Appendix B
Description of Research Methodologies

APPENDIX B

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ACRONYMS AND ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADF&G	Alaska Department of Fish and Game
ARGOS	Satellite and Information Data Collection System
ASLC	Alaska Sea Life Center
BIA	bioelectric impedance analysis
C	Centigrade
cm	centimeters
CO_2	carbon dioxide
DNA	Deoxyribonucleic Acid
DPS	Distinct Population Segment
ESA	Endangered Species Act
FA	fatty acid
ft	feet/foot
g	gram
GPS	global positioning system
kg	kilograms
khz	kilohertz
km	kilometers
lbs	pounds
LHX	Life History Transmittors
M	Midazolam
m	meter
mg/kg	milligrams per kilogram
ml	milliliter
mm	millimeter
MMPA	Marine Mammal Protection Act
NFS	Northern fur seal
nm	Nautical Mile
NMFS	National Marine Fisheries Service
NMML	National Marine Mammal Laboratory
NOAA	National Oceanic and Atmospheric Administration
P	pethidine
PCP	phencyclidine
psi	pounds per square inch
PTT	platform transmitter terminals
R max	maximum net recovery rate
Rs	resistance
SLR	single-lens reflex
SLTDR	satellite-linked time depth recorder
SPOT2	smart position and temperature
SSL	Steller sea lion
STT	stomach temperature transmitter
TDR	time depth recorder
U.S.	United States
VHF	Very High Frequency
Xc	reactance
Z	biological impendence

1.0 INTRODUCTION

1.1 Objective of Paper

Research on Steller sea lions (SSLs) (*Eumetopias jubatus*) and northern fur seals (NFSs) (*Callorhinus ursinus*) dates back to the early 1900s, but has intensified in recent years with the listing of the SSL under the Endangered Species Act (ESA) and the classification of NFS as depleted under the Marine Mammal Protection Act (MMPA). Many of the research methods used on these two species have evolved over time for several reasons including, but not limited to: availability of better instruments; better understanding of the animals and their behavior; efforts to reduce harm to animals; and improvement of techniques by trial and error. The objective of this paper is to provide an overview of the current research techniques used on SSLs and NFSs, summarize the potential effects of these techniques, and describe the types of information collected using different techniques and how that information may be used.

1.2 Purpose of Research on Steller Sea Lions and Northern Fur Seals

The purpose of the research on SSL and NFS, as stated in the Draft Revised Recovery Plan for the Steller Sea Lion, herein referred to as the SSL Recovery Plan (NMFS 1992a, NMFS 2006a) and Draft Conservation Plan for Eastern Stock of Northern Fur Seal, herein referred to as the NFS Conservation Plan (NMFS 1993, NMFS 2006b), is to promote the recovery of the species' populations to levels appropriate to justify removal from ESA listings and to delineate reasonable actions to protect the depleted species under the MMPA. The need for research is rooted in the fundamental need to understand the species' biology and ecology, especially factors that determine SSL and NFS population growth, such as; rates of reproduction and mortality, emigration and immigration, and incidence and types of predation, parasitism, and disease. These things are in turn functions of factors such as habitat availability and use, behavior, and energetics.

1.3 Information Sources – National Marine Mammal Laboratory, Websites, Literature

Information gathered for this report was collected from the following sources: applications and annual reports from National Marine Fisheries Service (NMFS) permit holders; websites hosted by the National Marine Mammal Laboratory (NMML), the Alaska Sea Life Center (ASLC), Alaska Department of Fish and Game (ADF&G), and Texas A&M University; and peer-reviewed literature.

2.0 RESEARCH METHODS

2.1 Aerial Surveys

2.1.1 Description of Methods

Aerial surveys are used to conduct counts of SSLs, but are not used to assess abundance of NFSs. The typical protocol used by ADF&G and NMML for aerial surveys involves flying over rookeries and haul-out sites at 100 – 150 knots air speed, a minimum of 150 - 200 meters (m) altitude, and 500 m (1/4 nautical miles [nm]) offshore in order to take 35-millimeter (mm) color photographs and Hi-8 video film for the purpose of counting non-pups present (Calkins and Pitcher 1982, Withrow 1982). Strong winds occasionally require flying at higher altitudes or farther offshore, whereas fog or low clouds sometimes require flying at a lower altitude or closer inshore.

The surveys typically include a single pass over each site, with additional passes made only when the photographers have reason to believe they missed part of the site. Replicate surveys on separate days are occasionally conducted to develop an estimate of the survey variance.

Sea lions are photographed using a 35-mm manual focus single-lens reflex (SLR) camera with motor drives and zoom lenses (70-210 mm or equivalent) and moderately fast (ISO 200 or faster) color transparency slide film. Where appropriate, sequential photographs overlap slightly to guarantee complete coverage of a site. Personnel also photograph each site using a high-resolution 8-mm video camera for back-up imagery. Following the surveys, adult and juvenile sea lions are counted on projected slides in the laboratory. Because altitude and orientation are known, the length of individual animals can be measured and animals are assigned to age and sex classes.

In the late 1990s, researchers began developing a new technique for aerial surveys, medium format color photogrammetry, which allows them to count pups and improve counts of non-pups using aerial surveys (Snyder et al. 2001, Fritz and Stinchcomb 2005). The medium format aerial surveys are conducted from directly above each rookery/haul-out site (vertical orientation) rather than from offshore (oblique orientation) as with 35 mm slides. The 2004 aerial survey conducted by ADF&G of non-pup SSLs was conducted using a 5-inch military reconnaissance camera with motion compensation mounted in the belly of an AeroCommander survey aircraft (Fritz and Stinchcomb 2005). Medium format color photographs were taken from directly above each rookery/haul-out site at an altitude of at least 700 feet (ft). The surveys were conducted between the hours of 0900 and 1700 local time when SSLs are most likely to be on land (Sease and Gudmundson 2002). Counts of SSLs were made from images placed on a high intensity light table with the aid of a dissecting scope (2X to 20X magnification). Comparisons of surveys conducted simultaneously with the 35 mm and medium format techniques (in 2000) indicated that the medium format technique yielded counts approximately 3.6 percent greater than those from 35 mm slides. Population trend analyses have been compensated to account for the difference in resolution from the different techniques (Fritz and Stinchcomb 2005). The ability of the medium format technique to provide counts of pups offers a much less disruptive technique compared to drive counts of pups conducted on land (Snyder et al. 2001).

2.1.2 Objectives of Research

The SSL Recovery Plan identified the need for Alaska-wide surveys of adult and juvenile SSLs every year, and a range-wide survey every fifth year. The status of the Alaskan SSL population is evaluated based on aerial surveys of adults and juveniles on observed rookeries and haul-outs during June and July.

2.1.3 Use of Data

Data from these surveys are used to determine the current status of the SSL population for evaluation relative to recovery criteria. These data are also used to evaluate trends by sub-area and site; to study causes of decline, and the efficacy of management actions.

2.1.4 Effects of Research

2.1.4.1 Steller Sea Lions

Disturbance from aircraft traffic has been observed to have highly variable effects on SSLs on land (Calkins and Pitcher 1982). Reactions range from none to complete departure from the haul-out site (i.e., a stampede). Researchers report that the sound change associated with banking of the aircraft increases the likelihood of disturbing the animals. Researchers also report that disturbances caused during aerial surveys are typically minimal; most SSLs appear unaware of the aircraft and less than 10 percent of the animals react at all. The few animals that are occasionally disturbed by the aircraft are usually found in remote regions that experience little aircraft or vessel traffic, or where the physiography of the site acts to amplify the sound of the aircraft.

When SSLs are disturbed off of rookeries, pups may be trampled or abandoned. Juvenile and adult animals can also be injured from running into each other or sliding or crashing into cliff facings or underwater rocks. In addition, excessive metabolic heat from the flight response can put the SSLs in jeopardy.

In two separate instances, captive SSLs jumping from elevations of 4-5 feet landed on the chest area, rupturing the brachiocephalic vein located in the left shoulder area, resulting in a serious or fatal injury (Sweeney 1990). Jaw fractures are also a common result of the flight response, which could affect feeding. Because the physiography of SSL habitat is characterized by rocky outcroppings and steep cliffs, the possibility of such injuries is high.

2.1.4.2 Northern Fur Seals

Although aerial surveys are not used to assess NFS abundance, studies have shown that NFSs could be adversely affected by aircraft noise. Insley (1992, 1993) suggested that aircraft activity could adversely affect NFSs because sound spectra of aircraft noise and airborne vocalizations are similar. He also noted that some NFSs oriented towards aircraft noise during overflights. Johnson et al. (1989) reported that aircraft caused a large stampede of bachelor bulls into the water on St. Paul Island and caused little disturbance on St. George Island. Attempts to reduce aircraft disturbance to NFSs include Aircraft Advisory Zones and Requested Aircraft Flight Paths which have reduced overflights of NFS rookeries on St. George and St. Paul Islands (NMFS 2006b).

2.1.4.3 Other Pinnipeds

In general, hauled-out pinnipeds react to airborne sound (and possibly sight) of aircraft by becoming alert and/or rushing into the water. Reactions tend to be most strong if the aircraft is flying low, passes nearly overhead, and causes abrupt changes in sound. Pinnipeds hauled-out for pupping or molting are the most responsive to aircraft. Partial habituation may occur under some conditions (Richardson et al. 1995).

Harbor seals (*Phoca vitulina*) often temporarily leave pupping beaches when aircraft fly over and do not always haul-out at the same site when they return to land (Johnson 1977). In Glacier Bay, harbor seals typically reacted strongly to small aircraft at altitudes below 61 m, but overflights above 76 m elicited minor reactions (Hoover 1988). However, harbor seals can also habituate to frequent overflights; for example, aircraft using Vancouver International Airport fly over a haul-out site and the harbor seals show little reaction (Johnson et al. 1989).

Ringed seals (*Phoca hispida*) hauled-out on ice often dive when approached by a low-flying aircraft or helicopter (Richardson et al. 1995). Born et al. (1999) indicate that the disturbance in ringed seals is related to the type of aircraft. Ringed seals entered the water in higher proportions and at greater distances to helicopters than fixed-wing aircraft.

