend surveys. Additional modeling conducted by Aaron Christ of the University of Iowa and Jay Ver Hoef (ADF&G) revealed that the highest variability in counts occurred at sites with greater number of seals hauled out, compared with haul outs with fewer seals. The current experimental design for aerial surveys to assess long-term population trends seals involves returning annually to count seals at the same haul-out sites (5-10 replicate counts per site). That methodology does not account for the potential that seals may shift their use of haul-out sites in response to shifts in the distribution of prey, or other factors unknown to researchers. Several survey routes conducted annually in each region, which cover a broader geographical area and return to sites with many seals for several replicate counts and sites with fewer seals only once per year may be more representative of population trends for harbor seals in Alaska. Moreover, varying the route for trend surveys each year may allow for discovery of new haul-out sites should seals shift their use of haul outs away from traditional sites.

Following through with the model developed by Aaron Christ of Iowa State University to determine what sites/factors contributed the most variability in trend estimates, ADF&G is collaborating with ESRI to use network analyst methodology (Arc View software) to design the most efficient route(s) for PWS surveys. Once optimal routes have been established for PWS, we will explore the financial feasibility of conducting a redesigned survey for our August 2003 trend estimates in that area. Revision of survey designs will be considered for other trend routes in Alaska in the future.

**Product:** A revised survey experimental design for harbor seal population estimation (e.g. trend and abundance) in the *Exxon Valdez* spill area, Alaska. Annual estimates of population trends for harbor seals in Alaska. Results of the initial modeling have been submitted for publication in a peer-reviewed journal (Adkison et al. *in review*) and modeling to determine what sites/factors contributed the most variability in trend estimates for harbor seals will be written up as a chapter in the Ph.D. thesis of Aaron Christ.

**Five-year project status:** Modeling efforts have been completed to assess factors contributing to variability in trend estimates. Results of that model are being incorporated into redesigning the trend route for PWS. No funding was available for consultation with Network Analyst specialists at ESRI; however that work is being conducted gratis and, if results are obtained in sufficient time, consultants there are using the project as a demonstration of GIS applications to study designs for a presentation at a 2003 meeting. In light of the reduction in funding for FY02, once optimal routes have been established for PWS we will explore the financial feasibility of conducting a redesigned survey for our August 2003 trend estimates. Unfunded for FY03 and beyond.

## B. STOCK IDENTIFICATION

### Overview

Scientists at the SWFSC have been using molecular genetic techniques to investigate population subdivision and movement patterns of harbor seals in Alaska. Variation in both mitochondrial and nuclear (microsatellite) markers is being examined to resolve population structure and estimate levels of dispersal which will provide the framework for delineating stock boundaries. The different properties of the two types of marker may also determine whether separate stocks are demographically and/or reproductively independent by distinguishing between actual (i.e., emigration) and effective (i.e., interbreeding) dispersal.

Patterns of mitochondrial DNA (mtDNA) variation in Alaska harbor seals indicate population structure on a number of spatial and temporal scales and clearly demonstrate that current management stocks are inappropriate and thus in need of revision. Heterogeneity in this marker is influenced by population size and is correlated with geographic distance, indicating that dispersal, when it occurs, is primarily among neighboring sub-populations. The fact that harbor seals are distributed almost continuously throughout their Alaskan range necessitated the development of new techniques for analyzing the genetic data in order to resolve population structure and identify management stocks. Two quite distinct, but complementary, approaches were used (Section I), both revealing subdivision on spatial scales of 150 to 300km. A complementary study on variation in 11 microsatellite markers (Section III)already showed structure on a broad geographic scale (500 - 800km).

The findings from a wide range of studies on the biology, ecology and demography of harbor seals in Alaska are all potentially of use in resolving population structure and identifying stocks in this species. Extensive investigations have been conducted on population genetic structure, geographic variation in morphology and reproductive physiology, movement patterns, foraging ecology, trends in abundance and habitat. There is a need to integrate these data if we are to understand the factors that influence population subdivision and dispersal patterns and, ideally, if we are to predict these patterns in areas where we currently have poor sample coverage (Section II).

The inability to reliably distinguish between harbor seals and spotted seals in the field has implications for the effective monitoring and management of both species. Molecular genetic analysis appears to offer the most useful means of not only distinguishing between either species but of also documenting the extent of hybridization (Section IV). Analysis of variation within mtDNA revealed a species-specific marker, at least for maternal lineages. What is needed now is a companion study that searches for a complementary nuclear DNA marker. The combination of both markers will not only add to our ability to distinguish among species but to also address questions of hybridization.

Despite the extensive number of samples and amount of genetic data that has already been collected, sample size is still small in some areas and there are (notable) gaps in our sample coverage. This has been alleviated, in part, by an initiative to establish molecular techniques to extract and analyze genetic material from alternative sample types including hair, scat, and formalin-fixed tissues (Section VI). Molecular genetic tools are also being used to estimate levels of genetic diversity (Section V) and investigate mating systems and patterns of dispersal (Section VI). Diversity indices may be informative indicators of a population's evolutionary history and current ability to deal with environmental change and disease, while the resolution of harbor seal mating systems will aid in estimating effective population size, $N_{e,}$ a parameter of relevance to estimates of rates of dispersal and the delineation of stocks.

Work needs to continue on the development of analytical methods to resolving population genetic structure in continuously distributed species. Sample collection and analysis needs to continue in order to fill in important gaps and increase sample size from key areas. Further development and optimization of laboratory protocols for the analysis of alternative sample types is required, final analyses of data sets need to be completed, and guidelines for the delineation of stocks formulated.

**B. 1. Population structure and stock identification - mitochondrial DNA**

**Objective:** (1) co-ordinate with the ANHSC and ADF&G to collect and analyze tissue samples from harvested seals in key areas where current sample coverage is poor; (2) conduct direct sampling of live seals in key areas in order to increase sample numbers and coverage; (3) sequence and analyze new material; and (4) explore more ways to estimate rates of dispersal from the genetic data.

**Background:** Genetic stock division studies on harbor seals in Alaska by the SWFSC were initiated in 1995, supported by the NMFS Office of Protected Resources with assistance from other sources. This research has also been supported by the ADF&G while samples have been provided by a number of institutions and agencies including ADF&G, ANHSC, and NMFS. As sample numbers and coverage increased over the years, a genetic picture has emerged of a species that exhibits population structure on a number of spatial and temporal scales. Analysis of macro-geographic patterns of variation within the mitochondrial genome's (mtDNA) control region revealed significant structure, indicating very low levels of dispersal, among centers of abundance along the distributional continuum (Westlake and O'Corry-Crowe, 2002). Heterogeneity was influenced by population size and correlated with geographic distance, indicating that dispersal, when it occurs, is primarily among neighboring sub-populations. Differentiation between the Kodiak archipelago and Prince William Sound, and between Bristol Bay and the Pribilof Islands indicated that current management stocks were inappropriate and highlighted the need for a more detailed analysis of population and stock structure.

As more samples were collected and analyzed and many of the sampling gaps were filled in we were able to investigate the detailed population structure of harbor seals in Alaska. Two main approaches were used to examine the patterns of genetic differentiation. The first approach was based on grouping geographically small initial groupings of sampling sites into larger and larger groupings based on estimates of genetic distance. The primary method used in this approach was Boundary

Rank, a recently developed method that applies the principles of classic cluster analysis to the analysis of population genetic structure in continuously distributed species (Martien and Taylor, in review). This method was developed in part to resolve population structure in Alaskan harbor seals and uses the estimated genetic relationships among sampling sites to nest groups of geographic strata into fewer, larger strata under certain geographic restrictions. The results of this analysis were compared with those of traditional distance-based cluster and phylogeny reconstruction analyses. The second approach, used hypothesis testing to assess genetic subdivision among the same initial strata as used in the Boundary Rank analysis. These analyses revealed that harbor seal populations in Alaska are subdivided on geographic scales on the order of 150 to 300km (O'Corry-Crowe et al., unpublished). Preliminary estimates of rates of dispersal based on the genetic findings indicate that demographically trivial levels of interchange are occurring between the differentiated areas. Collectively, these findings further indicate that current stock designations are inadequate and in need of revision.

While the analysis of patterns of variation within harbor seal mtDNA has revealed much information about this species' population structure and dispersal patterns in Alaska that will ultimately be used in the revision of stocks, there are still areas of the species range where we know little because sample coverage is poor. Lack of knowledge about the underlying pattern of subdivision and dispersal increases the risk of poor management decisions because the default is to define stocks too coarsely, as is the current situation with harbor seals in Alaska. The increased risk of making poor decisions may occur either through (a) the under-protection of areas where local depletions go undetected or (b) the over-protection of areas where entire regions are afforded greater protection due to severe depletions in just one sub-area. Such a decision may unnecessarily restrict take in healthy neighboring sub-areas.

Tissue samples are required from a number of critical areas. The ANHSC bio-sampling program offers the best chance of getting these much needed samples. In addition, specifically targeted direct sampling is required in order to resolve population and stock structure in areas where, for a variety of reasons, opportunistic sampling has been rare.

**Justification:** Variation within the maternally inherited mitochondrial genome (mtDNA) of Alaskan harbor seals is currently being analyzed. Samples for analyses have been provided from a number of sources including subsistence harvests, tagging studies, and State of Alaska and University of Alaska tissue archives. To date, a total of 881 seals have been analyzed for sequence variation in 435bp of the mtDNA control region.

Initial analyses revealed significant genetic structure over large geographic distances (>500km). Seals from Bristol Bay, for example, are genetically differentiated from seals in Southeast Alaska. Significant differentiation has also been found over distances as small as 150km, indicating that population subdivision may occur on a much smaller scale. However, this micro-stratification was only demonstrable in areas where sample size (n = 30-40) was relatively high and well distributed among haulout sites. Clearly, the potential exists in any area for harbor seals to maintain small, isolated populations for sufficient periods so that genetic differentiation can occur. This means that caution must be applied when drafting stock boundaries based on genetics in areas of inadequate sampling. It is important to emphasize that failure to demonstrate genetic subdivision may be just as likely due to having inadequate numbers of samples as to the fact that there was none to find.

**Methods:** The SWFSC has identified areas where more tissue samples are needed. These are: (1) areas of Southeast Alaska, including central Southeast from Sumner Strait to Stikine Strait, Chatham Strait, Lynn Canal, Icy Strait, Cross Sound and Glacier Bay, (2) the eastern Gulf coast including Lituya, Dry, Yakutat and Icy Bays, and the Bering Glacier, (3) the Copper River Delta, (4) the western Kenai Peninsula, (5) Kamishak Bay (6) the southern Alaska Peninsula, (7) parts of the Kodiak Archipelago, including eastern Kodiak Island, Afognak Island and western Kodiak Island, (8) the Aleutian Islands, and (9) southern Bristol Bay, including Izembek lagoon, Port Moller and Port Heiden.

Genetic samples can be gathered from harvest, live capture operations, directed biopsy programs and by non-invasive means (e.g. hair, feces). Many samples should be forthcoming from the current ANHSC bio-sampling program. Cooperation among harbor seal investigators and management authorities will be necessary to co-ordinate sampling from live capture studies and directed sampling operations, and to discuss modification of requirements due to the exigencies of collecting in remote locations.

Tissue storage and molecular techniques will be as described in previous reports and papers. Briefly, tissue samples will be stored in 20% DMSO and saturated salt. Total DNA will be extracted and archived using standard protocols. Following quantification of the DNA, a section of the mtDNA genome containing the highly variable control region will be amplified by the PCR. Both strands of the target region will then be sequenced and analyzed on an automated sequencer. The resulting 435bp sequence will then be aligned and serve as the raw data for subsequent analyses of stock structure.

**Product:** A series of reports and a scientific manuscript or manuscripts detailing the genetic stock structure of Alaska harbor seals based on patterns of mtDNA variation.

**Five-year project status:** The analysis of macro-geographic patterns of mtDNA variation across Alaska was recently published in November 2002 (Westlake and O'Corry-Crowe, 2002). A scientific manuscript describing the Boundary Rank method (Martien and Taylor, submitted) is currently in review. An Administrative Report on the detailed analysis of population structure and dispersal patterns of harbor seals in Alaska, based on the 881 samples analyzed to date, is expected to be completed by the end of June 2003. A scientific manuscript on this analysis is planned for September 2003.

**Project lead:** SWFSC

**B.2. Integration of multiple data types in the analysis of population and stock structure**

**Objective**: (1) gather and compile all available data of relevance to population structure and dispersal patterns of harbor seals in Alaska in a fully compatible GIS-format; (2) Integrate the different types of data into an analysis of population subdivision; (3) Develop detailed models that explain population structure and dispersal patterns of harbor seals in Alaska as revealed by geographic patterns of variation within mtDNA; (4) Develop models that can predict probable population structures in poorly studies areas.

**Background**: Over the past decade, a large body of research has been conducted on many aspects of harbor seal biology of relevance to their population structure and dispersal patterns in Alaska including long-term studies on distribution and trends in abundance, and directed studies on movement patterns and population genetic structure (e.g., Frost et al, 1999; Small et al., 2003; Boveng et al, 2003; Lowry et al., 2001, Westlake and O'Corry-Crowe, 2002; O;Corry Crowe et al., unpublished). Prior to these studies, research was also conducted on geographic variation in morphology and reproductive physiology (Bigg 1969; Burns and Golt'sev 1984; Burns et al. 1984; Kelly 1981; Shaughnessy and Fay 1977; Tempte et al. 1991) while more recently, research has been initiated on geographic variation in diet, contaminants (e.g., Iverson et al., 1997).

Whether a particular aspect of the biology or ecology of harbor seals directly influences or simply reflects population subdivision, a thorough review of all relevant aspects is required if a more complete understanding of population structure is to be gained. In a current analysis the SWFSC is comparing findings from a genetic (mtDNA) study of harbor seals in Alaska to other data of relevance to population structure and dispersal patterns, including distribution, movements and habitat use, diet and trends in abundance. This comparison, as with similar comparisons conducted in other species, is being done in a somewhat *ad hoc* manner, where the scientist uses his or her best scientific judgement when interpreting the findings of the different studies.

A more objective analysis, however, is required that involves the quantitative comparison and integration of different data sets. To begin with, all available data considered of relevance to population structure and dispersal patterns of harbor seals needs to be gathered in a fully compatible GIS-based format. Analyses that detect and measure concordance among different data types that appear to measure the same thing, i.e., the spatial partitioning of groups of seals, need to be performed, while models that explain the variation observed in the mtDNA data and provide insight into the relationship between population structure, dispersal patterns and other aspects of harbor seal biology and ecology will have to be developed.

Ideally these analyses will result in predictive properties, where predictions can be made as to the population subdivision and dispersal patterns of harbor seals in areas where there is currently poor sample coverage. As well as improving our understanding of harbor seal population structure in Alaska, this study is aimed at improving management policies, including further refinement of the methodology used in integrating different data types when defining population stocks.

**Justification**: The findings from a wide range of studies on the biology, ecology and demography of harbor seals in Alaska are all potentially of use in resolving population structure and identifying stocks in this species. Extensive investigations have been conducted on population genetic structure of harbor seals over much of the species range in Alaska. Similarly, studies have been done on patterns of geographic variation in morphology and reproductive physiology of harbor seals across their Alaska range. Detailed studies of movement patterns, foraging ecology and trend in abundance have been carried our at a number of locations. Range-wide estimates of abundance and distribution are becoming available, while detailed information is available on many aspects of seal habitat including bathymetry, oceanography, the physical characteristics of haul-outs and seasonal ice conditions. Many of these studies, however, have been done in isolation and little has been done to integrate different data sets. There is a need to integrate these data if we are to understand the factors that influence population subdivision and dispersal patterns and, ideally, if we are to predict these patterns in areas where we currently have poor sample coverage. Developing such an approach will also aid in the incorporation of various data sources into the stock revision process.

**Methods**: Access data from various sources including the published literature, and unpublished data from other institutions and research groups. Compile the different data sets through GIS-based methodology using ARC-View and ARC-Info computer packages. Research and test currently available methods on data integration. Develop mathematical models that incorporate and explain variation in a range of data types/sources, including genetics, distribution, abundance and movement patterns. Investigate the predictive properties of these model with regard to population structure and patterns of dispersal in poorly sampled areas.

**Product**: A series of reports and a scientific manuscript or manuscripts. A GIS-based interactive tool that allows the layering of different data sets in a geographic format. A model or series of models that explain the patterns of variation observed in mtDNA, and thus dispersal patterns and population structure, in harbor seals in Alaska.

**Five-year project status**: In the first year of this project, efforts will focus on assimilating different data sets into compatible GIS-based formats using ARC-Info and ARC-View. In the second year research will begin on reviewing available geo-spatial models and developing new models. In the third year reports and manuscripts will be written and timelines for future work will be reviewed.

**Project lead**: SWFSC.

**B. 3. Population structure and stock identification - microsatellites**

**Objectives:** (1) Use the geographic strata developed for the mtDNA study to look for evidence of genetic subdivision; (2) direct sampling efforts to increase numbers and coverage to fill in gaps in current catalog; (3) determine the relationship between interbreeding and dispersal patterns among sub-populations; and (4) analyze new material.

**Background:** Microsatellites are a class of highly variable nuclear markers that have revolutionized the study of breeding systems, social organization and population structure. In contrast to the maternal inheritance of mtDNA haplotypes, microsatellite alleles are inherited from both the mother and father. Thus, by combining the analysis of variation at these loci with that of mtDNA, a more complete understanding of grouping, mating, and movement patterns may be achieved. Furthermore, the unusually high level of haplotypic diversity found within mtDNA in Alaska harbor seals has somewhat compromised the utility of this marker in resolving population subdivision, thus highlighting the need to look at other markers. In 1996 a project was initiated to determine the utility of microsatellites in resolving the stock structure of harbor seals in Alaska and preliminary findings were promising. To date over 400 samples from the entire Alaskan range of the species have been analyzed for variation at between 7 and 11 independent loci.

**Justification:** As with mtDNA, microsatellite analysis has revealed structure on a broad geographic scale. Seals from Bristol Bay, for example, are genetically distinct from seals in the Gulf of Alaska. The primary objective at this stage in the study is to exhaustively analyze these data and write up our findings.

We have found that the number of samples greatly influences the reliability of estimates of genetic subdivision. Small sample size increases the variance in the test statistic thus increasing the probability of a type II error of falsely not rejecting the null hypothesis of panmixia. There is therefore a need to increase sample size from a number of areas. Boosting sample size and distribution will also enable us to investigate population structure on a micro-geographic scale and determine if stocks are demographically and/or reproductively independent by comparing findings from nuclear markers with those from mtDNA to distinguish between actual (i.e., emigration) dispersal and effective (i.e., interbreeding) dispersal.

Individual microsatellite loci vary in their ability to reveal population structure, a feature that is related, in part, to how polymorphic they are. It is therefore necessary to continue to screen for variation at a large number of independent loci with differing levels of polymorphism.

**Methods:** Many samples are already available at SWFSC but new samples are required from several areas. Samples will be gathered from harvest, live capture operations, and by non-invasive means (e.g. hair) where possible, and the collection of samples will be co-ordinated with the relevant agencies and institutions. In some areas, it will be necessary to have a directed sampling program where we will live sample seals and/or collect hair (and if nothing else is possible, faeces), preferably during the breeding or molting season when seals are most accessible and movement patterns are most restricted.

Tissue storage and molecular techniques will be as described in previous reports. Briefly, tissue samples will be stored in 20% DMSO and saturated salt. Total DNA will be extracted and archived using standard protocols. Following quantitation of DNA (and sequence analyses of mtDNA, see part 1), alleles at a minimum of 11 polymorphic microsatellite loci will be amplified by the PCR, separated on an automated sequencer, and sized with the aid of Genescan 3.1 software.

As with the mtDNA study, geographic strata to be tested with the microsatellite data will be based primarily on harbor seal distribution, abundance, and movement patterns. Other factors that may influence or reflect movement patterns are also being considered in this process. Frequency-based ($F_{st}$, $\chi^2$) statistics will be used to assess levels of genetic differentiation.

**Product:** A series of reports and a scientific manuscript or manuscripts detailing the population genetic structure of Alaska harbor seals based on patterns of microsatellite variation, and how this pattern compares with the mtDNA findings. The analyses will be based on the strata used in the mtDNA studies.

**Five-year project status:** The lab work on the macro-geographic structure has been completed. Interpretation and write up is planned for June 2004. Completion of lab work exploring the utility of microsatellites in uncovering micro-geographic structure within regions in Alaska is planned for march 2005. At that time decisions will be made regarding the future of this line of research and future sampling requirements will be presented to the responsible party(ies), and timelines will be refined at this point.

**Project lead:** SWFSC

**B. 4. The relationship between harbor seals and spotted seals in Bristol Bay**

**Objective**: (1) Search for a species-specific nuclear DNA marker(s) that can consistently distinguish between spotted seals and harbour seals; (2) Use this marker(s) in conjunction with the previously discovered species-specific mtDNA marker to distinguish between seals of hybrid origin (e.g., harbor seal father and spotted seal mother) and misidentified seals (e.g., a spotted seal misidentified in the field as a harbour seal); (3) compare findings from the genetic investigation with a study of skull morphology which has detected morphological intergrades suspected to be seals of hybrid origin; (4) re-examine data on seal movements and distribution in light of the genetic findings.

**Background**: The geographic area of sympatry between Pacific harbor seals (*Phoca vitulina*) and spotted seals (*Phoca largha*) is poorly understood, due in part to the paucity of definitive visual criteria distinguishing the two species. Northwest Bristol Bay has been considered the most likely area of sympatry in Alaska during summer and autumn when both species haul out on land. Up to now large concentrations of seals along the north side of the Alaska Peninsula in southern Bristol Bay have been assumed to be harbor seals and have thus been included in estimates of abundance and range for this species in this region. A previous genetic investigation determined that harbor and spotted seals are reciprocally monophyletic for mtDNA (O'Corry-Crowe and Westlake, 1997) and this has been confirmed by more recent analyses. Thus a molecular genetic marker has already been found that distinguishes maternal lineages of both species. Using this marker, a number of individual seals identified in the field as harbor seals on the basis of external morphology and location were determined to possess spotted seal mtDNA haplotypes. In one case, a number of animals in eastern Bristol Bay believed to be harbor seals were tagged with satellite transmitters were recorded making uncharacteristically long-distance movements for that species (Small, pers comm.). Later analysis of mtDNA data revealed that these individuals possessed spotted seal mtDNA lineages (O'Corry-Crowe and Small, unpublished). Assuming that harbor seals are not paraphyletic for mtDNA, these findings indicate that this sub-set of animals were either misidentified spotted seals, or hybrids (or descendants) of spotted seal females and harbor seal males. Resolving between these two possibilities has important scientific and management implications.

Although the analysis of MtDNA variation has detected inconsistencies between identifications made in the field and the genetic identity and origins of individual seals, its maternal mode of inheritance limits its ability to distinguish between misidentification and hybridization as the cause. In contrast to the haploid mtDNA marker, by-parentally inherited nuclear markers, such as isoenzymes, microsatellite loci and SNPs, contain two alleles or copies of each gene or locus, one copy inherited from the mother and the other from the father. Thus, information on both the maternal pedigree and paternal pedigree is contained in these types of genetic markers. If an individual seal is a true harbor seal (i.e., of pure harbor seal ancestry) then both alleles should be typical harbor-seal alleles. Similarly, if an individual seal is a true spotted seal, both alleles should be characteristic of spotted seals. By contrast, an individual seal that is of hybrid origin should contain one copy that is characteristic of one species (e.g., harbor seal) and one copy that is characteristic of the other (i.e., spotted seal). Thus the combination of an haploid marker (such as mtDNA or Y markers) that can distinguish between species with a similarly informative nuclear maker will enable the identification of hybrid individuals. Furthermore, this type of analysis may also shed light on the direction of hybridization.

In molecular genetic studies of hybridization among sister taxa, there are two avenues that can be pursued in the search for an informative nuclear marker(s), (1) the analysis of rapidly evolving neutral markers that quickly mutate and drift to fixation for different alleles in either species, or (2) the analysis of genes under strong selection that become fixed for different alleles in each species.

(1) The probable recent common ancestry of spotted and harbor seals may mean that there are few selectively neutral markers with fixed differences. Fixed differences do appear to exist within the mtDNA genome (O'Corry-Crowe and Westlake, 1997). However, the effective population size, *Ne*, of mtDNA is up to 4 times smaller than that of nuclear markers, and thus drift is up to four times greater.

(2) Markers under strong selection may be the most informative. Spotted seals occupy quite a different habitat to harbour seals with the likely consequence of strong selective pressures acting on a host of genes. An early electrophoretic study by Shaughnessy (1975), however, failed to find differences among the two species. A new class of genetic marker, Single nucleotide polymorphisms or SNPs, have been discovered recently, many of which occur within the coding regions of genes and thus theoretically should be under varying degrees of selective pressure. The characteristics of these sites lend them to automation where a battery of SNPs can be analyzed simulataneously.

We propose to run the eleven microsatellite markers that are currently being used to examine population genetic structure in harbor seals across Alaska (Section III) on a sub-set of spotted seals from across that species' range (excluding the area of species sympatry) to determine if any of these nuclear markers have fixed differences between species. Any marker displaying fixed differences will be cloned and sequenced to describe the molecular basis of these differences.

**Justification**: The inability to reliably distinguish between harbor seals and spotted seals in the field has implications for the effective monitoring and management of both species. Molecular genetic analysis appears to offer the most useful means of not only distinguishing between either species but of also documenting the extent of hybridization. Analysis of variation within mtDNA revealed a species-specific marker, at least for maternal lineages. What is needed now is a companion study that searches for a complimentary nuclear DNA marker. The combination of both markers will not only add to our ability to distinguish among species but to also address questions of hybridization.

**Methods**: The SWFSC already has DNA extracted from over 900 north Pacific harbor seals and 150 spotted seals. All of these animals have also been analyzed for variation within mtDNA. Furthermore, some 400 harbor seals from Alaska have also been screened for variation at 11 microsatellite loci (Section III). Forty spotted seals, 10 each from 4 areas across the species range(not including are of sympatry with harbor seals), will be screened for variation at these same 11 loci. Any marker displaying fixed differences will be cloned and sequenced to describe the molecular basis of these differences.

If none of the above markers display fixed differences between harbor and spotted seals, the search for a such a marker will continue, and may included screening isoenzyme and blood protein loci ans well as several SNPs. Once a marker with fixed differences has been found, the survey will be extended to include more samples in order to determine if the marker is indeed a species-specific marker.

**Product**: A series of reports and a scientific manuscript or manuscripts detailing the methods used and the results. If the search for a molecular marker is successful, a 'hybrid ID' kit will be developed that can rapidly test whether a given tissue sample is of hybrid origin or not

**Five-year project status**: <u>Year 1</u>: the analysis of a range-wide set of spotted seals for variation in 11 microsatellite markers, including data analysis and the production of a report. <u>Year 2</u>: If the search for a species-specific marker is productive, a more extensive survey of spotted seals and the analysis of all potential hybrids will be conducted. If none of the microsatellite markers currently in use in our lab turn out to be informative, the search for a species-specific nuclear marker(s) will continue with the analysis of SNPs and/or proteins. <u>Year 3</u>: Based on our findings, a user-friendly molecular method will be developed that can rapidly test whether a given tissue sample is of hybrid origin or not. <u>Year 4</u>: A scientific manuscript(s) detailing the methods used and the relevance of the findings to harbor and spotted seal management, cryptic species analysis and phocid taxonomy.

**Project lead**: SWFSC

## B. 5. Estimates of genetic diversity as indicators of population fitness

**Objective:** Establish whether estimates of genetic diversity at multiple loci are good indicators of population evolutionary history and fitness.

**Background:** As well as revealing population genetic structure, molecular genetic techniques can be used to investigate the consequences of population decline on spatial and temporal patterns of genetic variation. Rapid population declines can result in the loss of important genetic heterozygosity (variation) which may affect individual and population 'fitness' and compromise a population's ability to respond to environmental change.

**Justification:** The genetic consequences of dramatic declines in harbor seal abundance in certain areas of Alaska could be manifested as a reduction in reproductive capacity and lowering of the ability to deal with disease or environmental change. Caution is needed however, when using estimates of genetic diversity at neutral markers as indices of fitness, as low levels of genetic diversity may be due to natural spatial organization and mating systems instead of severe reductions in population size. Nevertheless, estimates of diversity at several independent loci may be informative when used with ecological data in determining the relative importance of environmental and genetic factors in not only causing population decline, but also inhibiting population recovery. A decline in population size in Prince William Sound over the past few decades (Frost et l., 1999) was accompanied by a discernable reduction in mtDNA diversity, manifested as a loss of rare haplotypes through random drift (Westlake and O'Corry-Crowe, 2002). A continued population decline will erode genetic diversity further, with potentially adverse effects on evolutionary potential and individual fitness.

**Methods:** We will continue to search various archives for historical samples, especially pre-decline samples from the Gulf of Alaska. These samples will be analyzed for variation within mtDNA and several microsatellite loci. Estimates of heterozygosity will be compared between sample sets collected in the 1970s prior to the recent declines in abundance and sets collected in the 1990s and today.

Once the collection of mtDNA and microsatellite data has been completed we will calculate various indices of genetic diversity. Estimates will then be compared among stocks with different abundances and trends, dispersal rates and gene flow (see parts 1, 2, and 3) in order to assess the relative contributions of evolutionary history, abundance, rate of decline, gene flow, and spatial organization and mating systems on genetic heterozygosity. We will also attempt to compare our values with those of studies on other harbor seal populations in the north Atlantic as well as north Pacific. In some cases it may be necessary to account for slight differences in the choice of markers among studies.

**Product:** A manuscript and a report on the analysis of the relationship between genetic diversity at several neutral markers and recent trends and current abundance in a number of harbor seal stocks in Alaska.

**Five-year project status**: Data on changes in mtDNA variation within Prince William Sounds were recently published in a scientific paper (Westlake and O'Corry-Crowe, 2002). Sufficient data on 12 markers should be available by December 2004 allowing preliminary results to be available by march 2005. The analysis will be constantly updated and refined as sample size increases and sample coverage improves.

**Project lead:** SWFSC

**B. 6. Alternative approaches to sample acquisition**

**Objective:** Continue to develop lab techniques to extract DNA from alternative sample types in order to increase sample coverage and numbers.

**Background:** Harbor seal samples for genetic analysis have, up to now, consisted almost entirely of tissues (skin, muscle, liver) taken directly from live or dead animals. These samples come primarily from subsistence harvest and tagging operations, and as such sample coverage is dependent on where these activities take place. Because of their typically skittish nature and their tendency to haul out on relatively inaccessible coastlines, harbor seals are often difficult to catch and sample. This difficulty in directly sampling harbor seals explains, in part, the gaps in sampling along their Alaskan range and prompted us to investigate a number of indirect methods to sample acquisition.

**Justification:** Beginning in 1997 a project was initiated to develop laboratory methods to extract DNA from three alternative sources of genetic material: scat, shed hair, and formalin fixed tissues. Since then, a protocol has been developed to extract DNA from seal scat of a quality adequate enough for amplification and sequencing of mtDNA control region and analysis of variation at several microsatellite loci. Secondly, we have been successful in extracting, amplifying and sequencing mtDNA from hair shed by seals while hauled out on glacial ice in Glacier Bay. Finally, the fixing of tissues in formalin has long been accepted as excluding such samples from genetic analysis, primarily because of the difficulty in extracting DNA of high enough quality in sufficient quantity. Problems may also arise at the amplification (PCR) and sequencing stages. Considering the wealth of marine mammal samples that have been collected over several decades and fixed in formalin and subsequently preserved in alcohol, SWFSC initiated a project to develop methods to extract DNA from formalin-fixed tissues. Initial results are promising. We successfully extracted and sequenced mtDNA from 9 harbor seal samples collected from the Pribilof Islands more than 20 years ago, more than doubling our sample from this area.

These three projects have expanded greatly our ability to collect samples for genetic analysis and fill in gaps in our sampling coverage of Alaska harbor seals. Such non-invasive approaches also avoid the possible disruptive effects of traditional sampling methods on harbor seal behaviour. Further optimization of protocols is required, however. In some tissue types, sequencing is possible but microsatellite amplification is proving problematical.

**Methods:** A range of standard and novel lab techniques are being used to extract DNA of sufficient quality and quantity for PCR and sequencing from shed hair, scat and formalin-fixed tissues. Detailed descriptions of protocols will be available upon completion of this work. Briefly, methods range from standard protocols to lyse cells and digest proteins with EtOH DNA recovery to the use of guanidium thiocyanate and multiple washes to extract DNA.

**Product:** A series of theses, reports and scientific manuscripts detailing protocols will be written up upon completion. Assessments of the efficiency of each method and thus utility of each sample type will also be made.

**Five-year project status**: A complete analysis of the methods used to extract and analyze genetic material from scat and hair samples will be complete by September 2005. Progress on the formalin-fixed tissues will be reviewed in September 2005 and written up December 2005

**Project lead**: SWFSC

**B. 7. Harbor seal mating systems and dispersal patterns**

**Objectives:** (1) Determine the mating system of Pacific harbor seals in Puget Sound and relate findings to the analysis of stock structure of the species in Alaska. (2) initiate a study on mating systems of harbour seals at one ore more sites in Alaska

**Background:** Little is known about the mating system of Pacific harbor seals, primarily because of the difficulty in observing mating in the wild and the limitations of using individual mating success in estimating reproductive success. Although female reproductive success can be measured directly in terms of pup production and survival, male reproductive success is impossible to determine from observation, particularly as mating frequency may not be a good index of the number of offspring an individual male fathered. With the advent of modern molecular genetic tools such as DNA fingerprinting it is now possible to accurately measure male reproductive success and thus determine the mating system of a species by estimating the variance in male reproductive success. This study of mating systems in Puget Sound may be applied to populations in Alaska.

**Justification:** Resolving the relationship between reproductive success and mating frequency can aid in the estimation of effective population size ($N_e$), an index of relevance to investigations of stock identity and dispersal in this species. $N_e$ is smaller than N, for example, if only a proportion of adult males contribute to next year's cohort of pups. A small $N_e$ in turn increases the rate of genetic divergence among strata due to the greater effects of genetic drift. We are currently using a molecular genetic approach to studying the mating system of Pacific harbor seals. The combination of behavioral observations during the pupping and mating season and molecular genetic analyses of mother-pup pairs and adult males has yielded the first clear evidence of variation in male reproductive success in this subspecies. Current efforts are directed at using these findings in estimating $N_e$ for application in genetic stock identification. As well as estimating the variance in male reproductive success by establishing paternity, the same techniques are being used to assess relatedness among all members of the population. This approach will hopefully help us identify immigrants and thus describe the nature of dispersal across stock boundaries. One year of data has been collected from one location in Puget Sound to date and ideally multiple years of data at a number of different sites need to be collected to address these questions.

**Methods:** The location or locations will be chosen based on a number of criteria including the accessibility of the seals, the size of the population, and the physical characteristics of the haul-out site and surrounding area. Behavioral observations will be conducted to determine population size, record associations, and assess the mating system. Samples will be collected either directly by biopsy during capture operations or indirectly from scat, shed hair, afterbirth, or shed blood.

Samples will be preserved in a 20% DMSO and salt solution or snap frozen and returned directly to the lab.

An array of laboratory procedures, many developed at our lab, will be used to extract, amplify and sequence an array of genetic markers, including mtDNA, microsatellites, and gender. (See sections 1, 3, and 5 above for details). Paternity assessment and relatedness will be estimated using a number of standard statistical packages as well as a number of techniques currently under development in our group.

**Product:** A series of theses and reports and a scientific manuscript or manuscripts detailing the findings of the current research as well as proposed research on harbor seal mating systems and how this relates to the analysis of population structure in this species in Alaska.

**Five-year project status:** A masters thesis on the current study of Pacific harbor seal mating systems was completed in July 2000 (DeAngelis, 2002). A manuscript of this work is expected by March 2004. Timelines for continued research will be refined once agreement has been reached on study sites and duration.