Walrus (*Odobenus rosmarus*) responses to overflights of haul-out sites vary with range, age, sex, and group size, as well as aircraft type and flight pattern. For example, adult females, calves, and immatures are more likely to enter the water than males (Richardson et al. 1995). Some walruses raised their heads when a helicopter was over 2.5 kilometers (km) away and some rushed into the water when it came within 1.3 km (Salter 1979). Brueggeman et al. (1990) found that 12 percent of walrus groups observed in open waters and 38 percent on pack rice responded to a survey aircraft at 305 m altitude.

2.1.4.4 Cetaceans

Reactions of toothed and baleen whales to aircraft are reported less often than pinnipeds, possibly indicating that airborne sounds from an aircraft are less relevant to marine mammals in the water than to pinnipeds hauled-out on land or ice. When reported, reactions vary from diving, slapping the water, or swimming away. Their overall behavioral state during the overflight influences the disturbance (Richardson et al. 1995).

2.1.5 Mitigation

Researchers believe approaching or departing from 1 km or more offshore without banking reduces the incidences of disturbance because the aircraft would only be in hearing range of the animals for one to two minutes. Limiting the frequency of the aerial surveys over individual rookeries and haul-out sites, limiting surveys to times of year when pups are older (less likely to be trampled), or requiring surveys to be flown at higher altitudes may reduce the possibility of adverse effects.

2.2 Vessel Surveys

2.2.1 Description of Methods

Counts of animals from vessels are typically not conducted for SSLs or NFSs because it is difficult to see the animals from a vessel, although researchers will attempt to conduct re-sighting of brands from vessels. Vessels are used to drop personnel onshore for ground counts and capture/restraint, scat collections, as well as to capture animals using a floating platform trap or underwater lasso (Section 2.5). At each site, a small group of biologists surveys the site from a skiff to determine the best way to approach, herd, and move animals. The skiff approaches the beach and the biologists come ashore as needed for collection of samples or capture of animals.

2.2.2 Objectives of Research

The SSL Recovery Plan identified the need for Alaska-wide surveys of adult and juvenile SSLs every year and a range-wide survey every fifth year. The status of the Alaskan SSL population is evaluated based on aerial surveys of adults and juveniles on observed rookeries and haul-outs during June and July.

2.2.3 Use of Data

Data are used to determine the current status of the SSL population for evaluation relative to recovery criteria. These data are also used to evaluate trends by sub-area and site, in order to study causes of decline and the efficacy of management actions.

2.2.4 Effects of Research

2.2.4.1 Steller Sea Lions

Researchers use a skiff to cause a large number of the adult animals to leave the rookery or haul-out for the water in order to count pups, by approaching and making noise to attract attention. This method allows the researchers to gauge the rate at which animals enter the water, to reduce the likelihood of a stampede. The types of impacts associated with stampeding animals are discussed in Section 2.1.4.1.

2.2.4.2 Northern Fur Seals

Few studies have described NFS responses to vessel traffic. Johnson et al. (1989) reported observations of NFSs approaching vessels at sea, but also reported that they avoided ships if the ships were engaged in seal hunting. Some evidence suggests that NFSs are curious in the water and may be attracted to vessels, but this behavior may be related to past experiences of individual animals (NMFS 2006b).

2.2.4.3 Pinnipeds

Walruses observed by Salter (1979) showed no response when boats with outboard motors approached the haul-out site at distances of 1.8 to 7.7 km. For walruses hauled-out on ice, the probability and type of reaction depends strongly on distance, ship speed, and sound (Richardson et al. 1995).

California sea lions (*Zalophus californianus*) in the water tolerate close approach by vessels and sometimes tend to congregate around fishing vessels. They are typically more responsive when hauled-out on land, but rarely react unless a boat approaches within 100-200 m (Richardson et al. 1995). Reactions appear to be more common if motor noise varies.

Harbor seals may be displaced from haul-out sites when boats approach within 100 m; less severe disturbance can cause alert reactions without departure (Bowles and Stewart 1980). Some harbor seals returned within an hour and others remained absent for over three hours after leaving a haul-out in response to a boat (Allen et al. 1984). In Alaska, most harbor seals pay little attention to fishing vessels at over 200 m away, become alert at 150-200 m, and vacate the haul-out site within 60 m (Johnson et al. 1989).

2.2.4.4 Cetaceans

Odontocetes exhibit tolerance of vessel traffic, but may react if confined (e.g., ice, shallow water) or if previously harassed by vessels (Richardson et al. 1995). Bottlenose dolphins (*Tursiops truncatus*) commonly approach boats, often swimming in the bow and stern waves (Shane et al. 1986). However, boats may alter dolphin behavior. Shane (1990) found that altered behavior was least common when dolphins were actively socializing.

Beluga whales' (*Delphinapterus leucas*) reactions to small vessel approaches range from approach to strong avoidance. The intensity of disturbance varied with the number and speed of boats, the activity and ages, and the location (Blane 1990). Beluga whales react strongly and at long ranges from ships and icebreakers during the spring (Finley et al. 1990).

In general, when baleen whales experience low-level sounds from vessels, there is little reaction. When vessels approach whales slowly and non-aggressively, whales tend to exhibit slow avoidance maneuvers. When vessels approach whales with strong or rapidly changing noise, whales often swim rapidly away (Richardson et al. 1995).

2.2.4.5 Other Marine Mammals

In Alaska, Udevitz et al. (1995) estimated that approximately 15 percent of sea otters (*Enhydra lutis*) along boat survey transects were not detected because they moved away from the approaching boat. Garrott et al. (1993) found that some sea otters on shore moved into the water from approach of a small boat traveling parallel to the shore.

2.2.5 Mitigation

By approaching cautiously and monitoring the rate at which adults enter the water for pup counts, the likelihood of a stampede is decreased, which decreases the possibility of pups getting trampled during a stampede. Disturbing the adults slowly also allows the pups time to move away from the water, reducing the amount of pups entering the water when researchers move on shore (NMML 2005 Annual Permit Report).

2.3 Ground Surveys

2.3.1 Description of Methods

There are instances where neither aerial nor vessel surveys are desirable or practical for SSL and NFS research. For example, except when using the newer wide-format photography, the resolution of photographs taken during SSL aerial surveys is inadequate to detect pups reliably. Thus, in some instances, personnel come ashore at rookeries to count pups in what are called "drive counts." Pup counts typically take place during the last week of June through the second week of July at rookeries throughout the range. Pups are counted first by clearing the rookery of most SSLs other than pups. A team of biologists experienced in herding SSLs slowly moves non-pups away from the pups. After the non-pups have retreated, two or more biologists make independent counts of the live (and dead) pups on the beach and in the water.

In some locations, SSLs can also be counted by observers positioned on cliffs above the rookeries at established observation points (Withrow 1982). Animals are then counted and sexed through high-powered binoculars without any disturbance of the animals. Observers typically scan the area and count the number of territorial males or bulls present. The observer next census the number of females, followed by immatures, and pups. A scan is then made to record the number and sex (if possible) of animals in the water.

NFS pup production estimates have been conducted using the shear-sampling method (York and Kozloff 1987). Pups are marked by shearing a small patch of hair from the top of their heads, which exposes the silver fur underneath and produces an easily identifiable mark. The number of pups marked on each rookery is approximately 10 percent of the most recent pup production estimate for each rookery, and the marking effort is allocated proportionately throughout the rookery according to the distribution of breeding males. After allowing a few days to pass for adequate mixing of the marked and unmarked animals, each rookery is observed from overhead vantage points twice by multiple observers. Recent pup production estimates have been conducted biennially and have included subsampling of rookeries during some years (Towell et al. 2006).

2.3.2 Objectives of Research

The SSL Recovery Plan identified the need for surveys of SSL pups at Alaska rookeries every year and range-wide every fifth year. Pup counts obtained during late June and mid-July provide supplemental information on population status, in conjunction with aerial surveys of non-pups. The NFS Conservation Plan identifies the need to continue adult male NFS counts and develop estimates of pup production and survival. Ground surveys are essential to developing census information on NFSs, particularly because they are not counted using aerial photography.

2.3.3 Use of Data

Data from these surveys are used to determine the current status of the SSL/NFS population for evaluation against recovery criteria. These data are also used to evaluate trends by subarea and site, in order to study causes of the decline, and the efficacy of management actions.

2.3.4 Effects of Research

2.3.4.1 Steller Sea Lions

The possible effects of a stampede are similar to those described for aerial surveys (i.e., serious injuries and mortality). Parturition in SSLs occurs from mid-May until Mid-July, with the highest frequency of births occurring in mid-June. Thus, the majority of pups on a rookery at the time that ground counts occur would be a few days to six weeks old. Because the motor skills of pups at this age are not as well developed as in older pups, they would be likely to be unable to avoid getting trampled or knocked into the water if adults stampeded. For those that are knocked into the water, they may not be able to climb the rocky cliffs common to rookeries. For those that are able to reach shore, they may suffer hypothermia and respiratory complications as a result of aspirating water. Pups injured during a stampede may not die from their injuries immediately, as is the case from hemorrhaging or infections.

If sufficient pre-disturbance monitoring is not conducted, it is not possible to identify mother-pup pairs and whether a pup has been abandoned. Because foraging trips of lactating females may last several days or more (Brandon 2000), there is no way to determine whether a pup has been abandoned as a result of the disturbance unless researchers remain to monitor the rookery for several days. Fostering is very rare in SSLs; thus the majority of abandoned pups will starve.

The disturbance associated with ground counts can result in aggressive interactions among SSL. When adult animals are displaced from the rookery during breeding season, at least some males are likely to have to re-establish their territories, increasing the likelihood of aggressive interactions among males and the possibility of injury (Lewis 1987). In addition, other SSLs on the rookery, including pups, may be injured during these aggressive competitions among males. Along with the possibility of physical trauma, heightened aggressive interactions, and resulting psychological effects can results in secondary disease manifestations (Sweeney 1990).

SSL mothers are very attentive, particularly when their pups are young. When under duress, as may be caused by research-related disturbance, they may carry their pups into deep water where pups can drown. Thus, it is important to avoid disturbance at the time of parturition to allow for maternal security.

The magnitude of the disturbance effects may be affected by the number of personnel who come ashore, the amount of time the rookery or haul-out is occupied by researchers, the frequency of these disturbances, and the timing of the disturbance. A recent study by Kucey and Trites (2005) determined that assessing the effects of disturbance on SSLs is extremely difficult, particularly when determining the recovery after disturbance. In addition, no studies have assessed long-term effects of disturbance on SSLs.