**Project lead:** SWFSC

## B. 8. Metapopulation viability analysis of harbor seals in Alaska

**Objective**: (1) to explore a range of metapopulation structures and dynamics for harbor seals in Alaska (2) to model the viability of sub-populations of harbor seals under a metapopulaton framework (2) to model the loss of genetic variability within sub-populations of harbor seal under a metapopulation framework.

**Background**: Although harbor seals in Alaska occur almost continuously throughout their Alaska range, their abundance and their distribution while hauled out is non-uniform. This heterogeneity in density combined with our recent findings on population genetic structure and dispersal rate indicate that harbor seals in Alaska are organized into a series of demographically 'discrete' sub-populations with low levels of interchange. On some spatial scales and/or in some regions of their Alaska range, however, the level of dispersal may be large enough to where the dynamics within one sub-population or area may affect that of neighboring sub-populations or area. Understanding such metapopulation dynamics is essential to conducting meaningful population viability analyses (PVA) and designing effective management strategies.

**Justification**: Harbor seals in Alaska appear to be organized into a series of demographically 'discrete' sub-populations with low levels of interchange. On some spatial scales and/or in some regions of their Alaska range the level of dispersal may be large enough to where the dynamics within one sub-population or area may affect that of neighboring sub-populations or area. Understanding such metapopulation dynamics is essential to conducting population viability analysis (PVA) and designing effective management strategies. In this study we propose to explore the metapopulation aspects of harbor seal population subdivision in Alaska and conduct PVA using both a metapopulation and isolated population framework.

**Methods**: The study will simulate metapopulation dynamics, population viability and changes in genetic variability in harbor seals in Alaska. We will use a number of currently available computer packages as well as custom designing, if necessary, new methods for population viability analysis and metapopulation analysis. A variety of models and parameter estimates, including dispersal rate, dispersal distance and sub-population size will be explored. Simulations will be run for a range of time horizons (e.g., 50 years, 100 years).

**Product**: A series of reports detailing the inputs, assumptions and behavior of the models tested and the findings from the simulations.

**Five-year project status**: In the first year a number of currently available packages for population viability analysis will be explored and some preliminary simulations on Alaska harbor seals conducted. In year two, more elaborate simulations will be run, incorporating more parameters and the most up to date data on genetic diversity, genetic structure, dispersal rate, etc. By the end of year two, timelines for future work will be refined.

**Project lead**: SWFSC

## B. 9. Mating Systems and Identification of Factors That May Contribute to Increased Reproductive Success

**Objective:** We will conduct a retrospective analysis of our archival genetic database (*n*>1,000) of DNA samples collected from seals across the state since 1993 to derive an index of reproductive success (i.e., parent-offspring relationships) and subsequently examine previously acquired data on dive behavior, diet, and condition for those individuals to explore which of those factors may be positively associated with increased reproductive success. We will test the hypothesis that individuals with offspring are in better condition than those without offspring, and will assess area differences in reproductive success. We also will compare this individual-based, bottom-up model of factors contributing to harbor seal population processes (particularly factors related to dive behavior and diet) with a spatially explicit top-down model (see section F6) that explores how prey availability and predators affect seal population processes. That model provides predictions of optimal foraging strategies for seals under predation risk. A comparison of our respective modeling results will assess whether seals that have offspring appear to have dive behaviors and diets more consistent with optimal foraging strategies than do non-parents. Further model comparisons will determine whether individuals in increasing populations more closely conform to the model predictions of foraging strategies that increase net energy gain while reducing predation risk than do seals in a declining population. Hypotheses to be tested are as follows:

I. Hypothesis: Reproductive success differs among populations that are currently experiencing growth and those that are declining.
    - Reproductive success will be greater in Kodiak than in PWS or SE Alaska.

II. Hypothesis: Survival and reproductive success is a result of better body condition.
    - Individuals identified as parents will have health and morphometric data indicative of better condition than do non-parents.

III. Hypothesis: Survival and reproductive success is a result of foraging strategies that reduce risk of predation and/or increase net energy gain.
    - Individuals identified as parents will exhibit dive-depth profiles and diets more consistent with DSV model predictions of optimal foraging under predation risk than do non-parents.
    - Parents that have multiple offspring (i.e., have greater reproductive success) will exhibit dive-depth profiles and diets more consistent with DSV model predictions of optimal foraging under predation risk than do parents with only one offspring.

IV. Hypothesis: Foraging strategies are a learned behavior.
    - Parents and their offspring will forage in similar manners.

V. Hypothesis: Individuals in increasing populations (Kodiak) exhibit dive-depth profiles and diets more consistent with DSV model predictions of optimal foraging under predation risk than do individuals in a declining population (PWS).

Although our main objective with this retrospective analysis is to determine what factors contribute to individual variability in reproductive success and whether those factors vary among areas with different population trends, our analyses also may provide some insights into philopatry. Our multiple years of genetic samples will allow us to assess whether individuals we sampled as pups in earlier years remained in the area (i.e., exhibited philopatry to their natal site) and survived to reproduce, as evidenced by genetic matches to individuals captured >5 years later, after the original pups would have reached sexual maturity.

**Justification:** Our genetic assessment of reproductive success (and the possible inferences of survival and philopatry) will allow an extensive exploration of archived data on a relatively short timeframe, potentially providing insights into factors contributing to differential survival and reproductive success that can then be empirically tested in our current and future research. Furthermore, the same microsatellite sequence data for each individual that will be required for our study can also be used by SWFSC to expand their assessment of stock structures for harbor seals in Alaska (Westlake and O'Corry-Crowe 2002). Their work likely will further elucidate the extent of gene flow among areas for harbor seals, which will aid in interpretation of our present analytical approach. Specifically, more microsatellite data with more polymorphic loci may permit an estimation of the probability of dispersal via an analysis of migrants per generation, and assignment tests that may determine the probability than an individual originated from the location in which it was captured (Blundell et al 2002). Those analyses could indicate whether absence of offspring in the area (particularly males) is more likely to be a result of dispersal of the adult or their offspring, or mortality.

**Background:** Microsatellites are nuclear DNA that do not undergo selective pressure, resulting in highly variable tandem repeats that are useful for studies of population genetics (Tautz 1989), including gene flow, sex-biased dispersal, reproductive success, and kinship (Blundell et al *in review*, Blundell et al 2002). Parent-offspring relationships have been successfully determined in a number of harbor seal studies using microsatellite (Coltman et al. 1998, Coltman et al. 1999, Schaeff et al. 1999) and minisatellite DNA (Perry and Amos 1998), and SFSC has successfully determined parent-offspring relationships for other harbor seal samples in their laboratory (O'Corry-Crowe, pers. com.) with as few as eight microsatellite loci.

Other studies have identified 19 polymorphic microsatellite loci that can be used in harbor seals (Davis et al. 2002), thus our ability to determine unique DNA fingerprints for each seal sampled in our extensive genetic archives will be greatly enhanced by the addition of more loci to our sequence data, and parents can be matched with their offspring with high confidence levels. With nine microsatellite loci, 68% of parent-offspring relationships for 53 river otters (*Lontra canadensis*) were assigned at a 95% confidence level and 32% were assigned with 80% confidence (Blundell et al. *in review*).

**Methods:** Using microsatellite DNA, we (SFSC and ADF&G) will determine identification of individuals through DNA fingerprinting and we will assess parent-offspring relationships. Once we have identified parents and offspring through analysis of microsatellite data using the program Kinship (Version 1.2 Goodnight et al. 1994, Queller and Goodnight 1989, Queller and Strausmann 1993), we will return to our extensive database and construct a multinomial logistic regression model that incorporates all available data (e.g., blood chemistry profiles, morphometrics, and other indices of health, analysis of diet, and dive data) for parents and non-parents to assess what variables may distinguish between seals identified as parents and those that are not. Additionally, we will use mixed-effect GLM analyses to compare data among parents and their offspring to assess similarities, particularly in dive profiles and diet, and to assess differences in number of offspring (reproductive success) among areas. We also will explore the use of more complex individual-based models to explore population-level consequences of factors contributing to individual variation in reproductive success.

**Product:** A manuscript with results of our archival data and associated hypothesis testing will be prepared for submission to a peer-reviewed journal. Additionally, results from this portion of our study will be compared with results from the dynamic state variable (DSV) model constructed in the study described in section F6, and our mutual results and associated hypothesis testing will either result in a separate manuscript, or will be included in with our results, or in a final manuscript associated with the DSV model.

**Five-year project status:** Not funded FY03

**Project lead:** SWFSC/ADF&G

**B. 10. Spotted Seal Sympatry With Harbor Seals**

**Objective:** Refine the methodology to extract and amplify DNA from the feces of harbor seals for the purposes of isolating a marker that allows species identification. This marker, which can be easily isoloated from the feces of seals can be used to assess seasonal distribution and potential extent of hibridization of harbor seals vs. spotted seals in areas of sympatry as determined by fecal DNA.

**Justification:** See above.

**Methods:** The laboratory methodology will be developed using fresh fecal samples collected on haul outs. Seasonal aspects of species overlap and an exploration of potential hybridization of spotted and harbor seals will be determined by isolating DNA from seal feces collected at haul outs in the Bristol Bay four times a year.

**Product:** annual report, Masters thesis chapter, and possibility of a peer-reviewed publication.

**Five-year project status:** Not funded FY03

**Project lead:** ADF&G – Masters study conducted at University of Alaska Fairbanks, co-advised by G. Blundell (ADF&G), G. O'Corry-Crowe (ASLC) involved as co-author on resulting publication or possibly as a committee member.

**B. 11. Fecal DNA**

**Objective:** Refine the methodology to extract and amplify DNA from the feces of harbor seals for the purposes of: a) species identification [seasonal distribution of harbor seals vs. spotted seals in areas of sympatry as determined by fecal DNA]; b) gender identification via fecal DNA paired with identification of prey remains in feces to assess gender differences in diet to address the question -- do changes in the composition of prey available to seals (i.e., a reduction in fish species preferred by females) have potential demographic implications?; c) identification of individuals through microsatellite DNA fingerprinting from fecal DNA. (Expanded DNA sampling via fecal DNA will facilitate stock structure clarification via assignment tests to determine the likelihood that an individual originated from the population in which it was "captured" vs. likely population of origin as a measure of dispersal); d) mark-recapture studies (survival estimates) using microsatellite DNA fingerprinting by repeated identification of individuals over time through isolation of DNA from their feces.

**Justification:** Because of their typically skittish nature and their tendency to haul out on relatively inaccessible coastlines, harbor seals are often difficult to capture and sample. DNA, amplified from seal feces collected at haul-out sites throughout Alaska, provides an avenue to expand the extent of DNA sampling without requiring that seals are live-captured or harvested. Species-specific and sex-specific primers have been developed in pinnipeds (Reed et al., 1997) and can be utilized to determine the extent of sympatry and hibridization of spotted and harbor seals. Because female reproduction is the primary regulating or limiting factor of population size it is important to explore dietary differences between the sexes of seals and establish the basis for examining gender-specific effects of changes in prey availability over time. These data will aid in our continued efforts to understand what factors may be contributing to the continued decline of some seal populations in Alaska.

The shorter DNA base pairs required for species and sex identification can be isolated from seal feces relatively easily, thus funding for this project will result in the success of objectives a and b. Isolation, from feces, of the longer DNA base pairs required for identification of individual seals via microsatellite DNA fingerprinting is a more complex process that will only be optimized if funding is devoted to developing the laboratory methodology and it is made the primary task of a devoted individual (e.g., a graduate student). The methodology for extraction of longer base pairs from feces has been successfully developed in many carnivores and primates (e.g., Ernest et al. 2002, Morin et al. 2001, Lathuilleire et al. 2001) but the process is labor intensive and does not easily fit into the work load of a busy DNA lab such as the Southwest Fisheries Science Center. Therefore we propose to collaborate with the University of Alaska Fairbanks, funding this project as a Masters study.

Optimization of the methodology for extracting DNA from the feces of harbor seals and amplifying the longer base pairs necessary for identification of individual seals through DNA fingerprinting holds the potential to increase our understanding of stock structure. Fecal samples can be collected in areas where live captures and subsistence harvest do not occur and DNA samples of individuals can be isolated from their feces to fill in some of the gaps in our DNA sampling. Moreover, if the methodology for this extraction can be optimized, we can explore the potential for studies of vital rates (survival and reproductive success) of individuals through repeated "recaptures" of individuals overtime via isolation of their DNA fingerprint from feces collected from haul outs. Given the many important applications for this methodology if individuals can be identified from their DNA fingerprints in feces, we believe it is very important to devote some funding specifically for this project.

**Background:** Polymerase chain reaction (PCR) amplifies selected portions of DNA extracted from small amounts of tissue using primers targeting specific regions of the molecule (Mullis and Faloona, 1987). Examination of this DNA can provide information on the genetic make-up of an individual, including determination of sex and identity. Recently, methods have been established to extract DNA from the intestinal cells shed in feces (Höss et al., 1992). These methods allow DNA from an animal to be studied without having to handle the animal and is, thus, a non-hazardous and economical method of DNA collection.

The sex of an individual also can be determined by amplifying a region of the Y chromosome. Because only males possess Y chromosomes, positive amplification of a region on this chromosome can verify male gender (Taberlet et al., 1993). The male sex-determining locus, *Sry*, has been widely used for sex determination and establishment of paternal lineages (Lundrigan and Tucker, 1994; Reed et al., 1997). The positive amplification of the *Sry* gene can be established by visualizing an aliquot of the PCR product in an agarose gel stained with ethidium bromide. Because the absence of the *Sry* fragment could be a false negative due to a failed PCR reaction, a second region of DNA, not specific to the Y chromosome can be co-amplified with the *Sry* gene as an internal positive control (Taberlet et al., 1993). These techniques have been used to reliably establish the sex of a variety of mammals including bears (*Ursus arctos*), seals (*Halichoerus grypus* and *Phoca vitulina*), and whales (*Physeter macrocephalus*) using hair, fecal and skin samples for the DNA source (Taberlet et al., 1993; Richard et al., 1994; Kohn et al., 1995; Reed et al., 1997).

Reed et al. (1997) determined the best method (i.e., best balance between effort and success rate) for isolating DNA from seal feces for the purposes of species differentiation between harbor and gray seals (*Halichoerus grypus*), and to determine whether microsatellite DNA isolated from feces could be used to identify individual seals and the sex of the seal using a male-specific *SRY* primer (Richard et al 1994). DNA was amplified from 85% of the fecal samples tested ($n = 173$) for at least one microsatellite loci and 82 of those samples yielded DNA for 4-5 of the five loci chosen for screening, providing unique identification (i.e., DNA fingerprinting) for individual seals and species differentiation. Of the 151 samples for which at least one loci was amplified, 53 samples were identified as originating from a male. Because negative results could signify failure to amplify DNA, rather than belonging to a female, results were cross-validated with samples in which 4-5 microsatellites were identified. Forty of the 82 samples for which multiple loci were amplified were identified as belonging to males. As a control, a blind test was conducted using blood and matched feces from eight individual seals. DNA amplified from seven of eight fecal samples were correctly matched with DNA from blood of the same seal; only two loci were amplified from the $8^{th}$ fecal sample corresponding to a matched blood sample, preventing cross matching (Reed et al. 1997).

**Methods:** The laboratory methodology will be developed using fresh fecal samples collected on haul outs. Accuracy of the identification of individuals through DNA fingerprints in their feces will be verified by matching DNA fingerprints obtained from blood and feces of the same individuals, primarily through blood and feces collected from individuals held in captivity.

**Product:** Annual reports, Masters thesis, peer-reviewed publications.

**Five-year project status:** Not funded FY03
**Project lead:** ADF&G

## C. HABITAT AND ECOSYSTEM STUDIES

**Overview**
These tasks integrate harbor seal abundance and distribution with relevant physical and biological information in a geographical framework. This integration provides a basis for examining multi-factor relationships and patterns that might not otherwise be detected.

### C.1. Characterize harbor seal habitat and prey aggregations

**Objective:** Construct a geographic information system linking harbor seal abundance and distribution, spatial data on human activities such as fishing and vessel traffic, and known aggregations and spawning runs of fish preyed upon by harbor seals.

**Justification:** Data on harbor seal habitat is collected in association with other studies; however, no comprehensive set of habitat characteristics for harbor seals is available. Habitat data are useful to develop both research and management activities. Information of this nature can be used in research to assess whether trend survey sites are representative of the overall trend area and areas outside the trend route. These data are also useful for management purposes to aid in evaluation of impacts from development activity and to develop conservation measures in sensitive harbor seal sites. A GIS format of habitat maps will also allow for better assessment of complex interactions involving other species and other resource concerns. Of particular interest is the role that known prey aggregations play in the distribution and seasonal changes in distribution of the seals.

**Methods:** Data will be compiled from existing sources. These data will include information on haul-out substrate, bathymetry, major fishing areas, vessel traffic lanes, glacial ice areas, estimated subsistence mortality level, estimated mortality incidental to commercial fisheries, major freshwater streams, locations of human coastal communities, and documented aggregations and spawning runs of known harbor seal prey. These data will be consolidated into a GIS format and related to the spatial distribution and abundance of harbor seals in Alaska, including individual haul-out and pupping locations.

**Product:** A GIS database of habitat characteristics associated with distribution patterns and abundance of harbor seals in Alaska.

**Five-year project status:** Statewide distribution and abundance data have been compiled. In some areas, such as Cook Inlet, more complete seasonal coverage will be provided in 2003-2004 as described in tasks F.3 and F.4. Sources and availability of remaining habitat characteristics are being identified. Acquisition of data and integration into GIS format will follow. Unfunded in FY04.