2.3.4.2 Northern Fur Seals

The possible effects of ground counts and disturbance on NFSs are similar to those described for SSLs. NFS mother-offspring pairs recognize each other's vocalizations during the course of the breeding season and are able to retain those memories for at least four years (Insley 2000). If mother and pup are separated due to disturbance before vocal recognition is established, there is a possibility that they will remain separated and the pup will die.

A detailed analysis of the influence of human disturbance on NFSs has not been undertaken, but experiments conducted by Gentry (1998) indicate that NFSs are resilient to extreme disturbances during the breeding season.

They often detect human scent and become more vigilant, but typically do not leave the breeding area. Outside the peak breeding season, mothers will separate from their young once human presence is detected but often return within a few hours (NMFS 2006b). Repeated displacement of females may result in permanent abandonment of sites. Juvenile males are less tolerant of human presence and are displaced from haul-out sites easily (Gentry 1998).

2.3.4.3 Other Pinnipeds

A study by Gazo et al. (2000) found that Mediterranean monk seal (*Monachus monachus*) pups washed from their beaches died from multiple skull fractures as a result of impacts against rocks, and those that managed to arrive back onshore still alive probably died shortly thereafter.

A study on the social calls of South American fur seal (*Arctocephalus australis*) mothers and pups revealed that the postpartum fasting period is a critical time for establishing mother-pup bonds (Phillips and Stirling 2001). They use individual calls to reunite and maintain contact in dense breeding colonies. Mothers must learn their pup's individual call during the days immediately following birth in order to assure recognition and reuniting following foraging trips to sea. Increased disturbance during this critical time period may affect the ability of mothers and pups to reunite after the disturbance or after foraging trips.

2.3.5 Mitigation

To minimize the impacts of pup counts, the following methods are included in the protocol:

- Surveys are not conducted until the end of the pupping season (late June or later) after the majority of mother-pup bonds are well established;
- Time occupying the beach is minimized; and
- Only biologists experienced in herding the adults out of the way and experienced counters are used to complete the surveys as quickly as possible.

Additional mitigation measures may include:

- Limiting the frequency of disturbance at individual rookeries (to reduce chronic disturbance) between years and within one year;
- Waiting until pups are at least two months old and more capable of avoiding injury when adults stampede; and
- Conducting pre- and post-activity monitoring.

2.4 Remote Video Monitoring

2.4.1 Description of Methods

Advances in video technology have made it possible to conduct behavioral studies of marine mammals in very remote locations over extended periods of time. The use of remote video recording, including animal-borne video cameras and surface-mounted, remotely operated cameras at rookeries and haul-outs has increased dramatically in the last decade (Loughlin et al. 2005) and has advanced the study of pinnipeds in many ways. Animal-borne cameras have been used to determine energy expenditure and prey ingestions in SSLs. However, there are limits to and biases associated with this technique. For example, commercially available portable video cameras are still fairly large and could potentially introduce a substantial amount of drag to the animals as they forage under water, thereby inflating the estimated cost of foraging. In addition, while it is possible to identify common prey species

from captured footage even with low light levels, setting the rate of recording to ensure such brief feeding events are captured is limited by the data storage and power supply capabilities of this technology (Andrews et al. 2005).

Remotely operated video cameras mounted on land have been used with very good success for collection of behavioral data at SSL rookeries in Alaska (Chiswell Island, Benjamin Island), Oregon (Rogue Reef and Orford Reef), and California (St. George Reef) (Loughlin et al. 2005) and at NFS rookeries in Alaska (Pribilof Islands). Remotely operated video cameras are typically deployed when most of the animals are absent, or in conjunction with other research, to minimize disturbance. Cameras are mounted at several vantage points to record all or a portion of the rookery or haul-out. Cameras can either record images for periodic retrieval (by removing the recording media) or deliver images in real time to a remote location. For example, the cameras used for the Chiswell Island projects are equipped with 12-18 power optical and digital zoom lenses, mounted in a waterproof housing that includes remotely controlled pan tilt, zoom, and windshield wiper/washer functions. These cameras can be remotely redirected by staff at the ASLC to scan the haul-out area or zoom in on animals of particular interest. Both audio and video signals are sent by cable to a central control tower, which transmits images and sound to a central headquarters at the ASLC in Seward, Alaska for viewing and storage of the data. The cameras and control tower are powered by a 12-volt battery system charged by solar and wind power (Andrews et al. 2005).

2.4.2 Objectives of Research

The SSL Recovery Plan and NFS Conservation Plan identified the need to monitor the health, condition, and vital parameters of SSLs and NFSs. More specifically, this included conducting intensive studies on rookeries, on vital parameters such as sex and age classes on rookeries, pup production and survival, and observations on material care of pups. Objectives of the remote video program are to provide basic information on SSLs/NFSs, the ecological and biological population aspects of SSLs/NFSs, develop predictive models on population, and provide data necessary for the conservation and recovery of SSLs/NFSs. The remote video camera technology allows for direct observation of marine mammals in their natural habitat to collect data on these vital parameters, both in real time and by recording for review at a later time. These methods minimize the need for human observers in the field.

2.4.3 Use of Data

Observations on rookeries focus on the breeding biology of these animals, including pup numbers, birth dates, suckling times, maternal care, parental period duration, aggressive interactions, foraging cycles, and rookery attendance (Maniscalco et al. 2002, 2005; Parker et al. 2005; Andrews et al. 2005). Changes in the population's vital parameters—such as sex ratio, age distribution, and pup production—provide information on the status of the population and source of the decline of the SSLs/NFSs.

2.4.4 Effects of Research

Because biologists are remote from the rookery or haul-out, and observation of SSLs/NFSs occurs from viewpoints overlooking the rookeries, no animals are taken by harassment, disturbance, or capture under remote video protocols.

An indirect effect of the remote video monitoring is primarily associated with installation of the camera and data transmission systems on the rookeries. Installation of cameras is typically done prior to the breeding season to minimize disturbance to the rookery. Maintenance of equipment during the breeding season can lead to some level of disturbance at the rookery, especially if helicopter access is required. Cameras are typically mounted above or on the periphery of the rookery for optimum visibility; therefore, unscheduled maintenance of the camera typically does not require walking through the main breeding areas.

2.4.5 Mitigation

Because there is no measurable disturbance effect of the cameras or transmission equipment on the animals on the rookery or haul-out, mitigation measures are not necessary. However, efforts should be undertaken to ensure that maintenance of the equipment is minimized during critical times of the year. Routes of access to cameras and other equipment for unscheduled maintenance should be determined in advance in order to limit disturbance should maintenance be required.

2.5 Capture and Restraint

2.5.1 Description of Methods

2.5.1.1 On-Land

SSL are captured by a variety of methods depending on their age, size, and location. Young pups on rookeries can easily be picked up by hand. Subadult SSLs and adult female SSLs can be captured on-land with a large hoop net (3 ft in diameter and 5 ft long handle). Subadult and adult SSLs of either sex can be captured by remotely darting with injectible immobilizing agents.

To capture SSLs using a hoop net, one or two biologists approach as close as possible to the target animal before entrapping it in the net. Once captured, the animal is transferred to a fabric restraining wrap used for weighing the animal. The animal may be restrained in this wrap during measurements and while collecting samples, or it may be sedated and/or anesthetized for collection of tissue samples or attachment/insertion of scientific instruments. See Section 2.6 for description of use of remotely delivered immobilizing agents used to capture SSLs. NFS are typically captured by herding into barricades.

2.5.1.2 In-Water

SSLs are captured in the water using the lasso technique developed by the ADF&G or floating cage trap developed by NMML. For the underwater lasso technique, two or three divers, supported by a skiff and a larger vessel, approach a haul-out underwater. The natural curiosity of young SSLs draws them to the divers. After a brief period of accustomization, SSLs will approach close enough that a rope lasso tended by personnel in the skiff can be placed around them by the divers, slightly behind the fore flippers. The lasso is tightened and the rope is retrieved by the skiff crew. Animals are wrapped in a restraining net and pulled into the skiff. Animals are placed into a restraint box aboard the skiff, where they are transported to a larger vessel where they will be immobilized with gas anesthesia for handling, sampling, and instrument attachment.

Researchers may also deploy a floating cage trap. The trap consists of a 12-ft wide buoy with a 12-ft by 12-ft platform for a haul-out surface. There are 6-ft high steel cage walls around the perimeter of the platform, with a wide trap door on one side. SSLs are able to haul-out and return to the water freely through the trap door. To capture SSLs, the trap door is dropped when SSLs are hauled-out inside. Captured SSLs are then transferred into a holding cage on a research vessel. They are moved one at a time from the holding cage into a stainless steel squeeze cage (analogous to handling runs and squeeze cages used for livestock). While in the squeeze cage, the animal is weighed, measured, tagged, branded, and samples are taken. When all the procedures are complete, the squeeze cage is opened and the animal is released. The average handling time is approximately 10 minutes.

2.5.2 Objectives of Research

The SSL Recovery Plan and NFS Conservation Plan identified the need to monitor the health, condition, and vital parameters of SSLs and NFSs. More specifically, this included developing indices of condition and obtaining measurements and samples using non-lethal techniques. The objective of capturing animals is to continue ongoing

studies of the physical condition of pups and juveniles outside of the breeding season, particularly during the winter. Assessing the condition, status, and foraging behavior of pups as they are weaned and of juveniles that are foraging for themselves is the most direct means to understand this critical time in the animal's life.

2.5.3 Use of Data

These data will provide information on the relative health of the population when compared to results from preceding years and known standards. These data particularly address seasonal changes in the physical condition of SSLs, which will contribute to assessing the potential impacts of commercial fisheries and environmental changes on the status of SSLs.

2.5.4 Effects of Research Methods

Restraint procedures constitute one of the most stressful incidents in the life of an animal, and intense or prolonged stimulation can induce detrimental responses (Fowler 1986). Each restraint incident has some effect on the behavior, life, or activities of an animal. A variety of somatic, psychological, and behavioral stressors can be associated with capture and restraint of wild animals. These include strange sounds, sights, and odors, the effects of chemicals or drugs, apprehension (which may intensify to become anxiety, fright, or terror), and territorial or hierarchical upsets associated with displacement of animals by researchers who come onto rookeries and haul-outs. Animals that are stressed can incur contusions, concussions, lacerations, nerve injuries, hematomas, and fractures in their attempts to avoid capture or escape restraint (Fowler 1986). The stress response can change an animal's reaction to many drugs, including those commonly used for chemical restraint, which can have lethal consequences.

As reviewed in Fair and Becker (2000), the physiological responses in dolphins to capture stress include responsive indicators such as decreased eosinophil counts, imbalances of thyroid hormones, responses to glucocorticoids, and elevations of other blood constituents such as glucose, iron, potassium, and several enzymes. Continuous stimulation of the adrenal cortex, as from stress associated with chronic disturbance or repeated capture, can cause muscle weakness, weight loss, increased susceptibility to bacterial infections, and poor wound healing, and can lead to behavioral changes including increased aggressive and antisocial tendencies (Fowler 1986).

Capture myopathy is a possible consequence of the stress associated with chase, capture, and handling in numerous mammal species (Fowler 1986). Capture myopathy is characterized by degeneration and necrosis of striated and cardiac muscles and usually develops within 7 to 14 days after capture and handling. It has been observed both in animals that exert themselves maximally and those that remain relatively quiet, and occurs with either physical or chemical restraint. Fear, anxiety, overexertion, repeated handling, and constant muscle tensions such as may occur in protracted alarm reaction are among the factors that predispose an animal to this disease. A variety of factors may function in concert or individually. The muscle necrosis is likely due to acidemia resulting from a build up of lactic acid following profound muscle exertion: once necrosis has occurred, the prognosis for recovery is not favorable. The number of times an animal is captured, the method(s) of restraint, as well as the age and general condition of the animal are all factors that will affect an animal's response to capture.

The annual reports from the current and previous permits held by NMML and ADF&G indicate that some animals showing distress and/or adverse reactions to drugs or handling that were not immediately released, subsequently died. The annual reports also state that most SSLs (pups, juveniles, and adults) typically struggle initially upon capture, but calm down quickly after being hand-restrained.

2.5.5 Mitigation

To minimize the impacts from capture/restraint, the following methods may be used:

- Pups are processed in small groups. Prior to handling, a small pod of pups (10-20) is rounded up. These animals are allowed to rest before handling, are watched over for signs of distress, are kept cool, and animals showing signs of distress are released.

- Pups are restrained by hand, not with a restraint board. The primary handler is always an experienced biologist who monitors the pup for signs of stress.

Methods and equipment for capture/restraint are constantly being refined to limit the amount of stress and reduce any potential pain and suffering associated with the capture. Underwater captures have greatly reduced the potential for injury compared to land-based captures and have facilitated faster handling times. Efforts to approach or handle a particular animal are immediately terminated if there is any evidence that the activity(ies) may be life-threatening.

2.6 Anesthesia Sedation, and other Drugs

2.6.1 Description of Methods

In order for drugs to be administered, including general anesthesia, to SSLs or NFSs, they must first be captured and restrained (Section 2.5). Occasionally, administration of anesthesia to animals, as well as other drugs, is done through the process of darting without, or prior to, the previous use of capture and restraint. In darting, animals are generally stalked and darted from a distance.

Delivery of drugs, including general anesthesia, to marine mammals can be difficult due to their particular anatomical and physiological specializations, as well as the operational logistics precipitated by the marine environment they inhabit. These intricacies are complicated further because determining the proper dosage, which is primarily a function of age, weight, and health, is often difficult. The proper dosage is vital to not only the success of the drug, but also the survival of the animal itself, as overdoses can have lethal consequences (Fowler 1986). Estimation of body mass, which is used to calculate drug dosage, can be done with an accuracy of ±20 kilograms (kg) by an experienced biologist (Loughlin and Spraker 1989; Heath et al. 1996). The effective dose of a particular agent varies with species.

Both the safest and most effective site for the insertion of darts, or projectile syringes, is in the deep muscle tissue areas of the hind limbs. For proper absorption and function, the drug should be injected into muscle tissue. However, the blubber layer of pinnipeds can make delivery of an injection to the muscle difficult. The usual induction time for most chemical agents is 10 to 20 minutes following an intramuscular injection (NOAA Fisheries 2005c)

2.6.1.1 Atropine (pre-anesthetic)

Atropine, a naturally occurring alkaloid of Atropa belladonna, is a prescription drug that can only be obtained from a veterinarian. Atropine is a premedication for anesthesia and is usually administered intramuscularly (at about a 0.02 milligrams per kilogram (mg/kg) dose for California sea lions) about 10 minutes prior to use of immobilizing agents (Haulena et al. 2000). Atropine reduces salivation and gastric and upper respiratory tract secretions (Anderson 1982), and in pinnipeds, is usually administered in doses of 0.005 mg/kg (Sweeney 1974). Atropine is a muscarinic-receptor antagonist, meaning that it blocks parasympathetic stimulus to reduce heart rate. This slow heart beat rate, or bradycardia, is an important element of the cardiovascular dive response that is sometimes mimicked during Telazol anesthesia. Thus, atropine can reduce the cardio-respiratory problems associated with the use of Telazol (Calkins 2004).

Effects

The administration of Atropine can result in the accumulation of fluid in the lungs and has been described as the cause of death in two immobilized gray seals (Baker and Gatesman 1985). Also, as with any procedure that breaks the epidermal layer, there is a risk of infection.

2.6.1.2 Telazol

Telazol, a proprietary (Fort Dodge) combination of tiletamine and zolazepam, is a prescription drug that can only be obtained by a veterinarian. It belongs to a class of drugs known as dissociative hypnotics, is similar to phencyclidine (PCP), and works by disrupting the central nervous system. Telazol serves as a general anesthetic (or to induce sedation prior to administration of anesthesia) that provides immobility and muscle relaxation, which results in a state suitable for various diagnostics interventions.

Effects

Telazol is generally safe, but can cause substantial side effects in some animals, like those with hypersensitivity, heart, lung, or kidney disease. Extending immobilization by administering repeated doses of injectable agents is associated with a high risk of mortality, and an additional dose of Telazol should never be given (Gage 1993). A study reported that out of 51 adult female SSLs that were immobilized with Telazol between 1992 and 1994, there were 5 deaths (9.8 percent) (Heath et al. 1996). Two SSLs drowned in pools of water on the rookery and another death was the result of a malfunction with the gas anesthesia machine. Only two of the mortalities were due to Telazol complications: 1) in February 1993, under permit No. 771 (64) issued to NMML, an adult female that was darted with Telazol died; and 2) under that same permit and timeframe, a pup died after it was mistakenly darted with Telazol when it moved in front of the target animal. This mortality was apparently the result of the unintentional intravenous injection of a drug intended for intramuscular injection in a larger animal (Merrick 1993). Also, in one study, about 10 percent of animals administered Telazol were observed to become apneic (stop breathing) within five minutes of administration (Gage 1993). Between 1995 and 1997, however, Calkins was involved in 31 Telazol immobilization attempts, and encountered only one (3.3 percent) mortality (R.B. Heath, personal communication). This reduction in mortality could be attributed to the addition of atropine sulfate to Telazol in the injection dart (see relationship between atropine and Telazol described in 2.6.1.1; Calkins 2004).

Another possible effect concerning the administration of Telazol is the effect on the fetus or pup, as it has been shown to cross the placental barrier (Telazol drug information sheet; CI 5129-1; Fort Dodge Animal Health, Fort Dodge, IA).

2.6.1.3 Midazolam

Midazolam is proprietary (Versed) benzodiazepine sedative that depresses the brain, likely through a reduction in serotonin levels. It is a prescription drug that can be obtained legally by a veterinarian as an extra-label drug, even though it is not approved for animal use by the Federal Drug Administration (FDA). Midazolam is often used with other drugs to ease an animal in and out of anesthesia. Midazolam (M) was administered with pethidine (P) (M:P = 0.22:1.1 mg/kg) in three leopard seals (*Hydrurga leptonyx*) and resulted in manageable sedation levels, but a combination of tiletamine and zolazepam (i.e., Telazol) appeared to be more effective (Woods et al. 1994a).

Effects

Midazolam is generally safe, but can cause side effects in some animals, such as those with a hypersensitivity to the drug. Midazolam usually will result in disorientation associated with sedation, but can actually induce a paradoxical reaction of excitement in some animals. Administration of midazolam (with pethidine) has resulted in apneic condition and eventual death of crabeater seals (*Lobodon carcinophagus*) in Antarctica (Tahmindjis et al.

2003). Although, one study reported several potential advantages of midazolam over diazepam (i.e., Valium): water solubility, high lipophilicity at physiologic pH, and rapid metabolism (Reves et al. 1985). Also, if injected, as with any procedure that breaks the epidermal layer, there is a risk of infection.

2.6.1.4 Isoflurane Gas

Isoflurane gas, a halogenated ether, is an inhaled general anesthetic that precipitates reversible depression of the central nervous system, which results in unconsciousness, voluntary muscle relaxation, and inhibition of reflex activity (Fowler 1986). Inhalation anesthetics, such as isoflurane gas, are used to induce anesthesia in animals that can be manually restrained and are often used to increase the depth of anesthesia in animals previously immobilized by an injected agent (NMFS 2005b).

Effects

The effects of inhalation anesthetics, like isoflurane gas, appear to be relatively predictable with increased doses, which is different than injectable agents that often are unpredictable and variable when used on animals (Fowler 1986). Heath et al. (1996) reported that one SSL died because of a malfunction in the gas anesthesia machine. Overall, animals in captivity have been observed to fully recover from anesthesia with isoflurane after 8 hours (Gage 1993). In general, isoflurane gas appears to have the best recovery characteristics, and it is safe and reliable in otariids (Haulena and Heath 2001).

2.6.1.5 Valium

Valium is a registered brand of diazepam that depresses the brain, probably through a reduction in serotonin levels. It is a prescription drug that can be obtained legally from a veterinarian as an extra-label drug, even though it is not approved for animal use by the FDA. Valium is usually injected intramuscularly (at a dose of about 5 milliliters (ml) /100 kg for SSLs) and is often used with other drugs to ease an animal in and out of anesthesia. It is metabolized slowly by the liver and excreted by the kidneys; clinical effects generally disappear within 60 to 90 minutes (Fowler 1986). Valium is often used during the capture and restraint, tagging, and blood and tissue collection processes (NMFS 2005b).