**Project lead:** NMML

**<u>C.2. Habitat Selection By Harbor Seals</u>**
Objectives:

1. Document haulout, movement, diving, and spatial-use patterns of Alaska harbor seals
2. Estimate home ranges and habitat availability [attributes within the 99% utilization distribution (UD) contour for each individual]
    a. Identify concentrated foraging areas (CFA's; >average observation density) and repeatedly used depths (seasonal TDR and SDR data)
    b. Develop indices and direct measures of habitat variables
    c. Estimate movement, fidelity, and dispersal rates of pups
3. Estimate habitat selection at multiple scales
    a. Haulouts vs. random sites along coastline (logistic regression)
    b. Used vs. unused sites, at-sea (logistic regression)
    c. CFA vs. 99%UD
    d. 99%UD vs. overall range
4. Characterize and identify seasonal habitat shifts
5. Develop spatially explicit, seal habitat prediction models
    a. Test predictive power of these models using past and future survey data
6. Determine if habitats at trend survey sites are representative of the overall trend area and areas outside the trend routes (i.e. are trend estimates representative of population trends?)
    a. Compare habitat variables for haulouts surveyed during aerial flights with those for all known haulouts
7. Investigate potential cumulative effects from a variety of factors (e.g. changes in prey availability, human-caused mortality through harvest or incidental take in fisheries, disturbance, diseases, pollutants, predation) on harbor seal populations
8. Develop spatially explicit models to predict and compare forage conditions and foraging behavior among regions, estimate inter- and intra- specific competition, and develop a multi-trophic model to evaluate the ecological constraints on harbor seal populations.

**Justification and Background:** Overall, this research will (1) fill a substantial gap in our understanding of harbor seal ecology, and (2) assist in developing effective management and conservation strategies. Data on harbor seal habitat (e.g.; haulout substrate, bathymetry) has been collected in association with other studies in the GOA. However, quantitative relationships between harbor seal distribution and habitat characteristics remain largely unknown. Such data are necessary for making sound management decisions and identifying environmentally sensitive areas. Moreover, historic changes in specific habitat characteristics (e.g.; predator and prey distribution and abundance) could have contributed substantially to the observed population decline of harbor seals in the western portion of the GOA.

In collaboration with personnel from the NMML, available information on habitat characteristics are being compiled with estimates of abundance and distribution in a GIS format. All known haulout sites and associated substrate, as recorded in NMML and ADF&G databases, have been combined for analyses. Other existing databases (e.g.; prey distribution and abundance estimates) will also be incorporated when available, relevant, and compatible.

**Methods:** Several habitat characteristics will be included in habitat selection analyses. Variables included must be quantifiable and biologically relevant. The following habitat characteristics will likely be included in the multivariate analyses: haulout substrate, an index of "bathymetric diversity" (e.g.; absolute change in depth, number of fluctuations in slope and depth direction), indices of oceanographic conditions (e.g.; sea surface temperature, monthly probability of front occurrence, upwelling indices, salinity), an index of coastline complexity (e.g.; length of coastline per unit area), presence of major fisheries, distribution (depth) and abundance of prey and predators, proximity to vessel traffic lanes, presence of glacial ice, distance to glacial haulouts, estimated mortality by subsistence hunters, estimated incidental mortality by commercial fisheries, distance to major freshwater streams, distance to major recreation areas (e.g.; glacier and pelagic boat cruises, sea kayak activity), and distance to human coastal communities.

**Five-year project status:** Partially funded FY03 (Salary money available but funds for software not available. Objectives are best accomplished with Arc Info software)

**Project lead:** ADF&G

**C.3. TEK Research**

Information unavailable at this time.

**Lead**: ANHSC

## D. HEALTH and DISEASE

### D.1. Contaminant Levels of Free-ranging Harbor Seals

**Objective:** To determine the body burden of contaminants in harbor seals throughout Alaska.

**Justification:** The potential exists for several environmental factors to impact the biology of harbor seals, resulting in poor survival, recruitment and reproductive rates. While the leading hypothesis is that changes in the availability of high quality prey have reduced the carrying capacity of the Gulf of Alaska, a contributing factor to poor survival and reproduction may include exposure to organochlorine contaminants (OCs), with associated endocrine and immune system impairment (Addision, 1989; De Swart *et al.*, 1994, 1996; Reijnders, 1986.) OCs, heavy metals, and polychlorinated biphenyls (PCB) have been shown to have disruptive effects on the reproductive system of both males and females, as well as severely affecting the sexual differentiation of the developing embryo (Gray, *et al*., 1999, Goo *et al*. 1999). Primarily due to their chemical structures, PCBs and OCs are lipophilic and persistant, bioaccumulated according to trophic level and are stored by the body in fat depots (Mulvad, *et al*., 1996). In the case of the harbor seal, OCs and PCBs are most often found in the blubber, significant in light of fasting bouts that both males and females of that species undergo during which blubber is mobilized as an energy source. Female harbor seals, during lactation, excrete OCs in milk (Reddy *et al*. 2001; Furst, *et al*., 1994; Addison, 1989). The intake of contaminated milk can have a profound effect on neonates in terms of survival and future reproduction due to "targeting" of several endocrine systems by environmental contaminants. For instance, Sertoli cells of males play a central role in sperm production, replicate only during fetal, neonatal, and prepubertal periods, and are directly related to sperm quality and count. The Sertoli cells of males are also targets of environmental contaminants (Monsees *et al*. 2000 Bonefeld-Jorgensen *et al*. 2001), many of which neonatal seals are likely to be exposed to through the intake of contaminated milk. The cumulative effects of embryonic exposure, ingestion through suckling, and later ingestion during active growth through contaminated prey may be subtle or subclinical, or may manifest themselves with symptoms such as, 'failure to thrive' or 'failure to reproduce'. The systems that typically respond to environment changes, including contamination of suitable prey, are the endocrine and immune systems.

**Methods:** The primary target chemicals are parent PCBs and DDT. However, PCB metabolites and other contaminants such as organochlorine pesticides may be also examined as biomarkers for toxicity, and for better understanding of possible toxic effects. In general, the methods that have been used successfully for the analysis of PCBs and organochlorine pesticides in marine organisms are well accepted in scientific communities. Immunochemical methods may also be used to facilitate the work where it is appropriate.

**Product:** The initial products will be a survey of contaminant loads on harbor seals throughout Alaska. An accompanying baseline of endocrine or immune system indicators for seals, where blood samples are available, will be used to correlate with contaminant concentrations.

**Five Year Project Status:** This study was initiated in 2002. The first year focused on obtaining archived samples from previous collections of harbor seals and analysis of a subset of those samples. Future work will be dependant on additional funding; priorities will be based on findings and data gaps.

**Project Lead:** ASLC

**Project partners:** ANHSC biosampling program, ADFG

## D.2. Endocrine, Immune, and Blood Chemistry Markers of Health and Condition

**Objective**: To assess normal seasonal variation in (1) thyroid hormones and corticosteroids in serum and fecal samples (2) immunoglobulin levels, and (3) blood chemistry and hematology values of healthy growing captive seals and contrast those expected levels with samples obtained from wild seals to detect unusual levels immune, endocrine, or blood chemistry values indicative of abnormal conditions potentially affecting health or survival of wild seals.

**Justification:** Regional differences in harbor seal population trends probably reflect varying environmental conditions over time. However, the mechanisms driving those changes (e.g., changes in fecundity, mortality, emigration, or immigration) are poorly understood and have important consequences for management decisions.

The endocrine system is a complex, highly integrated means through which an animal physiologically adapts to its internal and external environment in order to maintain homeostatic balance. Mechanistic changes in the physiology of harbor seals indicated by abnormal endocrine activity may contribute to increased mortality or lowered fertility. Metabolic and stress hormones including thyroxine (T4) and triiodothyronine (T3) and cortisol provide measures of metabolic activity and stress and help identify times when seals may be affected by adverse environmental conditions. For example, Atkinson and Oki (Oki, 2001) found a significant elevation in thyroid hormones in the winter versus the summer, indicating a substantial shift in metabolic rate due to natural seasonal cycles. Similarly, the normal circadian pattern of cortisol, the primary gluconeogenic hormone, was abandoned in the winter. These findings suggest that during the winter even well fed seals have a continual need to produce metabolic heat through gluconeogenesis. Likewise, higher levels of thyroid hormones in the winter also indicate increased metabolic activity serving as an adaptive mechanism to cope with the low winter temperatures. Increased knowledge of the factors controlling individual harbor seals will enable researchers and resource managers better understand the current physiological condition of seals and assess whether seals in specific regions or time periods show evidence of abnormal metabolic activity or stress that may affect health or survival.

Hormonal regulation of the internal milieu is dependant on tightly synchronized of negative/positive feedback and cascade events, thus analysis of a suite of serum hormones provides a more comprehensive description of physiological state at any given moment in time. In mammals, the glucocorticoids (GCs) cortisol (measured in serum) and corticosterone (measured in feces), secreted by the adrenal cortex, are among the primary initiators of the "fight or flight" response. An additional function of GCs is to suppress $T_3$ and $T_4$ -driven metabolism during response to a stressful event (Kacsoh, 2000). The healthy mammalian system can recover fairly quickly from acute stress events however; chronically stressed animals are exposed to elevated levels of GCs for extended periods which can result in severely altered physiological condition (St. Aubin and Dierauf, 2001). Hormones accumulate in the feces over time prior to defecation, and therefore provide a description of the animal's integrated response over time, rather than the "snapshot" value provided by serum collection. Comparisons of fecal and serum glucocorticoids allow the discrimination between acutely or chronically stressed individuals and/or populations. Differentiation between physiological state at sampling and overall state is essential to the correct interpretation of hormonal data.

**Methods:** Blood and fecal samples will be obtained from captive harbor seals on a monthly basis for baseline analysis. Samples will also be obtained of free-ranging harbor seals captured through collaborative efforts with the Alaska Dept. of Fish and Game and the National Marine Fisheries Service for contrast with baseline data.

The analyses for cortisol and the thyroid hormones have previously been validated for other pinniped species (Atkinson and Oki, in review) and for harbor seals (Oki, 2001). Concentrations of cortisol will be measured in unextracted plasma using a single-antibody radioimmunoassay (Atkinson and Oki, in review, Atkinson and Adams, 1988). The plasma will be heated at 60°C for 30 minutes to denature cortisol-binding proteins before assaying directly. Samples will be analyzed in batches to reduce inter-assay variation. Concentrations of total and free, $T_4$ or $T_3$ (Atkinson and Oki, in review). The standard curves of each assay will be log-logit transformed, enabling extrapolation of sample concentration (Robard, 1974). A profile of the variation in total and free $T_4$ and $T_3$ will be generated and statistically analyzed.

An ELISA protocol similar to that described by Suer *et al.* (1988) has been used to evaluate serum antibody levels in several species of marine mammals against several antigens. A "sandwich" ELISA protocol will be employed in an effort to determine general immunoglobulin levels in these samples. In the sandwich ELISA, a plastic solid phase matrix (polystyrene microwells) is coated with unlabeled antibodies against the antigen in question, i.e. in this case against on of the heavy chain isotypes (gamma, alpha, or mu for IgG, IgA, and IgM respectively) of immunoglobulins from the harbor seal (prepared via completion of Objective 2 above). The sandwich ELISA conducted in this manner will allow quantification of general immunoglobulin levels in samples by comparison with a standard curve generated using preparations made with known concentrations of immunoglobulins purified from the harbor seal. Blood samples will continue to be collected on a monthly basis from the permanently captive seals at ASLC. Aliquots of each sample (and aliquots of other samples of harbor seal sera which become available) will be quantified for isotype levels using the ELISA described above.

Blood chemistry and hematology values are used in conjunction with body composition to detect significant changes in health status that might alter water balance, cause anemia, or compromise basic metabolic status (Castellini *et al*., 2000, 1993). Blood urea, nitrogen (BUN) ketone bodies, and free fatty acids, as well as hematocrit, hemoglobin, and erythrocyte sedimentation rate will be measured in captive and free-ranging seals.

**Product**: Techniques and results will be published in peer-reviewed scientific journals. Annual reports will be produced in accordance with the requirements of funding sources. Techniques and findings from this study will be used to develop into a health screening program where assessments of endocrine and immune activity and blood chemistry levels from serum of representative harbor seals sampled throughout the State will be evaluated.

**Five Year Project Status**: The analyses of the above health and condition parameters of growing captive seals fed differing diets at the ASLC will be initiated in FY03 and conducted in conjunction with *Effects of Dietary Lipids on Harbor Seal Growth and Health* study described in this plan. It will last through the growth and sexual maturation of the captive seals (3-6 years). Concurrently, samples obtained by the ADFG from free-ranging harbor seals throughout Alaska will be analyzed.

**Project Lead:** ASLC

**Project partners**: ADFG , ANHSC biosampling program, NMFS

**D. 3. Effects of Dietary Lipids on Harbor Seal Growth and Health**

**Objective:** Assess the long-term effects of high and low lipid diets on the nutritional physiology, growth, maturity, endocrinology, immunology, metabolic development and clinical health of growing harbor seals.

**Background**: It has been proposed that decreased availability of high lipid forage fishes (e.g., capelin, sandlance, and herring) observed since the 1977 regime shift has adversely affected marine birds and mammals, including harbor seals (Van Pelt *et al*. 1997, Duffy 1999, Pitcher 1990, Jemison and Kelly 2001, Gotthardt 2001). It is not known, however, whether differences in the availability of lipids affect the health and survival of seals sufficiently to account for the observed population change or whether other factors such as prey-specific protein composition need to be considered. This study investigates the importance of lipids in the diets of harbor seals and the long-term effects of high and low lipid diets on the growth, development, maturity, and health of seals.

**Justification:** The effects of the 1977 regime shift and the associated reduction in high lipid forage fishes, on harbor seal growth, health, tolerance of environmental stresses, and rate of reproductive maturation are largely speculative. Long-term studies of the effects of dietary lipids on health and growth parameters are lacking. This study will provide insight into the consequences of harbor seals feeding solely on prey comprised of low dietary lipids. The findings will be important for determining whether differences in dietary lipid concentrations have measurable impacts on harbor seal growth, survival and reproductive maturation sufficient to explain population change and variation in body condition and maturation rates observed in wild populations. It will provide information useful for modeling the energy requirements of harbor seals throughout their growth period. In addition it will aid in assessing whether changes in lipid availability is sufficient to explain the observed population changes or whether other factors, e.g., changes in protein composition/availability or periods of starvation need to be considered.

**Methods:** To investigate long-term health consequences associated with diet we will capture, over a two-year period, 8 healthy, recently weaned female pups that have received full maternal care and nurturing from haulout sites in southern Prince William Sound. They will be from an area that has received focused multi-disciplinary ecological studies (e.g., Pitcher and Calkins 1979; Frost and Lowry 1993, 1994; Frost *et al.* 1994-1998, 1999a,b; Duffy 1999; Coyle *et al.* 2001; Foy and Norcross 1999; Iverson *et al.* 1997, 1999, Ver Hoef and Frost. 1999). Genetically, these seals will be from the stocks that have been strongly affected by the Gulf of Alaska decline (Westlake and O'Corry-Crow 2002, Frost *et al*. 1994, 1999b, Hoover-Miller *et al.* 2001) and thus represent seals adapted to conditions in the Gulf of Alaska but susceptible to decline-related perturbations. The pups also will receive full maternal care and should accurately represent a cross-section of seals that is being recruited into a population that is not thriving**.**

Seals will be assigned to one of two groups for long-term feeding studies. Both groups will receive an identical base diet that will include species with < 4% fat (pollock, flatfish, squid/octopus, and pink salmon) for dietary balance. Seals fed the high lipid diet (Group A) will receive the remainder of their diet from high fat herring (est. 16% lipids); seals fed the low lipid diet (Group B) will receive the remainder of their diet as low fat herring (<6% lipids). Proximate analysis will be conducted of all prey fed to the seals to provide lipid, protein, water and caloric content of each dietary component.

Group A will be fed to a level of satiation that will allow training methods to continue using routine animal handling protocols by the husbandry staff at the ASLC. The weight of food consumed as a proportion of the individual seal's weight will be determined and averaged among the Group A seals. Group B seals will receive the same mass of each species of foods (as a proportion of the seal's individual weight). The diets are not designed to be iso-caloric and the mass of food will vary throughout the experiment, however the two groups of seals will differ in the amount of lipids they receive from herring. Seals in Group B are expected to be more nutritionally limited than seals in Group A. This design allows the seals to adjust their intake naturally by season as determined by the appetite of Group A seals. Adjustments to the proportion of herring in the diet will be made as necessary (see above) to ensure that Group B seals do not show blubber thickness below that seen for healthy seals in wild populations (Bishop 1967, Pitcher and Calkins 1979, Frost *et a*l. 1995-1999*b*, Small *et a*l. 2001). Routine health monitoring of plasma concentrations of blood urea nitrogen (BUN), non-esterized fatty acids (NEFA), and Ketone Bodies (HBA) will be used to monitor the physiology of the seals and ensure that none of the seals begins metabolizing body protein to meet energetic needs, (i.e., as seen in fasting or starving animals). For training purposes, the animals will be handled everyday at feeding times and trained to be weighed, measured, bled and otherwise participate in other sampling and husbandry.

In conjunction with investigations described in *Immune and Blood Chemistry Markers of Health and Condition* and *Reproductive Rates* described in this plan, f*or* seals in each diet group, we will contrast age, seasonal, and dietary differences in: growth (standard measurements, 3-D morphometrics, bone growth, whisker growth), blood chemistry, blubber deposition and turnover, food assimilation efficiency, endocrine development and activity, including thyroid, cortisol, and reproductive hormones, immune system development and activity, metabolic condition and development, and sexual development and maturity.

**Product**: Data resulting from this study will used to interpret samples and measurements from past and present field studies and will be made available to other researchers. Several papers for publication in peer-reviewed scientific journals will result from this study. Annual reports will be produced in accordance with the requirements of funding sources.

**Five-Year Project Status:** This study will be initiated in FY03 and continued 3-6 years, or until all seals reach reproductive maturity.

**Project Lead:** ASLC

**Project Partners**: ANHSC

**D. 4 Sample acquisition**

**Objective**: Facilitate the collection of biological samples from live-captured seals. Initiate collaboration with other investigators of harbor seal biology in Alaska and distribute samples so that multiple hypotheses can be addressed for each seal captured. Facilitate further collaboration and synthesis of data to enable hypothesis testing at a larger scale (e.g., individual-based models with multiple parameters) or the testing of multiple hypotheses from the same data.