Effects

Valium is generally safe, but can cause side effects in some animals, such as those with an illness or a hypersensitivity to the drug. Possible side effects include bradycardia (slowed heart rate), respiratory depression, tremor, confusion, blurred vision, nausea, vomiting, depressed gag reflex, lethargy, and ataxia (inability to coordinate muscle activity during voluntary movement) (NMFS 2005c). Generally, Valium should not be administered long-term. It usually will result in disorientation and weakness associated with sedation, but can actually induce a paradoxical reaction of excitement in some animals. In one study, diazepam was safely administered with ketamine, but resulted in apnea and entire-body shaking (Woods et al. 1994b). The additional effects of injecting Valium are probably incidental, relative to the capture and restraint, but have the potential to be serious and as with any procedure that breaks the epidermal layer, there is a risk of infection.

2.6.1.6 Flumazenil (Anesthetic Reversal for Midazolam)

Flumazenil reverses the sedative effect of benzodiazepines (e.g., Midazolam). At a dose of approximately 0.001-0.003 mg/kg, flumazenil has been successfully administered to reverse sedation for crabeater seals in Antarctica (Tahmindjis et al. 2003).

Effects

The additional effects of injecting flumazenil are probably incidental, relative to the capture and restraint, and usually do not present the same potential risk as the administration of drugs that depress the brain (i.e., Valium) or inhibit the central nervous system (i.e., Telazol). However, flumazenil can result in convulsions and other side effects, if it is used to try to reverse the effects zolazepam (one of the two principal components of Telazol) without also incorporating tiletamine, an agent for the reversal (the other half of Telazol) (NOAA Fisheries 2005c).

Due to its nature as a benzodiazepine receptor antagonist, flumazenil has been suggested as an antidote to benzodiazepine (i.e., midazolam) overdoses. However, when flumazenil (as well as adrenaline and doxapram) was administered to two crabeater seals in response to an apneic condition induced by supplementary doses of midazolam and pethidine, the two seals did not respond positively and died (Tahmindjis et al. 2003).

2.6.1.7 Synthetic Adrenocorticotropic Hormone (ACTH)

Adrenocorticotropic hormone (ACTH) stimulates the adrenal cortex and is used to evaluate adrenal function and screen for problems with the adrenal glands (Gulland et al. 1999). ACTH causes the adrenal glands to produce the hormone cortisol, which helps to manage stress. Gulland et al. (1999) found that an injection of ACTH in free-living seals induced a significant increase in mean plasma cortisol, but not of mean aldosterone levels, 60 minutes after injection. Synthetic ACTH is in the form of cosyntropin, which has the tradename Cortosyn.

Effects

Administration of ACTH can result in side effects such as bradycardia (slowing of the heart rate), tachycardia (increasing of the heart rate), hypertension, and/or rash. Also, as with any procedure that breaks the epidermal layer, there is a risk of infection.

2.6.2 Objectives of Research Methods

Anesthesia sedation, and other drugs, are primarily used for immobilizing the animal so that vital statistics (i.e., length and weight) can be gathered. Immobilization of SSLs can also be used as an opportunity to perform other procedures such as ultrasound, tooth extraction, flipper tagging, or medical treatment.

2.6.3 Use of Data

The research methods associated with anesthesia, sedation, and other drugs will produce data that will make a contribution towards the recovery of SSLs and NFSs in particular, and advance the knowledge of marine mammals in general. Moreover, the ultimate reasoning for much of the current research is summarized as three objectives in the SSL Recovery Plan. The objectives are as follows: 1) the collection of information on status and vital rates, 2) research programs to collect information on the remaining threats to recovery, including fisheries and other anthropogenic factors, and 3) the implementation of conservation measures to remove impacts of remaining threats to recovery.

2.6.4 Effects of Research Methods

For the specific effects of a particular agent, please refer to the corresponding section of the previous discussion (Section 2.6.1). The effects of research methods may vary depending on which marine mammal receives the agents, but the differences are not likely to be substantial.

Thus, the following discussion of research methods effects, as well as the previous analysis of various individual drugs, can be applied to marine mammals in general. The administration of drugs is an additive process that

begins with the previously mentioned capture and restraint, and its corresponding effects, and then progresses to the injection process and its associated effects.

Under many circumstances, it is probable that the actual capture and restraint of the animal would have a greater proportional impact to aggregate effects than the administration of drugs. Also, sometimes the administration of agents is actually part of the capture and restraint process (Section 2.5). The stress response induced by the capture and restraint process can change an animal's response to many drugs, including those commonly used for chemical restraint, which can have lethal consequences (NOAA Fisheries 2005c). Unintentional injection of drugs into the blubber often results in aseptic necrosis, which is usually the result of the blubber not being well vascularized and can sometimes lead to abscesses (Geraci and Sweeney 1986; Fowler 1986). Subsequently, the subcutaneous administration is usually problematic in marine mammals. One problem associated with subcutaneous drug administration is the possibility of accidentally injecting drugs subdurally (beneath the dura, a fibrous membrane covering the central nervous system) when trying to inject into the extradural vein (Stoskopf 1990).

Also, in some situations an intravenous injection instead of intramuscular can mistakenly occur, which can be problematic. Injections into the chest cavity or stomach region can result in puncture of the lungs or stomach, which can lead to the death of the animal. Also, the lag between the time a drug is administered and when it takes affect (10-20 minutes for most agents) creates a dangerous situation because an animal can be startled by the darting and move into the water before the immobilization has taken affect. The animal then could drown after the agent has become fully active.

Hyperthermia can occur in animals under anesthesia because blubber can make heat dissipation a problem. Hyperthermia can even be a problem at ambient air temperatures which are comfortable to but at which otariids over 25 kg tend to become hyperthermic during anesthesia (Gage 1993). Because many drugs can affect thermoregulation, hypothermia is also a possible result of the administration of chemical agents.

2.6.5 Mitigation

There have been numerous steps taken to mitigate potential adverse impacts associated with administration of certain agents. Some of these attempted mitigation measures include:

- Adding atropine sulfate to Telazol in the dart has helped to reduce the cardio-respiratory complications of Telazol (Calkins 2004).
- Improving gas anesthesia machines, which have resulted in SSL deaths due to malfunction (Heath et al. 1996; Calkins 2004).
- Excluding SSLs close to shoreline, as they can be frightened into the water by the darting and then drown as they become immobilized (Calkins 2004; Woods et al. 1994b).
- Using photogrammetry to measure body length and assist in body mass calculations, which will help in administration of the proper doses of agents (Calkins 2004).
- Allowing only qualified veterinarians, or other personnel with necessary experience, to perform procedures and administer agents (Calkins 2004).
- If a SSL dies, ceasing subsequent research until qualified personnel can review the incident (Calkins 2004).
- Providing for a pup left behind due to the death of the mother. The pup should be handled in a humane manner in consultation with NMFS regional office and ASLC veterinary staff (Calkins 2004).
- Realizing that during target research months in which a female might be pregnant (June through August), the embryo will be at the arrested blastocyst stage and thus the effects from a drug like Telazol would be negligible (Calkins 2004).

2.7 Temporary Marking: bleach, dye, paint, and hair shearing

2.7.1 Description of Methods

The protocol for temporary marking of animals using paints, hair bleaches, and dyes has been used successfully to mark many species of wildlife including marking individual SSLs and other pinnipeds (Hobbs and Russell 1979; York et al. 2005). Bleaches remove pigments from hair whereas dyes add pigment to the hair. Temporary marking techniques, similar to permanent marking, initially involve the capture and restraint of animals, usually a pre-weaned pup (Section 2.5) The purpose of temporary marking is generally similar to permanent marking: to identify an individual from a relatively long distance without having to recapture or disturb it multiple times. However, because pinnipeds molt annually, these marks are only good for a period of several weeks to several months. In some cases, bleached hair will last for up to a year (Gentry and Holt 1982).

The techniques for creation of these temporary marks depend on the type of hair bleach, paint, or dye used. The time of year, weather during application, wetness of the pelage, and the amount of drying time before the animal enters the water, are all factors in the longevity of these temporary marks. The success of these substances to mark individual animals also depends on the ability of the substance to adhere to wet fur or hair. One of the advantages of using paints or dyes is that they can be applied by hand, or remotely by a carbon dioxide (CO_2) gun that propels paint-filled pellets. Paint-filled pellets can also be used on mature adults too large to capture and restrain.

A drawback of this type of marking is that it does not allow for using detailed alpha numeric characters and is very limited in application. The firing of pellets has potential drawbacks in that researchers need to be relatively close to the target animal to be accurate.

Shearing of hair or fur to create temporary marks is useful in situations where short-term identification of a few individuals or a group of individuals is needed. Hair in a prominent part of the body can be sheared in the form of letters or numbers, or a single patch can be removed. With NFS, hair shearing removes the outer guard hairs to expose the light inner pelage and leaves a lighter colored patch. A hot-branding iron is also occasionally used to create temporary marks on the pelage of NFSs by singeing the guard hairs to expose the lighter underfur (Gentry and Holt 1982).

If animals can be captured and restrained, paints, bleach, and dyes can be used to make unique alphanumeric marks on their fur. The marks need to be made large enough to be easily read from a distance. Large color marks of numbers and letters are painted on the sides of NFSs using human blue hair dye. Within 2-3 days, bright beige to dark orange color appears in the affected area of the pelage (Gentry and Holt 1982). Capture and restraint likely involves more stress to the animal than remote marking, and may cause incidental disturbance of other seals at the rookery.

2.7.2 Objectives of Research Methods

The SSL Recovery Plan identifies the need to monitor the health, condition, and vital parameters of SSLs. More specifically, this includes conducting intensive studies on rookeries in order to develop indices of condition and obtaining measurements and samples using non-lethal techniques. The NFS Conservation Plan identifies the need to continue census of adult males and estimates of pup production and survival. The objective of temporary marking by these methods is for short-term identification of individual animals for purposes of census activity, behavioral studies, and collection of data on vital parameters such as survival, reproductive success, and site fidelity.

2.7.3 Use of Data

2.7.3.1 Steller Sea Lions

Temporary marking of individual animals can be used for many of the same purposes as permanent marking techniques, but to a much more limited extent. For short term investigations at rookeries and in coordination with remote video camera studies, temporary markings of individuals can substitute for more permanent markings and cause less stress on the animals.

2.7.3.2 Northern Fur Seals

On the Pribilof Islands, highly visible temporary marks are placed on captured animals in addition to other more permanent marking such as tags (Gentry and Holt 1982). This allows for better re-sighting of tagged animals from a distance.

Census of NFSs up to the late 1950s relied on direct pup counts. Beginning in 1960, more sophisticated mark-recapture studies were developed to estimate pup production. Experimenting with different methods resulted in the use of marks and tags to help estimate numbers, and in 1963 the method of shearing a small patch of guard hair from the pup's head to expose the light underfur was instituted (Chapman and Johnson 1968). The sheared pups were used as a method for estimating total numbers of pups in the rookeries (Antonelis et al. 1988; York and Towell 1997).

During rescue of entangled two to four year-old male NFSs, animals captured are shear-marked, by cutting the guard hairs using hand-held shears to expose the lighter under-fur. These shear-marked NFSs can be resighted during subsequent searches and round-ups, and provide some evaluation of the success of the disentanglement efforts and overall degree of entanglement during the round-up period (Gentry and Holt 1982).

2.7.4 Effects of Research

Similar to other methods of marking, animals have to be initially captured and restrained prior to application of marking substance or use of shears. Effects are generally due to capture and restraint, similar to other marking methods (Section 2.5). Because these methods are noninvasive, no anesthesia is required.

2.7.4.1 Steller sea lions

Temporary marking of SSLs with bleach, paints, or dyes has not been identified as affecting survival of pups or adults. Toxicity of the marking substance is potentially an issue in that it can be removed during grooming and be ingested by the target animals or others nearby (Hobbs and Russell 1979).

For marking using shears, there have been no reported observations of physical injury or thermal stress in association with this technique (Gentry 1979).

2.7.4.2 Northern Fur Seal

With NFSs in the Pribilof Islands, quick-drying rubber-base highway paint or plastic resin naphtha-based paints have been used to create temporary markings (Gentry and Holt 1982). However, examination of the areas marked with naphtha-based paints shows that the growth of both guard hairs and underfur were affected by the procedure; therefore, this paint was not recommend for further use on NFSs (Gentry and Holt 1982).

Shearing has been extensively used for temporary marking at NFS rookeries in the Pribilof Islands. There is the potential for some degree of thermal stress in marked animals, although there have been no observations of this so far (Gentry 1979).

2.7.4.3 Other Pinnipeds

Using bleach paints or dyes to mark individual harbor seals is a common practice for many pinniped species such as harbor seal, Hawaiian monk seal (*Monachus schauinslandi*), and elephant seals (*Mirounga angurostris*) (Farrell and Jennings 1979; Gentry 1979; Henderson and Johanos 1988). No adverse effects of these temporary marking procedures were reported.

2.7.5 Mitigation

Mitigation of potential effects of temporary marking procedures is similar to that for other marking procedures requiring capture and restraint of the target animal. Efforts to reduce the direct effects of bleach, paints, dyes include:

- pups that are very young or in poor physical condition (under 20 kg) should be marked only with hair-clipping procedures;
- use of non-toxic substances is preferred; and
- CO^2 – use of fired paint or dye pellets should be limited to short distances to maximize success and limit contamination of the area from missed pellets.

2.8 Flipper Tagging

2.8.1 Description of Methods

Any animal captured (including pups) may be marked with plastic tags for future identification. Numerous plastic tags are available from commercial livestock sources in a variety of sizes, colors, and identifying symbols or numbers. In SSLs and NFSs, the tags are affixed through a fore-flipper, anteriorly, in loose skin, near the area where the flipper meets the body. In most cases, each animal receives two tags, one per flipper, to minimize the chance of losing the ability to identify the animal should one tag be lost. Flipper tags are subject to extreme physical abuse and are prone to high loss rates. Under ideal conditions, they can be expected to last four to six months. However, studies in captive pinnipeds suggest that tags can last one to two years (Dierauf 1990).

2.8.2 Objectives of Research

Flipper tagging positively identifies individual animals but requires recapture or recovery of the animal. The objective of temporary marking by these methods is for short-term identification of individual animals for purposes of census activity, behavioral studies, and collection of data on vital parameters such as survival, reproductive success, and site fidelity.

2.8.3 Use of Data

Because the tags are not typically visible from a distance, recapture is required to read the tag. This technique is useful as a secondary mark on animals tagged with other methods.

2.8.4 Effects of Research

2.8.4.1 Steller Sea Lions

These tags are best considered semi-permanent markers as they can and do pull out because SSLs use their foreflippers in both aquatic and terrestrial locomotion. In addition, due to the effects of capture and restraint (described in Section 2.5), it is likely that affixing these tags causes more than momentary pain. There is a

potential for infection at the wound site or when a tag pulls out of the flipper. In moving about a rookery or haul-out site, there is the potential for a tag to be torn out of the flipper. According to the permit reports, no tag-related mortality has occurred. Merrick et al. (1996) reported that flipper tags can become difficult to read as the colors/markings fade over time and that they are not readily visible from any distance. In addition, the gregarious nature of SSLs causes them to group together and obscure the flippers. There is no information on long-term tag retention, average retention rates, or rate of infection caused by flipper tagging in the annual reports from NMFS permit holders.

2.8.4.2 Northern Fur Seals

A study conducted by Trites (1991b) assessed the effects of tagging and handling on NFS pups and found that tagging too early does not alter growth rates, as had been previously suggested (Roppel 1984), but that smaller pups were selected for tagging because they were more easily accessible. The other potential effects of tagging on NFSs would the same as those identified for SSLs.

2.8.4.3 Other Pinnipeds

A study of the effects of flipper tags on Hawaiian monk seals in which tagged pups and untagged pups were treated in a similar fashion, revealed that the tagged pups experienced no increase in mortality over the other group and showed similar behavioral traits as the untagged pups after 32 weeks (Henderson and Johanos 1988).

2.8.5 Mitigation

Care is taken to avoid placing the tag in an incorrect location: if the tag is too low, the animal will walk on it, if the tag is too high, it will irritate the flank area. Only qualified personnel with sufficient experience in this technique should be allowed to perform these procedures. It is also recommended that only animals believed to be in optimal health be captured and subjected to this procedure.

2.9 Hot-brands

Hot-branding for permanent marking of pinnipeds has been successfully used since the early 20th century (Wells 2002). The practicality of hot-branding as a means of permanently marking pinnipeds in the wild has been demonstrated in several studies (Hobbs and Russell 1979; Calkins and Pitcher 1982). However, there has been insufficient re-sighting effort of the more than 15,000 SSLs branded by ADF&G and NMML since 1975 to validate the merits of hot-branding versus the potential for adverse impacts to individual SSLs (Merrick et al. 1996).

2.9.1 Description of Methods

The protocol for permanent marking of animals using hot-branding involves capture and restraint of animals and the application of a hot-branding iron to the skin of captured animals to kill the hair follicles and pigment-producing cells. This creates a permanent bald brand with large visible numbers or letters that can be seen and used to identify an individual from a relatively long distance without having to recapture or disturb them multiple times (NMFS 1993a). Hot-branding is a preferred method of permanently marking pinnipeds (Gentry and Holt 1982; Merrick et al. 1996; NMFS 2002).

Branding Irons: Commercial branding irons have been found to be unsuitable for SSLs; therefore, specifically designed brands have been developed to minimize injury to the animals and to maximize the readability of the marks (Merrick et al. 1996). Rounded steel (rolled steel stock) is used for the brand materials because it is believed that square steel used in commercial brands would tend to burn the edges of the mark, making the area slow to heal, scab over and produce a blurred brand mark (Merrick et al. 1996). The shaft of the brand is approximately ½ inch thick with a wooden handle. A pair of locking pliers on the shaft is also used as a second

handle. The branding irons are typically heated with a propane forge. These units are easy to transport and provide sufficient heat for the process. There is one letter or number per brand with characters approximately 5 centimeters (cm) wide by 8 cm high. Brands on SSLs in Oregon used numbers slightly larger (6 cm by 10 cm) (NMFS 1992). Brands of this general size have been found to be sufficient to be visible at 100 meters (m) with 7 X binoculars (Merrick et al. 1996).

The process of branding SSL pups on rookeries usually requires that the majority of juvenile and adult animals be driven from the rookery. Researchers round up a group of pre-weaned pups that are then corralled against a cliff or boulders and taken one by one to be weighed and measured. When they are ready for branding, one pup at a time is captured and restrained by two researchers; one sitting over the animals midsection and the other holding the hind flippers so that the animals is immobilized and movement will not blur the brand (Merrick et al. 1996). Gas anesthesia, preferably isoflurane, can be administered to promote safe handling and minimize pain. NMFS and ADF&G have been using anesthesia on pups for branding since 1993. The researcher doing the branding then extends the fore flipper and gently steps on it to hold it in place to ready the animal for branding (Merrick et al 1996).

The branding irons, which have been heated to red hot, are quickly applied to the skin on one or both shoulders. Exposure time is approximately three to four seconds at a pressure of approximately 5 pounds (lbs). Contact is maintained until the hair is burned off and the skin is lightly singed. This procedure is repeated for each brand character used (Merrick et al. 1996).

2.9.2 Objectives of Research

The SSL Recovery Plan identified the need to monitor the health, condition, and vital parameters of SSLs (NMFS 1992). More specifically, this included conducting intensive studies on rookeries in order to develop indices of condition and obtain measurements and samples using non-lethal techniques. The NFS Conservation Plan identifies the need for studies on the long-term survival and reproduction rate and on the general condition and health of NFS populations (NMFS 2006b). Permanent marking of individuals is an effective technique in these types of studies.

2.9.2.1 Steller Sea Lions

The purpose of hot-branding SSLs is to be able to uniquely identify individual animals in the populations and follow these individuals through re-sighting in order to understand the population dynamics, behavior, and movements (Merrick et al. 1996). The ability to identify individual animals over the long-term is considered important in determination of vital rates such as age-specific survival and age at first reproduction. Studies on seasonal movements, site fidelity, and dispersal are facilitated by the ability to identify individuals in a population (Mellish et al. in review).

2.9.2.2 Northern Fur Seals

NFSs do not generally have any distinguishable markings that can be used to identify individuals from one year to the next. Hot-branding is a method of permanently marking individuals to aid in census work and to collect data on vital parameters. Hot-branding of NFSs is complicated by the thick guard hairs and undercoat, which adsorbs heat from the branding iron and must be removed before branding the skin (Gentry and Holt 1982). Three or four applications of the hot iron are some times necessary before the brand contacts the skin directly (Gentry and Holt 1982). Bald brands (either cold or hot) could have serious consequences for NFSs, because they rely on their pelage for thermoregulation and have a small surface area compared to SSLs, so a proportion of skin left without hair would be greater to produce a same size mark needed for detection from a distance. However, permanent marking methods (branding pre-weaned pups) and reliable techniques for later re-sighting these marked pups as two- or three-year-olds have had limited success in the 1980s and are not currently used (Gentry 1998).