**Justification:** Harbor seals are difficult to capture and the capture of these animals is not without risk thus the NMFS permit office issues only a few permits to capture seals and obtain biological samples. To minimize stress and repeated harassment of seals, it is imperative to maximize collaboration and sharing of biological samples; working collectively to achieve the independent and mutual goals of all the investigators involved. The quality of science is greatly improved by collaboration, bringing investigators with different expertise together to more fully address hypotheses.

**Methods:** ADF&G arranges vessel charters and the live-capture and biological sampling of seals, generally under the ADF&G permit. ADF&G invites collaborators to participate in capture trips, often sharing expenses associated with the fieldwork. ADF&G distributes samples among researchers and arrangements are made for data sharing and cost-sharing for some analyses.

**Five-year project status:** ADF&G is working closely with ASLC, conducting fieldwork in Prince William Sound in 2003 for a vital rates study (ADF&G) and a study of contaminants (ASLC), endocrinology (ASLC), and immunocompetence (ASLC). ADF&G also has initiated collaboration with researchers at the University of Alaska Fairbanks (UAF) and the University of Alaska Anchorage (UAA), providing blood samples, and blubber and muscle biopsies for Masters studies currently underway at each university. In 2002 ADF&G also initiated collaborative research in Kodiak conducting a pilot season for the vital rates study as well as obtaining samples for ASLC, and initiating a study conducted by a UAF student in conjunction with the Gulf Apex Predator project in Kodiak. Funded for FY03 at reduced levels, no funding for FY04 and beyond.

**Project lead:** ADFG

**D. 5 Disease screen**

**Objective:** Determine baseline exposure to disease in Alaska harbor seals.

**Justification:** The presence of disease in harbor seals could potentially have a significant impact on survival and reproduction, and thus population status. Few published data are currently available on disease exposure and occurrence in Alaska harbor seals. Studies indicate that harbor seals have been exposed to phocid herpesvirus, phocine distemper virus, *Brucella* spp., *Toxoplasma gondii*, and *Clamydia psittaci* (Sheffield *et al*. 1997). Although stranded animals are tested for some disease agents, a comprehensive study designed to determine the prevalence of disease in Alaska harbor seals has not been completed.

**Methods**: During 1978-1999, ADF&G obtained more than 450 sera from harbor seals collected and captured in the Bering Sea, the Kodiak Archipelago, PWS, and SE Alaska. These sera were collected to determine the antibody prevalence of selected microbial disease agents. A preliminary summary of the analyses to determine antibody prevalence was reported by Sheffield *et al.* (1997). Additional results have since been made available, and samples are currently being analyzed for phocid herpesvirus and phocine distemper virus. Once results are available from these most recent tests, retired ADF&G disease specialist (R. Zarnke) will complete his interpretation of all results to date and then work in cooperation with ADF&G harbor seal biologists to complete a manuscript for publication. Alternatively, the recently hired ADF&G state veterinarian (K. Beckmen) may assume responsibility for completing the manuscript. Additional sera samples, from all possible sources, will be collected and archived for future analyses.

**Product**: A peer-reviewed manuscript published in the scientific literature that documents evidence of Alaska harbor seal exposure to eight disease agents: canine distemper virus, phocine distemper virus, phocid herpesvirus 1, *Toxoplasma gondii,* influenza A, *Brucella* spp., *Chlamydia psittaci,* and caliciviruses. Subsequent reports providing current evidence of exposure to disease agents.

**Five-year project status**: Analyses of all sera samples collected during 1978-1999 completed in 2001 and reported in an annual report (Zarnke et al. 2001). Analysis of additional sera samples collected after 1999 conducted on an as-needed basis. Unfunded FY03 and beyond.

**Project lead**: ADF&G

**D.6 Blood chemistry**

**Objective**: Use blood parameters as a potential indicator of health status and develop statistical criteria to detect perturbations in blood parameters in populations of harbor seals.

**Justification:** Previous research on declining numbers of pinnipeds in Alaska waters elucidated the potential for detecting environmental perturbations using blood chemistry or blood proteins (Fadely et al. 1997, Zenteno-Savin et al. 1997). Most current hypotheses concerning the decline in the Alaska harbor seal population include a lower nutritional prey base. Recent studies that compared physiological and pathological parameters between stable and decreasing adult harbor seal populations in Alaska have shown some correlation between individual seal's health condition and changes in prey availability (Fadely and Castellini 1996). To determine an adult harbor seal health index using blood chemistry and morphometrics, Fadely et al. (1998) developed a method to interpret potential population "outliers" in adult harbor seals between areas of concern. To expand this concept, the health status in harbor seal pups will be examined by documenting blood parameter differences, along with morphometric measurements, in an area of chronic decline (Prince William Sound) and an area with slightly increasing numbers (Tugidak Is). It is well documented that there is a great amount of maternal transfer in harbor seals, thus using pups would provide important information on the health of pups and breeding females. Statistical methods will enable detection of developmental changes and potential "outliers" and also determination of clinical trends on the population level (Trumble et al. 1999). It must be stressed that these techniques will not be used to clinically diagnose individual harbor seals as unhealthy, but rather used to detect possible health trends in populations based on blood chemistry perturbations.

**Methods**: Develop specific baseline blood chemistry and hematology reference ranges, to be used as a health indicator, for areas of concern (i.e. declining populations) in Alaska waters. Blood from harbor seals will be drawn into various Vacutainer blood container tubes (heparinized, EDTA, and serum). Whole blood will be centrifuged and the plasma separated and frozen at -80C. Blood smears made from EDTA tubes will be used for differential counting. Approximately 1 mL heparinized plasma will be used for determination of standard plasma chemistries: sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphate, cholesterol, glucose, protein, blood urea nitrogen (BUN), albumin, creatinine, globulin, bilirubin, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), gammaglobulin transferase (GGT), and alkaline phosphatase (AP).

Novel statistical methods will be applied to determine potential health outliers in populations of harbor seals. These models are new applications of outlier theory, principle component analysis and survivorship statistics to marine mammal management issues. They will build on the work of Fadely et al (1999) with harbor seals, Bowyer et al. (1999) with river otters and Wells et al. (in development) with dolphins. (This work is the primary focus of the Ph.D. thesis of Steve Trumble).

**Product**: Reference ranges for blood chemistry parameters in spatially distinct harbor seal populations in Alaska waters. Statistically sensitive methods for determining environmental or human based perturbations in blood chemistry and hematology values, which would subsequently be used as a potential health indicator on the population level.

**Five-year project status**: Steve Trumble successfully defended his thesis in May 2003 and has a paper in press reporting on blood parameters for harbor seals. Three years' baseline data (FY00-02) were collected to establish meaningful blood chemistry parameters but no analyses have been done on those samples. Renewed collaboration between ADF&G and UAF was established in 2002-03 and a masters student will follow through with comparisons of blood samples collected in PWS from 2000 through 2003 with reference ranges established by Fadely and work done by Trumble. Project is expected to be completed in 2004-05. Subsequently blood parameters will be collected biannually in areas of concern. Partially funded in FY03 (sample acquisition but no funding for analyses).

**Project lead**: ADF&G/UAF

**D.7 Sample analysis**
Information unavailable at this time.

**Project Lead:** ANHSC

**D.8 Contaminant sources**
Information unavailable at this time.

**Project Lead:** ANHSC

## E. FOOD HABITS

**Overview**
Although harbor seals are known to eat a variety of fish and invertebrates (Imler and Sarber 1947, Wilke 1957, Pitcher 1980a), a complete understanding of diets throughout Alaska is lacking. Diet varies seasonally, regionally, and probably annually (Imler and Sarber 1947, Pitcher 1980a), but data on these variations are largely incomplete (Hoover-Miller 1994). The most comprehensive food habits study was conducted in the Gulf of Alaska where 548 seals were collected (269 of which had food remains in the stomach) during the 1970s (Pitcher 1980a), the largest number of samples came from the Kodiak area (n=102) and Prince William Sound (n=83). Overall in the Gulf of Alaska, the five top-ranked prey were walleye pollock, octopus, capelin, eulachon, and Pacific herring (Pitcher 1980a). The current studies listed below include complementary analyses of diet from scat and stomach contents and from fatty acid profiles extracted from blubber samples.

**E. 1. Food item analysis**

**Objective:** Determine temporal and spatial dietary prey composition for harbor seals in Alaska, through analysis of stomach and scat contents.

**Justification:** The food limitation hypothesis (expressed as reduced availability of prey or differences in prey quality and composition) is one of several that are being investigated to elucidate ultimate cause(s) of a decline in harbor seals numbers over the last 20 years. Sporadic diet composition studies have been conducted since the mid 1940s (e.g., Imler and Sarber 1947, Wilke 1957, Pitcher 1980a). Current and comprehensive diet data, however, are lacking and for some regions, non-existent. Investigation of harbor seal diet in regions of stable or increasing populations (Southeast Alaska) and of depressed populations (Prince William Sound, the Kodiak Archipelago, and northern Bristol Bay) would allow for comparisons among areas of differing population response as well as some comparisons with historical diet data. Seasonal comparisons of primary prey are limited. Prey analysis can be accomplished through examination of scats and stomachs, which yield information on recent dietary intake.

**Methods:** Scats will be obtained from harbor seal haulout sites and stomachs will be collected from subsistence-harvested animals. Scat will be collected in Southeast Alaska, the Kodiak archipelago, northern Bristol Bay and the north shore of the Alaska Peninsula. Scats will be collected from each of the winter, spring, late summer/fall seasons. Stomach samples were collected opportunistically from subsistence harvested animals beginning in 1995. In 1997, efforts increased to work with hunters in Southeast Alaska to obtain stomach samples. Stomach sample collection will be expanded to other areas of the state, principally the north shore of the Alaska Peninsula. Subsistence harvested animals are typically taken in the fall through the spring in most parts of the state; therefore, stomach samples will be representative of diet composition during this time period. Temporal analyses will also be conducted comparing stomach contents from recently collected samples with historical samples from the 1970's. Samples will be sent to Pacific Identifications in Victoria, B.C., Canada for prey identification.

**Product:** Manuscript(s) published in the peer-reviewed literature that describe the spatial and temporal diet of harbor seals in Alaska, and compare primary prey in the central-western Gulf of Alaska between the 1970s and the 1990s.

Sample collections will continue, with collection efforts focusing on geographic areas and/or seasons where data are limited. Preliminary analyses of the percent occurrence of the primary prey species and of prey "categories" were estimated in summer 2000 and reported in an annual report (Jemison 2001). Final analyses will use split sample frequency of occurrence to measure the importance of different prey categories (following Olesiuk et al. 1990 and Merrick et al. 1997) and will begin in summer 2003 in collaboration with Dr. Andrew Trites (Jemison et al. in prep). In collaboration with UAF and the Gulf Apex Predator (GAP) project, approximately 200 stomachs collected from seals that were subsistence harvested in 2000-01 are currently being prepared for analysis of prey remains. Stomach contents will be washed and prey remains collected for submission to a laboratory for analysis of prey content. Costs of sample preparation and laboratory analysis will be shared in a collaborative effort between Kate Wynne (GAP) and ADF&G. Plans were discussed for a future manuscript in which data from prey remains in seal feces collected in Kodiak would be compared with stomach contents of seals harvested in that area. A similar comparison will be made between stomach contents and analysis of fatty acid signatures of seals sampled in PWS. For all areas for which we have matched samples, data from prey remains in seal stomachs will be matched with age data from the same seal (obtained from counts of cementum annuli of teeth) to evaluate age-specific variation in diet as well as regional differences in diet. Preparation of stomach contents for analysis should be completed and samples should be submitted to the laboratory for identification of prey remains by fall 2003. Partially funded FY02 for stomach sample analyses. Partial funding for scat analyses FY03.

## E. 2. Fatty acid analysis

**Objective**: Determine temporal and spatial prey composition in harbor seal diets through analysis of blubber fatty acid signatures.

**Justification:** Studies to elucidate the suite of prey species consumed by pinnipeds have focused, to a large degree, on scat and stomach content analysis. These techniques can yield useful information on recent dietary intake and on the types of prey that are consumed. However, information from these samples may be limited in that stomach and scat contents may not provide a comprehensive profile of prey consumed. Prey is digested differentially, and some diagnostic hard parts may be retained in the stomach and thus over-represented in stomach contents (Pitcher 1980b, Harvey 1989). Also, stomach and fecal material likely only reflect the contents of a recent meal from a particular area and may not represent the temporal or spatial variation of foraging efforts and prey consumption. Recent techniques of fatty acid signature analysis have been applied as an additional, and complementary, method of studying the diet composition of harbor seals (Iverson *et al*. 1997). Fatty acid signatures in prey species have been shown to be reflected in the lipid profile of higher trophic level predators. Core samples of blubber from harbor seals may yield more detailed information on diet that might not be obtained from other methods. In addition, studies in the Gulf of Alaska (Iverson and Frost 1997) indicate that seals can be characterized to area and age class based on blubber fatty acid signatures. An understanding of shifts in diet composition over time and space and in specific cohorts may contribute to an understanding of area-specific population declines.

**Methods**: Blubber samples have been collected from harbor seals live-captured in Prince William Sound, the Kodiak region and Southeast Alaska. Additional blubber samples are obtained through the biological sampling program. Prey samples will also be collected to obtain prey fatty acid profiles for comparison to fatty acid profiles found in harbor seal blubber. Blubber samples will be processed and fatty acid signature analyses will be conducted in the laboratory of Sara Iverson, Dalhousie University, Canada or in a laboratory in Anchorage, Alaska.

**Product**: Manuscripts published in the peer-reviewed literature describing geographic differences in the diet of seals based on fatty acid signature analyses. This work complements and enhances the work that has been conducted in Prince William Sound (Iverson et al. 1997); Sara Iverson and Kathy Frost are currently collaborating on this manuscript. A manuscript or report comparing stomach content analyses and fatty acid signature analyses as methods to determine the diet of harbor seals is also being pursued by the Iverson laboratory and a similar report or manuscript will be prepared by Blundell and Wynne comparing results from prey remains in stomachs and feces in Kodiak, with results of fatty acid signatures vs. stomach contents in PWS for samples collected in 2000-2003.

**Five-year project status**: Blubber sample collection by subsistence hunters and during seal captures are ongoing. A prey collection program is underway in Southeast and is expanding to the Kodiak area to obtain prey samples for comparison with the prey library developed for PWS. Fatty acid analyses of harbor seal blubber and prey samples from Southeast and Kodiak are conducted as time and funding allows. Blubber samples are collected from seals live-captured through out Alaska and archived pending funding for analyses. A collaborative study between ADF&G and UAA will compare PWS data on seasonal changes in histochemistry and biochemistry of muscle fiber with data on diet (from fatty acid signatures) and condition (from deuterium oxide [D2O] samples assessing total body fat).

Costs of fatty acid analysis and D20 analyses will be split between UAA and ADF&G; both are currently unfunded for this project. Unfunded FY03 and beyond.

**Project lead**: ADF&G

## E.3. Stable Isotope analysis

**Objective**: Determine temporal and spatial prey composition in harbor seal diets through analysis of stable isotope signatures of hair.

**Justification:** Stable isotope analysis permits a rapid, inexpensive method ($26/sample) of determining diets of individuals, and would provide a comparison with fatty acid analyses of diet. Additionally, that comparison may afford a seasonal assessment of diets for an individual from samples obtained in a single sampling event, depending upon when blubber samples were obtained. Stable isotopic analysis of hair samples reflects the diet consumed by the individual during the growth period for the hair. Although stable isotope analysis generally cannot distinguish between prey species that forage at the same trophic level, $\delta^{13}$ carbon signatures can distinguish between prey groups. For example one can determine whether diets of harbor seals are composed primarily of forage fishes (e.g., sand lance, herring, capelin, and salmon) or of intertidal and demersal fishes such as pollock (Blundell et al. 2002). Mixing models are also sometimes used to determine the relative proportion of various prey groups in the diet of individuals with stable isotopic ratios (Ben-David 1997).

**Methods:** Hair samples (20-30 hairs) will be collected from all live-captures and subsistence harvested seals and stored in whirlpak bags. Samples will be submitted to the mass spectrometer lab at University of Alaska Fairbanks for stable isotope analysis. Additionally, samples of prey for which stable isotope signatures have not been established in the study areas (e.g., octopus) will need to be submitted to the laboratory for determination of stable isotope signatures.

**Product:** A manuscript or report comparing stable isotope analysis with results from analysis of fatty acid signatures and stomach contents.

**Five-year project status:** Hair and whisker samples are collected opportunistically from all seals live-captured in Alaska under the ADF&G permit. No prey items for which we do not have area-specific stable isotope signatures (e.g., octopus) have been collected in any area for stable isotope analysis. Partially funded FY03.

## F. LIFE HISTORY

### Overview

Information on some aspects of harbor seal life history in Alaska is limited. In particular, detailed data on the timing of pupping and molting are not available from some areas of the state. Additionally, in Southeast Alaska, the location of major terrestrial pupping sites is not well documented. Knowledge of critical habitat areas (such as large pupping sites) as well as pupping phenology is necessary to track changes in the timing and/or location of parturition and to establish a site in Southeast where disturbances can be monitored during this critical period.

Harbor seals undergo an annual cycle of regeneration and shedding, or molting, of hair. Shedding generally occurs after the pupping and mating periods in adults (Scheffer and Slipp 1944, Stutz 1967, Ling 1972) but prior to implantation of the embryo in females (Bishop 1967, Boulva and McLaren 1979). Typically, population trends and size estimates in Alaska are based on aerial surveys conducted during the molting period when presumably the largest number of seals are hauled out. Data on both regional and sex/age differences in the timing of molting should be considered when determining optimal survey periods. The following studies will provide important information on critical pupping sites in Southeast Alaska as well as detailed information on pupping and molting phenology.

### F.1. Pupping and molting phenology

**Objective:** Determine timing of pupping and molting in different regions of the state by detailed studies at representative haulout sites.