2.9.3 Use of Data

2.9.3.1 Steller Sea Lions

Data from re-sighting of branded animals are useful in determining seasonal use and movement patterns, dispersion from natal sites, site fidelity and distribution and dispersal of SSLs. Collecting useful data on each of these parameters requires marking animals at several sites over multiple years. Consistent efforts for re-sighting marking animals over many years is also an important factor. Data from re-sighting of individual animals have been instrumental in the separation of the western and eastern distinct population segments (DPS), documenting seasonal movement, immigration and emigration of animals between haul-out and rookeries, site fidelity, and in determining age-specific survival rates.

2.9.3.2 Northern Fur Seals

Hot-brands have been used to permanently mark NFSs for behavioral studies on the rookeries and some branded animals have been followed for five years (Gentry and Holt 1982). Limited use has been made of hot-branding of NFSs in recent years. Because NFSs move offshore after the breeding season, the potential for re-sighting animals away from the colonies is limited.

2.9.4 Effects of Research

2.9.4.1 Steller Sea Lions

Effects of hot-branding on individual marine mammals include brief acute stress from which the animal recovers over a period of time. This acute pain is reduced with the use of anesthesia. This procedure also produces burns that penetrate the entire outer layer of the skin and into the inner skin layer. These burns are characterized by formation of blisters, swelling, and fluids seeping from the burned area and are accompanied by severe pain due to damage of capillary blood vessels in the skin (Mellish et al. in review). However, the total area affected is a small percentage of the animal's skin surface (NMFS 1992). Further, Merrick et al. (1996) state that studies of branded SSLs on Marmot Island in Alaska suggest branding may lead to increased mortality.

The hot-branding procedure (without anesthesia) probably causes more than momentary pain, and there is the potential for infection of the burned area, especially with the unsanitary conditions on rookeries and haul-outs (NMFS 1992). Captive SSLs were used to document the effects of branding on the health of SSLs. Results showed statistically significant increases in white blood cell count, platelet levels, globulin and haptoglobin concentration up to two weeks after branding. No significant differences were found in serum cortisol levels. The changes in health parameters after branding were consistent with minor tissue trauma and probably not physiologically significant, as all parameters were indistinguishable from baseline levels after seven to eight weeks (Mellish et al. in review).

There is risk of injury or death from the process of capture, restraint, and anesthesia of animals before branding. In order to brand a large number of pups, researchers gather them into large groups for processing. Moving pups into groupings for branding can lead to injuries or death of the smaller and weaker pups. After branding, some injuries to pups can occur from trampling when adults return to the rookery or when a pup comes too close to a lactating female that is not its mother and is rejected (Mellish, et al. in review).

Mellish et al. concluded that while branding may present a statistically significant, short-term immune response in juvenile SSLs it does not appear to have any lasting physiological effects that might lead to impaired function or mortality. The physiological response to branding they observed is consistent with common low level, acute trauma that result from a variety of natural occurrences.

No mortalities have been observed during branding operations (NMFS 1993a). At Marmot Island between 1987 and 1988 and at Lovushki Island in Russia, only 2 dead marked pups were found out of 1000 pups marked. No difference was observed between survival of branded pups and pups marked with tags only (Merrick, et al. 1996). Survival of marked (branded and tagged) and unmarked pups at Marmot Island were similar (1.5 vs 1.4 percent, respectively) (Laughlin et al. 1996).

2.9.4.2 Northern Fur Seal

Little work has been conducted on the effect of hot-branding of NFSs. However, in studies of a similar species, New Zealand fur seal (*Arctocephalus forsteri*), in which 435 adults and pups were hot-branded; examination at 12 weeks showed the brands on 10 out of 27 pups had healed. In 2 pups, healing was > 95 percent; in 3 pups 90-95 percent of the wound had healed, and in 6 pups, 80-90 percent of the brand had healed (Wilkinson et al. 2001). There was no effect on pup growth when compared with unbranded pups. After one year, adults were examined for degree of brand healing. In the adults branded, 63 of 94 (64 percent) had healed, and 28 showed >95 percent healing of the brand (Wilkinson et al. 2001). Survival of the branded females appeared to be unaffected by the branding.

2.9.4.3 Other Pinnipeds

Hot-branding is a common method of permanently marking several species of pinnipeds around the world including harbor seal, elephant seal, gray seal (*Halichoreus grypus*), California sea lions, New Zealand fur seals, Weddell seals (*Leptonychotes weddelli*), and Cape fur seals (*Arctocephalus pusillus)* (Carrick and Ingham 1962; Hobbs and Russell 1979). The effects of hot-branding of elephant seals (*Mirounga leonina*) on Macquarie Island have undergone extensive review to determine the long-term effects to the seals (Gales 2000). Hot-branding has been conducted in the United States (U.S.) on harbor seals and SSLs on Rogue Reef and Orford Reef in Oregon, and St. George Reef in Northern California (NMFS 1992).

Studies of fitness in Southern elephant seals have indicated that branding does not negatively influence survival in the short- or long-term (McMahon et al. 2005, 2006).

2.9.5 Mitigation

Researchers have found that hot-branding, in comparison to freeze-branding, requires less application time and less pressure, with less chance of infections (Carrick and Ingham 1962; Warneke 1979). Branding irons should be hot enough that wounding does not occur (Merrick et al. 1996). Other measures to minimized harm to animals during branding operations include:

- To reduce the stress on the animals and possible increased risk of mortality, do not brand pups that are very young or in poor physical condition (under 20 kg).
- Allow only highly experienced and well-trained personnel to perform invasive procedures (including branding) according to their skills and qualifications.
- Process animals in groups small enough that all animals can be adequately monitored and to minimize handling/restraint time.
- Separate and monitor pups when collected, to ensure that they are not suffocating, being crushed, or aspirating milk.
- Immediately cease research-related procedures if an animal shows signs of acute or protracted alarm reactions that may lead to serious injury, capture myopathy, other disease conditions, or death; and monitor or treat the symptoms as determined appropriate.
- Restrain pups by hand, without using either a restraint board or drugs (except where the use of gas anesthesia is indicated for branding) and minimize handling time.

Additional mitigation measures for capture/restraint and anesthesia are found in Sections 2.5 and 2.6.

2.10 Freeze-Branding

2.10.1 Description of Methods

The protocol for permanent marking of animals using cryobranding (freeze-branding) is similar to hot-branding in several aspects. Initially it involves the capture and restraint of animals, usually pre-weaned pups, and the application of branding irons with large visible numbers or letters that can be seen and used to identify an individual from a relatively long distance without having to recapture or disturb it multiple times. Cold-branding involves application to the skin of a branding iron that has been cooled well below freezing (requiring a substantially longer exposure time than hot-branding). Freeze-branding can create two different types of marks: short contact kills the pigment producing cells (melanocytes), longer contact kills the hair follicles and pigment producing cells to create a permanent bald brand (NMFS 1993a; Merrick et al. 1996). Melanocytes can return on some brand marks and make the mark less readable (Keys and Farrell 1979).

2.10.1.1 Branding Irons

Branding irons used for freeze-branding are generally similar to those used for hot-branding but are often made of copper, lead, brass or stainless steel (Farrell 1979; NMFS 1993a). There is typically only one letter or number per brand with characters large enough to be seen at a distance. The size of brands has been found to be sufficient if they are visible at 100 m with 7X binoculars (Merrick et al. 1996).

The branding iron is chilled in a liquid coolant of alcohol and dry ice (–67°C to –77°C) or liquid nitrogen (-190°C) (Cornell et al 1979, Freeman and Lee 1989, Whittenburg 1987). The refrigerant materials can be dangerous to the researchers and impractical in the field, and safety equipment is often required to use them (Hoover 1988). The cooled brand is placed against the skin for 25 to 60 seconds per numeral at approximately 10-15 pounds/square inch (PSI) to produce a bald brand. This compares to only two to four seconds per character for a hot-brand (Merrick et al. 1996). There is some potential for smudging the brand since it has to be held in place under greater pressure for an extended period. Anesthesia with isoflurane gas is preferable in order to not smudge the brand from animal movement due to the extended time required to keep the branding iron in contact with the animal (NMFS 2005b). Anesthesia functions as a temporary anesthetic to reduce the pain of freeze-branding and also reduces the chance of blurred brand marks from sudden movements (Cornell et al. 1979).

There can be more preparation required for producing bald freeze-brands than hot-brands if animals are clipped or shaved and the skin swabbed with methylated spirits (an alcohol/glycerin mixture) to produce a bald brand rather than an unpigmented brand (NMFS 1993a). However, if animals being hot-branded need to be dried prior to branding, the preparation time may be roughly equivalent to that needed for a freeze-brand. Therefore, freeze-branding could take several minutes longer per animal than hot-branding due to the longer contact times required for a bald brand (Farrell 1979).

2.10.1.2 Liquid Spray

Another method involves spraying a specially formulated liquid, such as Freon 22 or combinations of chlorodifluoromethane and dimethyl ether, from an aerosol can through a set of uniquely designed stencils. The fur or hair is clipped to expose the skin, which is then wiped with alcohol. The stencil is placed against the skin and the liquid is spayed over the open markings. Evaporation results in freezing the outer layers of the skin, killing the pigment producing cells in the hair follicles. The time required is approximately 10-20 seconds (NMFS 2002).

2.10.2 Objectives of Research

The SSL Recovery Plan identified the need to monitor the health, condition, and vital parameters of SSLs. More specifically, this included conducting intensive studies on rookeries in order to develop indices of condition and obtain measurements and samples using non-lethal techniques. The NFS Conservation Plan identifies the need for studies of the long-term survival and reproduction rate and on the general condition and health of NFSs. Permanent marking of individuals is an effective technique in these types of studies.

2.10.2.1 Steller Sea Lions

The objectives of freeze-branding are similar to hot-branding in that there is a need to identify individual animals over a period of years for the purposes of determining seasonal use and movement patterns, dispersion from natal sites, site fidelity, and distribution and dispersal of animals.