**Justification:** Harbor seal numbers declined about 90% on Tugidak Island from the mid-1970 through the early 1990s (Pitcher 1990, Small *et al.* 1998). By monitoring pupping and molting phenology on Tugidak Island we have documented important changes in the timing of these annual events between the 1970s and the 1990s. The timing of pupping was 7–18 days later in the 1970s than in the 1960s and 1990s, suggesting that females may have had difficulty obtaining adequate food resources during the period of decline (Jemison and Kelly 1997). The date of the maximal count during the molting period on Tugidak Island was 11 – 33 days later in the 1970s than during 1997 – 1999 (Jemison unpub. data). The timing of the molt also varied by sex and age class and that the peak count for each sex/age class corresponded to the early stages of active molting.

Counts of harbor seals conducted during aerial surveys are used to estimate population trends and abundance; ideally, these surveys are conducted during a peak in the molting period, when the largest number of seals are on shore. The precise timing of molting, however, is not well known throughout Alaska, although there is evidence that it does vary among regions. For example, the maximal number of seals ashore during the molting period in 1998 occurred on 2 August on Tugidak Island and on 2 September in Nanvak Bay (northern Bristol Bay) (Jemison unpub. data). Abundance surveys not conducted at a similar stage of the molt among regions may not be directly comparable. Additionally, evidence from Tugidak Island suggests that the timing of the molting period can change across years. Such a change in the timing of molting could confound comparisons of abundance estimates, and increase the variation associated with trend estimates. Trend analyses of aerial counts of seals have found that certain environmental covariates (e.g., date, time of day) significantly affect counts (Small *et al.* 1998). Incorporating these covariates in the analysis reduces the variation in the trend estimate.

Land-based studies conducted during the molting period at trend or index sites in different regions of the state will provide detailed information on the timing of the molt, identifying which sex/age classes are most abundant during surveys and helping to determine the best survey window. Regional information on the timing of pupping will document shifts during this critical time period.

**Methods**: Baseline monitoring of pupping and molting phenology of harbor seals on Tugidak Island will continue during late May – June (pupping) and late July – August (molting). When additional sites are found in Southeast Alaska and possibly in Bristol Bay, these sites will be monitored following the protocol, described below, that was established at Tugidak Island.

Observations will be made from 30 m bluffs overlooking the haulout sites. Surveys, using a 15 - 60 spotting scope and binoculars, will be conducted daily during the pupping period and 1-2 days per week during the molting period. Seals will be categorized according to sex and age class. Sex is determined by examination of the ventral side of the animal, when exposed, and by association with a pup. Those animals for which neither approach is available will be noted as "sex unknown." Age-class will be assigned as pup, yearlings, subadults, and adults. During the molting period, each seal will additionally be categorized by molt stage (see Jemison *et al.* 1998).

If and when a new site(s) is found, the methods will follow as closely as possible to those developed at Tugidak Island, although the molting phenology data will need to be collected more frequently (3-4 times per week). At a new site, the use of time-lapse photography to extend observations across a broader time period will also be evaluated.

**Product**: Detailed information on the timing of pupping and molting in several regions of Alaska will be ascertained, with the ability to detect annual shifts in these life history events over time.

**Five-year project status**: Due to reduction in funding and shortage of ADF&G harbor seal personnel, the 2003 pupping season was cancelled at Tugidak but a field season during the molt is scheduled to take place. Funded at reduced levels FY03.

**Project lead**: ADF&G and NMML

**<u>F.2. Distribution of Cook Inlet harbor seals in relation to key life history events</u>**

**Objective**: This project's main objectives are to: 1) assess the abundance and distribution of harbor seals throughout Cook Inlet, 2) identify and document the harbor seal haul-out sites that are most important for the key life history events of pupping (June) and molting (August), and 3) to compare the pupping and molting situations to other periods (October and March) when these important life history events do not constrain the seals' behavior.

**Justification**: Reliable estimates of harbor seal abundance are needed to develop sound plans for conservation, management, and response to environmental impacts. There are two seasonal peaks in the numbers of harbor seals hauled out in Alaska, one during May - June associated with pupping, and the other during July - September associated with molting (Ashwell-Erickson *et al*. 1986, Jemison and Kelly 2001). In Alaska, aerial surveys have generally been conducted during the molting period when the number of seals hauled out was thought to be highest and the weather conditions were likely to be favorable for flying. Patterns of abundance at other times of year are not well known except at a few selected sites, such as Tugidak Island, where long-term, land-based studies have been conducted.

This study will use aerial surveys to provide information about the numbers and distribution of harbor seals found ashore in Cook Inlet at key times in the seals' life history. At present, surveys in Cook Inlet are scheduled only once every five years during the seals' molt period (August); the most recent surveys were conducted by the National Marine Mammal Laboratory in August of 1996 and 2001. To provide a more comprehensive picture for assessment of potential risks to harbor seals from human activities in Cook Inlet, we will supplement what is known about seals in the molt period with surveys conducted during pupping (June) and periods when seals are not as constrained by major life history events (such as October and March).

**Methods**: All surveys will be conducted following an established protocol used successfully for harbor seals throughout Alaska (*e.g.*, Task A.1; Frost *et al*. 1999, Boveng *et al*. 2003). Six to eight repetitive counts on separate days are planned for each major haul-out site within the Cook Inlet study area during each 2-week survey period in June, August, October and March. Harbor seals will be photographed with digital or 35 mm cameras equipped with a 70-210 mm lens, and counted from the digital or film images. The counts will be adjusted for covariate conditions (date, time of day, tide height, weather) to increase the precision of seasonal comparisons between pupping, molting, and winter situations.

**Product**: Detailed seasonal data on abundance and distribution of seals in Cook Inlet, a major site of petroleum leasing and development.

**Five-year project status**: Surveys will commence in June, 2003 and end in August 2004. Analysis and reporting will be complete by end of FY05. Funded from non-NOAA sources, FY03-FY05.

**Project lead**: NMML

**F.3. Seasonal variability of haulout patterns in Cook Inlet**

**Objective**: The primary objective of this project is to identify factors that impact the haul-out behavior of harbor seals at various sites in Cook Inlet. Specific questions to be addressed are:

1) How do changes in specific covariates (weather conditions, tidal height, time of day, date in seasonal life-history cycle) impact the haul-out behavior of harbor seals, and what is the combined impact of all the covariates taken together? 2) What is the relative importance of the various covariates on the haul-out behavior of harbor seals? 3) Does the relative importance or impact of specific covariates on harbor seal haul-out behavior change over the course of the seals' seasonal life-history cycle? For example, are seals more, or less, sensitive to changes in weather conditions during the molting season (August - September) than during other times of the year?

**Justification:** Harbor seals are particularly vulnerable to acute and chronic environmental impacts, such as those that could result from industrial accidents such as oil spills (Frost *et al.* 1994a, Frost *et al*. 1994b, Spraker *et al*. 1994, Hoover-Miller *et al.* 2001). If an oil spill were to occur, harbor seals would be at risk for direct exposure to oil both at sea and ashore when they haul out (Lowry *et al.* 1994). Although at-sea locations of seals can be quite hard to predict, harbor seals tend to haul out at specific sites, which can be identified via aerial surveys. The number of harbor seals that haul out at various sites, and would thus be at risk if the site was oiled, varies considerably through time and between sites (*e.g.*, Huber *et al.* 2001).

Much of this variability in haul-out numbers may be explained by life history and environmental factors that alter the haul-out behavior of seals (Watts 1996, Frost *et al*. 1999, Boveng *et al*. 2003). The number of harbor seals hauled out varies seasonally, generally peaking during pupping and molting seasons (*e.g.*, Brown and Mate 1983, Calambokidis *et al*. 1987, Jemison and Kelly 2001). This seasonal effect can be quite dramatic over short time periods. For example, the number of seals hauled out can decrease by 85% in the last three weeks of the molt season (Mathews and Kelly 1996).

**Methods**: We propose to assess harbor seal haul-out behavior year-round using time-lapse photography collected by autonomous camera systems deployed at haul-out sites in Cook Inlet. These units will record digital images of haul-out sites throughout the year, allowing us to count the number of seals hauled out at the monitored sites under all tidal and weather conditions throughout the year. These data will provide the basis for analyses of the relationships between haul-out patterns and the factors that affect the numbers of seals ashore, such as date, tide height, time of day, and weather. Our basic approach will be to utilize autonomous camera systems (digital camera, data logger, solar panel and/or wind generator, and external battery, housed in a weather-proof case) to collect images at particular haul-out sites (*e.g*., one image every hour during daylight hours). Camera systems will be deployed at three important haul-out sites in Cook Inlet. A weather station and up to three camera systems will be deployed at each haul-out site to improve coverage of haul-out habitats.

The resulting images will allow us to relate the haul-out patterns of harbor seals to relevant covariates, such as weather conditions (via data from the weather station deployed at each site), tidal height, time of day, and date in the seals' seasonal life-history cycle. The number of seals hauled out in each image will be counted. We will utilize a regression approach, similar to that used in other recent studies of haul-out behavior (*e.g*., Boveng *et al.* 2003, Simpkins *et al.* in review), to describe the relationships between these covariates and haul-out patterns.

**Product**: The use of time-lapse photography will provide reliable, accurate data; the digital images provide a permanent record, and the number of seals can be counted by independent observers to verify the results. Time-lapse photography will also provide information on seasonal haul-out patterns at fine temporal scales (*e.g.*, hourly data) more cost-efficiently than other methods. The results of the covariate regression models should be valuable components for assessment models of the number of seals at risk from potential industrial accidents such as oil spills.

**Five-year project status**: Camera system development and testing will start in 2003, with camera development expected to start in 2004 and conclude in 2005. Funded from non-NOAA sources FY03-FY05.

**Project lead**: NMML

**F.4. Pupping colonies in SE Alaska**

**Objective:** Locate large pupping colonies of harbor seals in Southeast Alaska.

**Justification:** Whereas large numbers of harbor seals are known to pup on glacial ice at several tidewater glacial fjords in Southeast Alaska, including Johns Hopkins Inlet in Glacier Bay and in Tracy and Endicott Arms, information on the location of other large pupping sites and the timing of pupping in Southeast is limited. It is important to obtain information such as the location of large (i.e, 50 or more pups) pupping sites, and where possible, document when pupping occurs.

**Methods**: Distribution data, which have largely been collected during the molting period, and local knowledge, will be examined for locations of large concentrations of harbor seals. Selected sites will be investigated either by boat or aerial survey to determine whether pups are present. Based on current information, surveys should ideally be conducted from approximately 20 May through 10 June in northern Southeast Alaska.

**Product:** Information on the location of large pupping sites in Southeast Alaska, and when possible, documentation on the timing of pupping at these sites.

**Five-year project status**: Ongoing during May and June of 2003-2004 depending on funding. In June 2003, the NOAA ship R.V. Cobb will be used to survey potential pupping sites in Southeast Alaska. Funded FY03.

**Project lead**: NMML

**F.5. Modeling Optimal Foraging Strategy For Seals Under Predation Risk in PWS** ("Effects of prey availability and predation risk on the foraging ecology and demography of harbor seals in Prince William Sound: development and test of a dynamic state variable model")

**Objective**: We will examine the contributions of prey availability and predation risk to the population dynamics of harbor seals (*Phoca vitulina*) in Prince William Sound. This work has great conservation value because seal numbers have been declining since 1984. Although available prey changed in the late 1970's, food limitation alone cannot explain the current population trajectory; body condition of pups is presently good, suggesting that mothers are not nutritionally stressed. Harbor seals are preyed on by transient killer whales (*Orcinus orca*) and may be under increasing risk from sleeper sharks (*Somniosus pacificus*). We hypothesize that these predators affect survival and reproduction through both direct mortality and sublethal costs in which seals compromise energy intake while avoiding predator encounters. We hypothesize further that individuals vary in their ability to balance energetic and antipredator demands, and only a subset of those that follow an optimal foraging strategy survive and reproduce. A proportion of the population might consist of poor foragers that initially compromise energy intake to avoid predation and—once energetically stressed—take larger risks to avoid starvation and consequently have greater predation rates. To test these hypotheses, we will model optimal foraging strategies that maximize lifetime reproductive output under different predator and food distributions, and field-test predictions in Prince William Sound. We will expand inferences by comparing our research with existing and ongoing studies in adjacent regions that have stable or increasing seal populations. Because seals and fisheries might compete for prey, computer simulations will explore how alternative fisheries scenarios might influence the foraging ecology and demography of seals.

Two specific hypotheses will be addressed:

1) Sleeper sharks and transient killer whales affect the survival and reproduction of seals not only through direct mortality, but also through sublethal costs due to seals compromising energy intake to reduce predation risk.

2) Individuals vary in their ability to balance energetic and antipredator demands, and only a subset of those that follow an optimal foraging strategy survive and reproduce. A proportion of the population might consist of poor foragers that initially compromise energy intake to avoid predation and—once energetically stressed—take larger risks to avoid starvation and consequently have greater predation rates.

**Justification:** Predation of seals is one of the hypotheses posited to explain the continued decline of some seal populations in Alaska. Testing hypotheses of predation in aquatic species is extremely difficult thus the role of predation in harbor seal population dynamics remains largely unexplored. We will use a novel approach, with a combination of modeling and field tests of model predictions. A basic premise of this work is that predators may affect prey not only through death or wounds to the captured, but also through the sublethal costs of antipredator behavior to those who managed danger (e.g. Abramsky et al. 2002; Heithaus et al. 2002). Sublethal costs include increased energy expenditure or lower rates of energy gain associated with vigilance, fleeing, or avoidance of habitats where predator encounters are likely. To optimize costs and benefits, investment in antipredator behavior should track changes in perceived risk. Thus, for a given food density, energy intake rates are lowest when perceived risk is highest. The corollary is that abundant and high quality resources may become functionally scarce or unavailable if associated with high risk (reviewed in Frid & Dill 2002; Heithaus & Dill 2002). If perceived risk is sufficiently high and long term, the energetic costs of antipredator behavior could impact body condition and reduce survival and reproductive output. Furthermore, animals in poor condition may experience greater predation rates when trying to avoid starvation by taking greater risks to find additional food (Sinclair & Arcese 1995 ). Thus, predators may affect the population dynamics of prey more strongly through interactions between sublethal costs of perceived risk and direct mortality than by direct mortality alone (Brown et al. 1999; Peacor & Werner 2001; Frid & Dill 2002).

**Methods:** Dynamic State Variable (DSV) models of optimal foraging assume that fitness (lifetime reproductive success) is a function of energy level discounted by mortality rate. Based on this function, they predict behavior from an initial period *t* to a terminal time horizon *T* (Clark & Mangel 2000). In the DSV application proposed #1, *T* represents the end of a foraging bout (series of dive cycles). Time, depth, oxygen stores, and energy level are the state variables, and prey availability and predation risk are the factors varying with depth. The optimal policy tells the animal whether to dive deeper, remain at the current depth, or surface at each point during a dive; it also tells the animal when to leave the surface to initiate the next dive. The optimal policy is derived by backward induction such that fitness is maximized at the end of the foraging bout. A policy matrix is defined by the state variables, and assumes probability distributions for prey availability and risk. It is stochastic variation in prey availability and risk that causes individuals to differ in fitness during simulations. Season and age of the individual are additional variables defining the matrix. The models incorporate spatial data from NMFS studies of sleeper sharks conducted by Lee Hulbert and available data on orca distribution and foraging behavior, along with data on prey availability in PWS.

To relate behavior to reproductive output and population dynamics (#2), the fate of a population of seals of a specified age following the optimal policy is simulated from the onset of the first foraging bout of the season until pupping time. For the population of seals (e.g., n = 1000) with a given fitness at *t*, multiple foraging bouts are modeled sequentially such that fitness at the end of a bout becomes the initial fitness for the next. At pupping time, the model estimates the proportion of individuals that survived (i.e., avoided predation and did not starve), and their likelihood of producing offspring based on accumulated energy level. These simulations provide the basic life table information from which population trajectories can be predicted (see Clark & Mangel 2000).

**Product**: Two products will be provided

1) A Dynamic State Variable (DSV) model predicting optimal diving by seals during foraging bouts under different predator and food distributions.

2) A second and expanded DSV model that builds on #1 to predict survival and reproductive rates and population dynamics of seals in relation to changing prey and predation risk.

**Five-year project status:** An initial, simplified model was developed in spring 2003, funded by a cooperative agreement between ADF&G and Simon Fraser University to provide a student stipend to Alejandro Frid, Ph.D. candidate, supervised by Dr. Lawrence Dill. The model will be further refined and predictions from the model will be field tested in fieldwork conducted in PWS beginning in July 2003 through March-April 2004, funded in FY03 (NPRB grant). Following the two seasons of fieldwork, the model will be further refined and used to predict population level consequences of particular foraging decisions under predation risk.

**Project lead**: ADF&G in collaboration with Simon Fraser University

**F.6. Movements and Haulout Behavior**

**Objective:** Document the haulout, movement, diving, and spatial-use patterns of Alaska harbor seals.

**Justification**: Knowledge of diving behavior and the spatial and temporal patterns between at-sea foraging areas and haulout sites will provide a better understanding of several basic life history characteristics of harbor seals, including foraging ecology, general movement and haulout use patterns. This knowledge is required to assess the possible impact of various perturbations on harbor seal populations.

**Methods:** Satellite-linked time depth-recorders (SDRs) deployed on harbor seals provide estimates of seals' locations and whether they are hauled out or at sea. These data can be summarized to provide numerous indices relative to general movements (e.g., mean distance traveled between haulouts, maximal distance between any two haulouts, mean distance to at-sea locations, size of at-sea 'foraging' areas, etc.) and haulout behavior (e.g., number of haulout sites used, haulout site fidelity, haulout timing and duration, etc.). Concurrently, information on the diving behavior (e.g., number of dives, maximal depth of any dive, proportion of time at-depth, etc.) of seals is collected continuously and summarized in 6 hour 'bins'. Over 100 adult and subadult seals were tagged with SDRs in Kodiak, PWS, and SE during 1993-1996.

Over 100 adult and subadult seals were tagged with SDRs in Kodiak, PWS, and SE during 1993-1996, 53 pups were tagged at Tugidak Island and in PWS during 1997-1999, and 20 non-pups were tagged in Bristol Bay in 2000 and 2001.

Additionally, ADF&G deployed Time Depth Recorders (TDRs) on seals in Alaska in SE Alaska in 2002 in a collaborative study between ADF&G and NMML, and in Kodiak (2002-03) as part of a recently established collaboration with UAF and the Gulf Apex Predator (GAP) project. TDRs can be programmed to record data every 5 seconds, if desired, thus providing detailed data (e.g. depth, time, temperature, light, and salt resistance) not only on individual dive profiles but also on exact timing and duration of haul-out behavior as compared with SDR data, which provides histograms of 6-hour time bins. TDRs do not provide location data, however, and must be used in combination with other telemetry devices if locational data are desired. In SE Alaska and in Kodiak, seals outfitted with TDRs also received VHF transmitters. Seals with TDR/VHF equipment were radio tracked from boats as well as from land-based data computer collection (DCC) systems. TDRs are data loggers and must be recovered once they fall off the seal during the annual molt. All 15 TDRs were recovered when deployed in SE Alaska in 2002 and those units are currently deployed on 15 seals in Kodiak. TDR data on timing of haulout behavior and matching location data specific to the haul out times (obtained from

VHF data) will be analyzed to assess fine-scale timing of haul out behavior and location of haul outs relative to other factors in the areas of deployment.

**Product**: Manuscripts published in the peer-review literature that describe the general movement and haulout patterns, and diving behavior of harbor seals in Alaska. Results will be pertinent to genetic research designed to identify stock boundaries, and elucidate potential interactions with commercial fisheries.

**Five-year project status:** During 1997-1999, 25 pups were tagged with SDRs on Tugidak Island and a similar number in PWS; data acquisition from these SDRs was completed in August 2000, with analyses conducted in winter 2000-2001. Adult and subadult seals were captured and tagged with SDRs in Bristol Bay in fall 2000. NMML has summarized findings from a study of glacial fjord haulout patterns and habitat use conducted in Tracy Arm during spring 1999. TDRs were recovered from 7 of 9 seals and an analysis of diving behavior will be conducted winter 2000/2001 in collaboration with the Canadian Department of Fisheries and Oceans. In August 2000, NMML deployed VHF tags and a remote receiving station in Nanvak Bay (Cape Newenham, NW Bristol Bay) to measure the haulout behavior of molting seals using sandy substrate; subsequent aerial surveys will assess, on a broad scale, any regional movement that may occur.

Several manuscripts describing movements and dive behavior of pups, adult and subadult harbor seals and comparisons among sex- and age-classes and between geographic areas are in various stages of preparation. Internal review and revision of a manuscript on adult and subadult diving behavior is near completion (Hastings et al. *in prep*), and the manuscript should be submitted for primary publication in summer 2003. Final analyses are completed on a manuscript assessing pup movements and a draft manuscript is nearing completion (Small et al. *in prep*), analyses of movements of adult and subadult seals (Small and Ver Hoef) are currently underway, and analyses of pup diving behavior (Frost et al.) are nearly complete. Data collection for seals tagged in Bristol Bay in 2000 and 2001 was completed in 2002; these data will be tabulated and analysis will commence in 2003-04. Partially funded FY02, unfunded FY03.

With the implementation of the vital rates study in PWS, utilizing subcutaneously implanted VHF transmitters that are duty cycled to transmit signals for 5 years, long-term movements of individual seals can be assessed. Those data will be compared with short-term data available from telemetry equipment attached to the pelage that is lost during the annual molt.

**Project lead:** ADF&G

**<u>F.7. Biosampling</u>**
Information unavailable at this time

**Project Lead:** ANHSC

## F.8. Populations Of Concerns – Patterns Of Continuing Decline

Assessing Patterns of decline in Glacier Bay

**Objective:** Conduct research in collaboration with the National Park Service and University of Alaska Southeast to evaluate factors that may be contributing to the precipitous decline of harbor seals in Glacier Bay.

**Justification:** Monitoring of seals has been conducted since 1992 in Glacier Bay with the main focus of research occurring in John Hopkins Inlet (JHI), as well as conducting aerial surveys to assess population trends for seals throughout that large bay. Seal numbers in that area have been declining since monitoring began, but recently the rate of decline has increased. In JHI seal numbers (excluding pups) were decreasing at a rate of 5.7%/year from 1992 to 1999. When survey data for 2000 and 2001 were incorporated the rate of decline increased to 9.6%/year. Similarly the aerial data for trend estimates for seals throughout the bay documented an alarming decrease of 14.5%/yr (1992-01).

Glacial areas may represent important pupping grounds, given the constantly available substrate -- ice bergs -- on which lactating seals and their pups can haul out on during the short 4-6 week lactation period. Availability of bergs as a haul out is not tidally influenced, thus seals have continuously available haul-out substrate and can allow pups to nurse at any time of day. Additionally, the array of icebergs in the water may reduce the efficiency of seal predators in locating their prey, either through echolocation (e.g., Orcas) or visual searching (e.g., sleeper sharks), although no formal test of this hypothesis has ever been conducted, to our knowledge. If glacial areas represent a safe haven for parturition and lactation, it is also possible that females from nearby areas may travel to glacial areas to raise their pups. Thus it is important to gain a greater understanding of factors that influence population dynamics in that area because the area could effectively function as a source population if it is also a birthing ground for non-resident females.

Although research has been conducted in Glacier Bay to monitor population trends and the effects of vessel traffic on seal behavior, additional research is needed in that area to evaluate other factors that may be contributing to the sharp decline of seals that has been documented in Glacier Bay. The relatively small area of Glacier Bay may also provide a unique opportunity to serve as a model system to evaluate effects of global warming on ecosystem processes, trophic interactions, and population dynamics.

**Methods:** VHF implants, duty-cycled to transmit signals for 5 years, would make it possible to monitor survival of individuals over an extended time period. Biological samples would be collected at the time of capture to assess health and condition of all individuals receiving long-term transmitters for later interpretation of what factors may contribute to differences in survival and reproductive success of individuals. Radio tagging females with long-term transmitters would also enable an assessment of whether lactating females captured in the bay are resident, or may have traveled from other areas to give birth in the bay (as indicated by some females leaving the bay once pups are weaned). Additionally, sonic gates may soon be installed at the narrow mouths of inlets in Glacier Bay to record the passage of sonic-tagged halibut. In collaboration with the National Marine Fisheries laboratory at Auke Bay, sleeper sharks and seals could also be sonic-tagged to record the passage of individuals, to determine whether sharks spend more time in areas where seal densities are higher.

**Product**: Annual reports and peer-reviewed manuscripts.

**Five-year project status**: Unfunded FY03

**Project lead:** ADF&G

## G. VITAL RATES

**Overview**
The vital rates of survival, maturation, reproduction, emigration, and immigration are the fundamental elements of the dynamics of a population. Estimates of the vital rates are the fundamental building blocks of demographic models and they can provide valuable diagnostic information about managed populations. These estimates are often difficult to obtain for wildlife populations, requiring long-term efforts and large samples of animals. The research described below to estimate some of the vital rates of harbor seals in Alaska attempts to satisfy the demands for large sample sizes by making efficient use of samples collected from the subsistence harvest and by developing new photographic techniques that will allow large numbers of seals to be individually identified and monitored.

### G.1 Reproductive Rates

**Objective**: Assess the temporal and regional reproductive rates of harbor seals using histological examination of female reproductive tracts from seals obtained through the biosampling program to determine if ages of sexual maturity and pregnancy or parturition rates have changed over time. In addition, the pregnancy status indicated by serum progesterone levels and ultrasound measurements of living female seals captured and released between December and May will be used to assess pregnancy status of living seals.

Regional differences in harbor seal population trends probably reflect varying environmental conditions over time. Breeding, pregnancy and successful production of healthy offspring depend upon hormonal controls. High progesterone levels after the implantation of the blastocyst are indicative of sustained pregnancy and can be used to reliably detect pregnancy several weeks after implantation (Grieg *et al*. 2001).

Most phocid seals are assumed to be monoestrous, and the ovaries of such seals produce one corpus luteum per year (Bigg and Fisher 1974, Noonan 1989). If harbor seals are monoestrous and the corpora lutea (CL) or albicantia (CA) remain visible, the number of CL and CA will indicate the years post puberty (Hayama *et al.* 1986) or the number of offspring produced in a lifetime (Ivashin 1984). However, Hawaiian monk seals appear to be polyestrous (Pietraszek and Atkinson 1994, Iwasa and Atkinson, 1996). The purpose of this study is to assess ovulatory cycles by examining the ovaries collected form harbor seal harvested by subsistence hunters. In collaboration with the Alaska Native Harbor Seal Commission's biosampling program we propose to assess the age of sexual maturity and conception or parturition rates in free-ranging harbor seals from Alaska. Analyses of the structures of corpora will be used to correlate with the reproductive histories or age (as determined by tooth sectioning) of these seals. Results will be contrasted with previous studies including Bishop (1967), Pitcher and Calkins (1979) for the Gulf of Alaska and Bigg (1969) for seals in British Columbia. Samples from Bishop (1969) and Bigg (1969) were taken from stable or increasing populations while those from Pitcher and Calkins (1979) were from seals experiencing precipitous population declines.

**Methods:**
*Histology*: Gross morphology including the shape, color, and weight of both ovaries from all seals will be examined at the time of excision. The ovaries will be sliced into sections 5-6 mm thick, and the corpora and follicles observed under a dissecting microscope. Histological processing will follow the methodology used by Iwasa and Atkinson (1996), and will be performed on the ovaries and sections of the vaginal epithelium. Any life history information will be collected. Teeth will be sectioned to determine age, which will be used to correlate with the gross morphology and histology.

*Serum progesterone*: Serum progesterone concentrations will be determined using solid phase radioimmunoassay (RIA) (Diagnostic Products Corporation, Los Angeles, CA), as previously described and validated for use in harbor seals in the ASLC endocrinology laboratory. Thawed, unextracted serum samples will be analyzed in batches to reduce inter-assay variation. Standard curves will be log-logit transformed, enabling extrapolation of sample concentration (Robard, 1974). A profile of the variation in progesterone will be generated, analyzed, and incorporated into a data base. Hormone concentrations will then be compared; incorporating morphometric and contaminant load data, and possible correlations between hormones, morphometrics, and pregnancy data will be investigated using multivariate analysis.

Assessments of endocrine levels from samples of serum and fecal materials from harbor seals sampled statewide will be contrasted with reference reproductive rates of seals in declining and increasing populations obtained through histological studies (e.g., Bishop 1967, Bigg 1969, Pitcher and Calkins 1979) and from samples obtained through the ANHSC biosampling study (see *Female Reproductive Biology* in this plan).

**Product**: The initial product will be a general survey of reproductive morphology and histology of harbor seals. As these initial samples will be from archives, the sample sizes will dictate the ability to perform statistical analyses. Reproductive histories, including age and parity will enable the morphological and histological data to be related back to population-level questions. This study is expected to develop a screening program where assessments of endocrine levels from statewide samples of serum and fecal materials from harbor seals will be contrasted with reference levels obtained through histological studies (e.g., Bishop 1967, Bigg 1969, Pitcher and Calkins 1979) and from samples obtained through the ANHSC biosampling program.

**Five-year project status**: A pilot season was completed successfully in October 2002 to validate efficacy of surgical procedures in wild-caught seals. Four seals underwent surgery to receive VHF implants and were released within hours of surgery after recovery from anesthesia. Limited funding in FY03 resulted in a scaled-back field season. Collaboration and cost sharing between ASLC and ADF&G will allow for 2 capture trips April and June/July 2003 in which we will attempt to deploy as many as possible of the 50 transmitters we are authorized to implant in 2003. During the April field season, 19 seals (10 females and 9 males) received VHF implants, and 13 of the 19 animals were relocated 2 weeks later during a short boat-based radio-tracking trip. All seals appeared to be in good health. Seals receiving implants in April also were equipped with VHF head mounts that will facilitate initial tracking as researchers learn how best to locate seals from weaker signals projected from VHF implants. Additional radiotracking via aerial, boat-based tracking and land-based data computer collection (DCC) systems will be conducted monthly if possible during summer/fall 2003. Radiotracking to assess seal survival and dispersal will continue for the duration of the VHF implant battery life as funding allows. During the May tracking trip, three DCC stations were established at haul out sites where seals were captured in April to allow for continuous determination of presence/absence of radiotagged seals.

**Project Lead:** ASLC

**Project partners:** ADFG, ANHSC biosampling program, ADFG – archived samples, UAF – archived samples, NMFS

## G.2. Age Estimation/Population Age Structure

**Objective:** Our objective is to compare age structures among populations that are stable or increasing, and those that are declining to obtain a better understanding of the demography of those populations. We will obtain age estimates for individual seals via counts of annual growth rings in cementum annuli of their teeth. In an effort to obtain as close as is possible to representative samples from various populations, we will collect samples of teeth from individual seals in multiple populations through a combination of collection of skulls and teeth from subsistence-harvested seals, and extraction of teeth from live-captured seals.

**Justification:** A comparison of age structures among populations that are stable or increasing, and those that are declining will help us to better understand the demography of those populations. Such data may indicate whether the decline of some populations is due to reduced survival and/or failure to successfully recruit pups born into that population, differential mortality due to human-caused disturbance, or age-specific mortality for individuals in populations where prey availability may be lower or contaminant levels higher. We recently validated the use of incisors to age harbor seals. From 193 subsistence-harvested seals and two seals of known age, we submitted (blind) a canine, premolar, and incisor tooth to Matson's Laboratory for age estimates using cementum annuli. The laboratory is well established for their expertise in aging teeth for other species. As has been noted in other species, canine teeth are likely to provide the most accurate estimate of ages for seals because annuli tended to be more distinct and regularly arranged for counting than in the other tooth types. Extraction of canines from live individuals, however, is not feasible, thus a smaller tooth must be used to age live animals. Age estimates of premolars and incisors for harbor seals were highly correlated with canine ages ($r$ = 0.9830485 and 0.9752055, respectively). Given the smaller size of incisor teeth compared to premolars and the high correlation between canine and incisors, we will use incisors to obtain age estimates for live seals.

**Methods:** We will extract an incisor tooth for age estimates as part of the normal processing routine for harbor seals captured for other purposes, that will be anesthetized as part of the handling procedure. Incisors from each individual will be submitted to Matson's Laboratory (LLC, Milltown, MT) for estimates of age by counting growth rings in cementum annuli. For all samples obtained from a subsistence harvest, we will submit a canine and incisor for continued comparison of age estimates from these two tooth types. Once we have sufficient estimates of ages from seals across the state through aging of teeth from live-captured and subsistence-harvested seals, age structures of seal populations will be compared among regions where the population trend is increasing, stable, and decreasing.

**Product:** Results will be reported in annual reports and incorporated into peer-reviewed manuscripts.

**Five-year project status**: Teeth for validation of aging with incisors were submitted to the laboratory in late September, results were received and analysis of were completed in April 2003. Partially funded FY03.

**Project lead**: ADF&G

**G.3. VHF Implants**

**Objectives:** Subcutaneously-implanted VHF transmitters to assess

1. Survival (age class and sex)
2. Age of first reproduction
3. Reproductive success
4. Dispersal

TRANSMITTERS ARE DUTY CYCLED FOR LONG-TERM DATA – 5 YEARS

Vital Rates – Hypotheses and Predictions

I. Age-specific survival differs between populations with increasing versus decreasing trends in abundance.
    - Survival will be lower, especially for pups and subadults, in PWS than in Kodiak or Southeast Alaska.

II. Reproductive success differs between populations with increasing versus decreasing trends in abundance.
    - Reproductive success will be lower in PWS than in Kodiak or SE.

III. Age of first reproduction differs between populations with increasing versus decreasing trends in abundance.
   - Females will have pups at an earlier age in Kodiak than in PWS or SE.

**Justification:** Dramatic declines in harbor seal numbers occurred since the 1970's in the Gulf of Alaska but no clear cause of the declines has been established. Estimates of vital population parameters are required to determine how two depressed populations (Kodiak Archipelago – now increasing; and Prince William Sound [PWS] – still decreasing) are responding to their respective marine environments; i.e., how differing reproductive and mortality rates drive changes in population abundance. Furthermore, vital rates of a population that has exhibited long-term stability (e.g., northern Southeast Alaska in the Sitka region) should be included as a "control" for this study. We will equip seals with subcutaneousy implanted VHF transmitters to assess vital rates over 5-yr periods. We will evaluate health and contaminant-load for those individuals to assess factors that contribute to differences in survival and reproductive success among individuals and populations.

An auxiliary benefit of our use of long-term VHF transmitters in seals is that it allows for an assessment of site fidelity over a period of years – a factor that has potential implications for assessment of population trends. Available data obtained from short-term attachment of telemetry transmitters to the pelage of seals, indicates that adult seals are relatively sedentary and do not move great distances from their capture site. Thus estimates of population trends, that rely upon seal counts at the same haul outs over an expanse of years, are expected to be indicative of population trends in that area. Our current methodology for trend surveys does not evaluate the possibility that movement of seals to new haul out sites may occur, or account for the potential effects of those movements on estimates of population trends. Therefore, deployment of long-term subcutaneously implanted VHF transmitters has an added benefit of assessing seal movements. We will attempt to relocate all seals implanted with VHF transmitters for the duration of time that those seals emit radio signals (5 years) to assess long-term fidelity to haul out sites or a broader area, as well as documenting haul out behavior of individuals over time.

**Methods**: We will conduct fieldwork PWS in 2003 to deploy 50 subcutaneous VHF transmitters in 2 capture cruises (April and June/July). Depending upon funding we hope to launch a similar study in Kodiak in 2004 (50 transmitters, 3 capture cruises), and expand to conduct field seasons in Southeast Alaska in 2005 and/or increase the number of VHF implants we have deployed in both locations in future years to bring the total number of seals with VHF implants at each location to 100 individuals. We will monitor individual seals with implanted transmitters in several populations over a period of years to assess survival, and for females, their subsequent reproductive success. Survival will be determined by relocating individuals over time. During the pupping season, the reproductive success of relocated females can be determined by observing females to document presence/absence of pups. Alternatively, the ability to relocate individuals also may allow recapture of some females over a period of years to obtain blood samples for progesterone assays of reproductive condition when pups are not present.

Additionally, we plan to track dispersal (if possible with VHF transmitters) and assess survival of juveniles and sub-adults of both sexes, and determine reproductive success (or age of first reproduction) for females. Data on differences in vital rates among populations (e.g., a reduction in survivorship or reproductive success) may provide insight as to the cause of decline in some populations. Those data will be combined with other data for that individual, obtained at capture, assessing general health and condition (with morphometrics and blood chemistry), diet (with stable isotopes and fatty acid analyses), contaminant load (assessment of organochlorines, heavy metals, and polychlorinated biphenyls), metabolic condition (with $D_20$), presence of stress hormones (e.g., corticosterone levels), and reproductive condition (progesterone assays), and interpreted in light of current and historic population trends for that area and existing data on prey availability.

**Background**: The Marine Mammal Center (TMMC) in California has successfully implanted subcutaneous VHF transmitters in harbor seals and is currently radio tracking 6 animals, several of which have been radio tagged for approximately one year (Lander pers. comm.). Assisted by TMMC staff veterinarian, we conducted a pilot season in October 2002 in Kodiak using identical surgical techniques to implant transmitters subcutaneous VHF transmitters that are duty cycled to transmit for 5 years. Product: Annual reports and peer-reviewed manuscripts.

**Five-year project status**: Pilot season completed successfully in October 2003 to validate efficacy of surgical procedures in wild-caught seals, with seals released within hours of surgery after recovery from anesthesia. Limited funding in FY03 resulted in a scaled-back field season. Collaboration and cost sharing between ASLC and ADF&G will allow for 2 capture trips April and June/July 2003 in which we will attempt to deploy as many as possible of the 50 transmitters we are authorized to implant in 2003.

**Project lead:** ADF&G

## G.4. Individual identification

**Objective:** To estimate survival and reproductive rates using photo-identification.

**Justification:** Population trends of harbor seals are monitored in several key areas in Alaska. While these data provide information on population status they do not provide specific information on more detailed population parameters such as survival and reproductive rates. Estimates of survival and reproductive rates would allow further investigation into factors that may directly affect these parameters, and ultimately population trend. Survival estimates and reproductive rates can be obtained through mark-recapture studies, whereby an animal is uniquely identified either by being "tagged" with an external marking or by identifying a unique mark that will enable the observer to resight the animal at future time periods. Unique identifiers have been used on humpback whales by photographing the distinct pattern of notches, scars, and pigmentation on the undersides of these animals' flukes (Mizroch et al. 1990). Unique identification of pinnipeds by some intrinsic marker is more problematic. However, a technique has been developed by Lex Hiby of Conservation Research Ltd. (CRL) whereby a photograph of the unique pelage pattern of gray seals (Halichoerus grypus) is taken, digitized and catalogued for comparison to other images in the database (Hiby and Lovell 1990). ADF&G has begun preliminary investigation of this technique for harbor seals hauled out on the beaches of Tugidak Island. If successful, the research will lead to estimates of age-specific survival and reproductive rates.

**Methods**: Seals will be photographed on Tugidak Island using Nikon D1 digital cameras and Schmidt-Cassegrain telescopes (Celestron C5). Observers will systematically survey seals hauled out on two beaches: Southwest Beach and Middle Beach, covering the whole beach on each survey day. Each beach will be surveyed on two consecutive days at approximately weekly intervals during the breeding season (May 20 – July 15), and every three days during the molting season (August 1 – September 30). Surveys will be conducted in the following manner: observers will move systematically through the beach photographing the ventrum of all seals showing a proper ventrum view (head, tail, and both foreflippers visible; and angle of body slight to moderate from the camera) will be photographed. Seals will only be skipped if they are not showing a proper ventrum view, are completely covered in sand, or are in molt stage B2 or C (completely or nearly completely bleached), but will not be skipped based on extent of patterning. Seals that are showing a proper ventrum view but are skipped because they are bleached are tallied on a form based on group, molt stage, age, and sex. Data collected for each seal photographed includes: location (GPS position), temporary animal id, estimated age-class, sex, association of mother and pup (if applicable), color phase, molt stage of the whole body and in the two fingerprint regions, pattern diversity in the fingerprint regions (ranks 1-4), distance to the mid-point of the seal from the camera body (measured with a laser rangefinder), photographer, recorder, and whether the seal was wet, dry, or wet and dry. Presence of scars or tags and identity of scarred or tagged animals is also recorded. Scarred and tagged seals are photographed from all views (left and right sides of head and body, dorsum and ventrum) if possible. Seals are identified as within or outside of groups, and groups are counted before and after photographs are taken; weather data is recorded at approximately 2 hour intervals. All image and seal information, skip tallies, weather data and group counts are entered into linked tables in the relational "Tugidak" database (ACCESS). All scarred and tagged individuals photographed, whether identified in the field or not, are checked against the known seal photo database to determine true identities and to check for misidentification error.

Images will be sent to Dr. Hiby of CRL for matching. The best images taken for each "temporary" seal will then be fit to a 3-dimensional ventrum model (Hiby and Lovell 1990) to correct for posture and viewpoint. The pelage pattern will be extracted from the core area of the fore cell and hind cell, described numerically, and entered into the fingerprint library. Software developed by CRL will be used to compare and rank fingerprints and to visually check for matches. Opportunistic left and right head views available from ventrum photos and from deliberate head photos taken of known seals will be analyzed using the 3-dimensional head model. Incorporation of the head view data may allow data from 1998-1999 (when only the head view was used) to be included in future analyses. These fingerprints will be compared using the same procedure as the ventrum data. Results of photo matching will be provided to ADF&G in an ACCESS database. Mark-recapture models will be used to estimate annual survival, and potentially reproductive rate, population size, and pup survival over the nursing period.

**Product**: This project will provide estimates of annual survival, and potentially provide estimates of reproductive rate, pup survival over the nursing period, and population size during the breeding and molting seasons. Age-specific estimates of life-history parameters will be available for those seals photographed as pups. Contingent on development of an accurate photogrammetry model, annual and age-specific estimates of growth and body size may be provided by this project.

**Five-year project status**: Images of over 5,900 "temporary" seals (including resights of the same individuals) have been successfully catalogued from 1998-2001. Numbers of temporary seals (not considering multiple resightings of the same seal) "marked" (photographed) per season has increased from 250-800 for head photos (1998-1999) to 1000-2800 for ventrum photos (2000-2002). With a four person spring team and 2 person fall team, the maximum number of "temporary" seals markable on Tugidak Island was ~2,500-3,000 per season (5,000-6,000 per year). If after testing and determining pelage patterns of individuals are consistent from weaning into adulthood, data from seals first photographed as pups will be used to estimate age-specific survival and reproductive rates. Seals will be photographed according to the survey design during pupping and post-molt annually. Photographs are provided to CRL for matching in July (breeding season data) and in October (molt season data) of each year. Results of matching are provided to ADF&G by CRL by March of the following year.

Intensive analyses are currently underway to determine the error in matching of photographs of "known" individuals (i.e., number tagged or distinctively scarred) that are submitted blind to CRL for photo matching. Revisions are underway by CRL to determine if modifications to the current matching system can improve the system's ability to rank matching photographs within the top 0.5% of the library. Several revisions already completed appeared to have improved performance including adjustment of the size and location of hind cells such that hind cells score as well as fore cells, and inclusion of more cell regions from various areas on the ventrum. Ranking failures that result from sparse pattern in the cell regions may be improved with a new algorithm similar to that used for cheetah flanks which doesn't depend on dense pattern in the cells center. We are also testing the efficiency and accuracy of a more manual matching procedure modified from Crowley et al. (2001), as well as evaluating whether a combination of the two methods may yield the most accurate and efficient system.

To accomplish this, an automated ACCESS form has been created for fast, efficient manual categorization of seals in photographs. Various categorization schemes (containing sex, color phase, and 16 types within each color phase based on spot/ring density and spot/ring size in 3 standard regions on the ventrum and the foreflippers) and sorting procedures are being tested to determine the maximum ranking efficiency of the manual system when used on its own and when used in conjunction with the CRL system. We are also currently conducting simulations to determine the effect of photograph matching error on estimates of vital rates when using open-population mark-recapture models.
Survival analysis will be conducted after 4 to 5 years of data collection (2004-2005 for ventrum data). Funded FY02, reduced funding FY03.

## H. HUMAN INTERACTIONS

**Overview**
The research categories addressed above focus on harbor seal biology and ecology. The following research tasks examine direct anthropogenic impacts to harbor seals. Tasks 2 and 3 reflect the specific need, as mandated in the MMPA, to obtain estimates of human-caused mortality and injury to harbor seals. The two sources of direct human-induced harbor seal mortality and injury in Alaska are subsistence removals and, potentially, commercial fisheries interactions. The first task reflects the growing concern about the potential impacts to harbor seals from vessel disturbance.

### H.1. Disturbance

#### H.1.1. Tracy Arm/Endicott Arm

**Objective:** Conduct a study on the effects of vessel disturbance on seal behavior, comparing the effects of that disturbance in an area of heavy vessel traffic with seal behavior in an area of light vessel traffic in the adjacent Endicott Arm.

**Justification:** Two years of data have been collected in this area, documenting the behavior of seals to the approach of vessels. Although disturbance has been noted (e.g., seals abandoning icebergs) no data exist on the behavior of specific individuals. For example, seals may be observed diving into the water from icebergs as a vessel approaches, and seals may be observed hauling out on icebergs after a vessel has passed. Nonetheless it is not possible to determine whether the seals that haul out after the vessel has passed were those that abandoned the haul out as the vessel approached. Without identification of individuals, and an assessment of how long the disruption of haul out behavior lasts for a given seal, it is not possible to calculate the energetic costs of evasive behavior at the approach of a vessel.

Because the lactation period for harbor seals is short (4-6 weeks), if nursing boats are regularly interrupted during this critical period, a pup may not obtain sufficient nutrition, prior to weaning. Small body size and low nutritional reserves for weaned pups may reduce the survival of those individuals as they learn to forage on their own. Similarly, seals of all ages haul out, likely to conserve energy when they are not foraging. If seals that are disturbed by the approach of vessels abandon their haul out for extended periods, or significantly reduce the amount of time that they would normally be hauled out, that disturbance may represent substantial energetic costs to the individual. The cumulative effect of repeated disturbance may result in reduced reproductive success or lower survival. Thus the effects of vessel disturbance may have demographic consequences to seals via disruption of nursing pups and repeated energetic costs of prematurely abandoning a haul out when seals are disturbed by the approach of a vessel. Long-term radio tagging of individuals with subcutaneously implanted VHF transmitters will allow documentation of the duration of disturbance from vessel activity. Furthermore, survivorship of seals in areas of high vessel traffic (Tracy Arm) can be compared with survivorship in an area of low vessel traffic (Endicott Arm).
**Methods**: Approximately 15-20 seals (preferably adult females) will be implanted with subcutaneous VHF transmitters in each area (Tracy and Endicott Arms). Behavioral response of individuals to the

approach of vessels will be monitored in each area for a minimum of one season. Survival of those individuals will be monitored for the duration of the battery life of those transmitters by radio-tracking several times per year in the area. Survival will be compared between areas.

**Product**: Annual reports and peer-reviewed manuscripts.

**Five-year project status**: Unfunded FY03

**Project lead**: ADF&G

**H.1.2 Aialik Bay Harbor Seal Disturbance Monitoring**

**Objective:** Determine the frequency, types, and levels of disturbance harbor seals experience in response to tourism and commercial fishing activities in upper Aialik Bay and compare results with baseline studies conducted from 1979-1981.

**Background:** With the creation of the Kenai Fjords National Park and the expected increase in tourism traffic, the National Park Service funded studies of the status of harbor seals in Aialik Bay from 1979-1981, when vessel and air traffic were infrequent. From May-September, multiple tour-boats, kayaks, and other vessels visit upper Aialik Bay on a daily basis. Aircraft traffic also has increased in frequency. Brief surveys conducted by the National Park Service have shown a large increase in the number and frequency of vessel traffic with potential effects on haulout attendance. This study provides a multi-year data set for comparison with baseline data from 1979-1980.

**Justification**: Disturbances from resting or pupping sites can increase the energetic requirements of seals. During pupping and molting many seals are nutritionally stressed and need to minimize unnecessary energetic loss. Frequent disturbance may alter the location and time that seals haul out, although some seals appear to become habituated to common sources of disturbance. Disturbances occurring while females are giving birth and shortly thereafter have the potential for causing permanent mother-pup separations resulting in the death of newborn pups (e.g., Bishop 1967).

**Methods:** A remote-controlled video camera located on Squab Island will be operated at the Alaska SeaLife Center (see above). The frequency of vessel traffic, approach characteristics, and the responses of seals will be recorded. Interactions will be evaluated using sequential images from the video that are time and date stamped. Distances between vessels and seals showing different levels of response will be determined whenever possible. The behaviors of seals that have not been disturbed will be sampled throughout the day in conjunction with population monitoring surveys and fixed observations of groups of seals. These data will be important for evaluating how the seals are currently responding to disturbances and how frequently they are disturbed. It also will allow detailed review of interactions to help develop effective navigation recommendations for minimizing adverse human impacts.

**Product**: A report will describe changes in (1) the behavior of seals since the 1979-1980 baseline studies and (2) the frequency, type, and level of disturbance seals are currently experiencing. Variation in response behaviors will be associated with different types of disturbance. Recommendations to minimize the effects of human activities on harbor seals will be made.

**Five-Year Project Status**: 2002 was the first year of the study. Surveys will be conducted annually from June through September each year. Longevity of the project is dependent on funding. Funded FY02 and FY03

**Project Lead**: ASLC

**Project Partners**: NPS (Oceans Alaska Science and Learning Center), Port Graham Corporation, USFWS

## H. 2. Harvest monitoring and mortality estimation

**Objective**: Determine the total number, including the proportion of struck and lost animals, as well as sex and age, of harbor seals harvested by Alaska Natives.

**Justification:** The MMPA requires an estimate of the annual human-caused mortality and serious injury of marine mammal stocks by source. The subsistence harvest of harbor seals represents one source of human-induced mortality or serious injury. Harbor seals are a traditional subsistence food of Alaska Natives in many coastal Alaska communities. In addition to being a food source, harbor seals represent a significant part of the cultural and spiritual basis of Native communities. Alaska Natives may take marine mammals for subsistence use under both the MMPA (Section 101(b)) and the Endangered Species Act (Section 10(e)). Native takes for subsistence or handicraft purposes are generally not subject to regulatory control unless a stock is depleted (MMPA) or unless Native takes are substantially disadvantaging the stock (ESA). Although Native subsistence harvest of harbor seals is not the subject of direct management action, monitoring of all mortality, including subsistence removals, is necessary to ensure that the harbor seal population(s) does not fall below an optimum sustainable population.

**Methods**: Subsistence harvest levels will be estimated using either direct hunter reporting or retrospective survey techniques. The methods to be used will be determined and implemented in close coordination with the ANHSC.

**Product**: A time-series of the total subsistence takes of harbor seals in Alaska including the number of animals taken and struck and lost, by sex, age class and geographic region.

**Five-year project status:** Ongoing

**Project lead**: ANHSC

## H.3. Incidental take by commercial fisheries

**Objective**: Determine the level of incidental take of harbor seals in commercial fisheries off the coast of Alaska.

**Justification:** The Marine Mammal Protection Act (MMPA) requires that a species or population stock not be permitted to diminish below its optimum sustainable population and that measures be immediately taken to replenish any species or population stock which has already diminished below that point. Section 118 of the MMPA specifically mandates that the incidental mortality or serious injury of marine mammals, including harbor seals, occurring in the course of commercial fishing operations be reduced to insignificant levels approaching a zero mortality and serious injury rate. Fisheries are classified according to the degree of interaction with marine mammals. Should the level of human-induced mortality exceed the potential biological removal (PBR) level and the stock be declared "strategic" the commercial fisheries which interact with that species would be required to reduce the incidental mortality and serious injury of that stock taken incidentally in the course of commercial fishing operations to a level below the PBR calculated for that stock. As a result data must be collected on the level of incidental serious injury and mortality occurring in commercial fisheries. This information is also required in the annual stock assessment reports. Currently few data are available on the incidental take of harbor seals in commercial fisheries.

**Methods**: Current methods include reporting via a fisher self report system and directed observer coverage in some commercial fisheries. Federal groundfish fisheries have varying observer coverage depending on vessel size and fishing targets; selected state fisheries are observed on a periodic basis. Estimated observer coverage levels in state fisheries are calculated based on a statistical model that incorporates fishing effort and PBR levels of a reference species.

**Product**: An estimate of the number of harbor seals taken incidental to commercial fisheries in Alaska.

**Five-year project status**: Five-year project status: The fisher self report system and groundfish observer program continue on an annual basis. The marine mammal observer program is currently focusing on Category II fisheries (those fisheries having occasional incidental mortality and serious injury of marine mammals) in Alaska on a rotating schedule. Observers were placed in the set and drift gillnet salmon fisheries in Cook Inlet in 1999-2000 with coverage levels ranging from 2% to 5%. No harbor seal mortalities or serious injuries were observed. Observer coverage moved to the set gillnet fishery in the Kodiak in 2002, where approximately 5% of fishing effort was observed. No harbor seal mortalities or serious injuries were observed. Observer coverage in the Kodiak set gillnet fishery will continue again in 2004. All incidental takes of marine mammals, including harbor seals, will be documented. Pending funding beyond 2003, fisheries in other areas of the state, including Southeast Alaska, Yakutat, Bristol Bay, and the Aleutian Peninsula will be observed.

**Project lead**: Alaska Region

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## Appendix 1
## Budget: Total Project Cost

| AGENCY | ESTIMATED EXPENSE |
|---|---|
| National Marine Mammal Laboratory | $504,000.00 |
| Alaska Department of Fish & Game | $900,000.00 |
| Southwest Center | $191,500.00 |
| Alaska Sealife Center | $735,000.00 |
| National Marine Fisheries, Alaska Region | $100,000.00 |

| | |
|---|---|
| Alaska Native Harbor Seal Commission | Amount Unavailable |