2.10.3 Use of Data

2.10.3.1 Steller Sea Lions

Data from freeze-brand re-sightings, similar to hot-brands, have been very useful in determining important life history and vital parameters of the SSL. Brand re-sighting data in general is used for determining vital parameters such as age-specific survival and documenting immigration and emigration of animals between haul-outs and rookeries.

2.10.3.2 Northern Fur Seals

Attempts to use freeze-branding on NFSs were conducted between 1966 and 1978 for the purpose of mark and recapture studies to determine pup numbers (Keyes and Ferrell 1979). The pigment cells of the hair follicles and skin of adult NFSs seem to be insusceptible to cryogenic treatment during the spring and summer but were more responsive in the fall with the onset of molt (Keys and Farrell 1979). However, freeze-branding pre-weaned pups has had limited success as a reliable means for re-sighting marked pups as two- or three-year-olds as they return to the rookeries (Gentry and Holt 1982, NMFS 2006b).

2.10.4 Effects of Research

As with other marking techniques including hot-branding, capture and restraint is the major stress-inducing activity (Section 2.5). Anesthesia for restraining animals for freeze-branding also adds risks for the animals.

Freeze-branding is considered by some to be more acceptable for marking wildlife than hot-branding because, if done correctly, there is a negligible risk of infection (Cornell et al. 1979; Day et al. 1980; Harkonen 1987; Boyle et al. 1994; Troy et al. 1997). Other researchers have found that freeze-branding requires greater time and increased pressure of application, with a greater chance of infections, than hot-branding (Carrick and Ingham 1962; Warneke 1979; McMahan et al. in press). Daoust et al. (2006) found that freeze-brands tend to heal faster than hot-brands, although legibility over time was an issue in freeze-brands. In the NMFS Environmental Assessment on the effects of branding pinnipeds, hot-branding was said to be preferred over freeze-branding because freeze-branding required longer restraint times that could result in increased stress on the animals (NMFS 1993a).

2.10.4.1 Steller Sea Lions

Since 1993, both NMML and ADF&G have been using isoflurane gas to anesthetize SSLs during hot-branding or freeze-branding. There also has been concern about the safety of using anesthesia to restrain the SSLs. Because the animals being hot-branded under existing permits are anesthetized, a longer restraint time for a freeze-brand

would not necessarily result in more stress (Hobbs and Russell 1979). However, the use of anesthesia is not entirely without risks, and the risk of adverse effects increases with the duration of use.

Freeze-branding was not considered a viable alternative to hot-branding in the 1993 Environmental Assessment because:

- Freeze-brands require longer contact time with the animal which could result in additional stress.
- Animals would have to be anesthetized to obtain legible brands, and the use of anesthesia was cautioned against because of the potential for overdose and overheating.
- The equipment needed for freeze-branding was considered too cumbersome and logistically difficult in the field.
- The unpigmented skin produced by a freeze-brand could be difficult to distinguish from the light pelage of harbor seals and SSLs.

2.10.4.2 Northern Fur Seal

Freeze-branding has been used to permanently mark NFSs over a several-year period, between 1966 and 1978. Effects of freeze-branding on this species are generally similar to freeze-branding in SSLs. The amount of time required for each brand was a limiting factor for large numbers of pups. Effects of freeze-branding of NFS pups was not observed to adversely affect survival of the individual animals, although pups did appear to be sensitive to the super-chilled temperatures during the process (Keys and Farrell 1979).

2.10.4.3 Other Pinnipeds

Freeze-branding has been used to create permanent marks on a number of other pinnipeds, such as harbor seal, gray seals, elephant seal, California sea lions, and New Zealand fur seal (Hobbs and Russell 1979; Troy et al. 1997; Harkonen 1987). Researchers found that in freeze-branding of New Zealand fur seals, brands were not legible after the first molt but were after the second molt. This suggests that if re-sighting during the first year after branding was necessary, freeze-brands should be accompanied by other markings such as tags (Troy et al. 1997).

2.10.5 Other Marine Mammals

Freeze-branding has been effectively used on bottlenose dolphins in the past (Irving et al. 1982). Application time was 15 seconds and brands were recognizable at a distance for several years. Freeze-branding has been used on spinner dolphins (*Stenella longirostris*) in Hawaii with few negative effects (White et al. 1981). Tissue response from the freezing varies among individual animals, and is also related to the skill and experience of those applying the brands.

2.10.6 Mitigation

Researches have found that freeze-branding, in comparison to hot-branding, requires greater time and increased pressure of application, with greater chance of infections. Mitigation measures to minimize this potential for injury to animals during freeze-branding operations are similar to those for hot-branding and include:

- To reduce the stress on the animals and possible increased risk of mortality, do not freeze-brand pups that are very young or in poor physical condition (under 20 kg).
- If anesthesia is used, isoflurane gas is the preferred method.
- Allow only highly experienced and well-trained personnel to perform invasive procedures (including branding) according to their skills and qualifications.

- Process animals in groups small enough that all animals can be adequately monitored and minimize handling/restraint time.
- Separate and monitor pups when collected to ensure that they are not suffocating, being crushed, or aspirating milk.
- Immediately cease research-related procedures if an animal shows signs of acute or protracted alarm reactions that may lead to serious injury, capture myopathy, other disease conditions, or death; and monitor or treat the symptoms as determined appropriate.
- Restrain pups by hand, without using either a restraint board or drugs (except where the use of gas anesthesia is indicated for branding) and minimize handling time.

2.11 Venipuncture and Blood Collection

2.11.1 Description of Methods

Venipuncture and the subsequent collection of blood from SSLs, NFSs, and other marine mammals involves the capture, restraint, and anesthesia of the animal prior to collecting the sample (Sections 2.5 and 2.6). Each capture and restraint event will have some effect on the behavior, life, or activities of the animal and can create a variety of somatic, physiological, and behavior stressors (Section 2.5). Therefore, capture and restraint, and the use of anesthesia, adds to the potential effects of the venipuncture and blood collection procedures.

Once captured and sedated, the animal is restrained on as smooth a surface as possible. An attending veterinarian or other qualified personnel monitors the respiration, heart rate, and temperature of the animal. If the animal continues to struggle or shows signs of stress, it is released immediately. Blood samples are generally taken by venipuncture from a rear flipper or the caudal-gluteal vein while the animal is restrained. In pups, blood samples are drawn from the pelvic venous plexus (Castellini et al. 1993). After the blood is collected, along with any other experiments, the animal is monitored until it can be released.

2.11.2 Objectives of Research Method

The objective of conducting venipuncture is to collect blood samples that can be used to determine animal health and condition. This information can then be used to determine the current status of the SSL or NFS population for evaluation relative to recovery criteria.

2.11.3 Use of Data

Analysis of components of blood can give insight into the general health of animals. Bishop and Morado (1995) examined blood characteristics of SSL pups captured live on rookeries in Southeast Alaska and the Gulf of Alaska. White blood cell counts suggested mild physiological stress responses that were perhaps due to capture and handling. Red blood cell counts were suggestive of anemia, especially in animals sampled in the Gulf of Alaska. Zenteno-Savin et al. (1997) found higher levels of haptoglobin in SSL blood in the Aleutian Islands than in Southeast Alaska sea lions. In other animals, elevated haptoglobin levels are known to be associated with stress (e.g., trauma, infection), but no explanation was suggested for the results in SSLs. The recovery actions outlined in the SSL Recovery Plan provide the following recommendations related to blood and serological samples:

- Examine blood and tissue samples for evidence of contaminant-linked endocrine effects.
- Monitor health, body condition, and reproductive status.
- Collect blood samples for archiving, and for health and condition studies.

2.11.4 Effects of Research Method

The effects of venipuncture (*not* including the effects of capture, restraint and possibly anesthesia that are discussed elsewhere in this document) on SSLs, other pinnipeds, and cetaceans, are similar across the groups and minor overall. However, multiple attempts to obtain a blood sample are stressful to the marine mammal and likely cause some degree of pain. If improperly conducted or conducted too frequently on the same animal, venipunctures can result in damage to the vein, clotting, and infection or abscess. Removing too much blood relative to the animal's size may result in fatigue, anemia, and weakened immunity. Problems with clotting and excessive bleeding can also occur. However, these risks are greatly reduced by following the mitigation recommendations provided in Section 2.11.5; when the procedure is performed by qualified experienced personnel, following accepted standards, the risk is negligible.

2.11.5 Mitigation

Mitigation techniques specifically related to venipuncture include the use of sterile, disposable needles to reduce the risk of infection and cross contamination. The volume of blood taken from any individual animal should not exceed 10 ml of blood per kg of body mass, either as a single blood draw or over the course of several days.

2.12 Skin, Blubber, and Muscle Biopsy

2.12.1 Description of Methods

2.12.1.1 Capture

The collection of skin, blubber, and muscle samples from pinnipeds usually involves the capture, restraint, and possible anesthetizing of the animal prior to collecting the sample (Sections 2.5 and 2.6). Once captured and sedated, the animal is restrained on as smooth a surface as possible for collection of the samples. Skin and blubber samples are typically collected near the hind flippers using a surgical biopsy punch 7 mm in diameter.

For various reasons, some researchers use remote sampling techniques to collect skin and blubber samples from certain cetacean species and some pinnipeds including South American fur seals and SSL (Best et al. 2005; Hooker et al. 2001; and Gemmel and Majluf 1997). A typical biopsy dart has a cylindrical punch that is about 2.5 cm long and fitted with a barbed filament to hold the sample (Hooker et al. 2001). The dart is then attached to a standard cross-bow bolt. To allow recovery of the biopsy dart, a spinning reel is attached to the stock and a monofilament retrieval line (11-kg) is attached to the dart and wound on the reel. The use of the monofilament tether greatly impairs the flight of the dart; however, with practice it is easy to compensate and a high level of accuracy can be obtained (Gemmel and Majluf 1997). The dart can be shot from on board a vessel or while observing animals on the ground. The main disadvantages of remote sampling compared to sampling restrained animals are the increased potential for injury if a dart hit off target and the inability to ensure the dart has collected the appropriate amount of tissue.

2.12.2 Objectives of Research Method

The objectives of collecting tissue samples including skin, blubber, and muscle from SSLs and NFSs is to analyze the samples to determine general condition, nutrition, reproductive state, contaminant load, and other aspects of marine mammal health. This information can then be used to determine the current status of the SSL and NFS population for evaluation relative to recovery criteria.

2.12.3 Use of Data

Biopsies can often provide data that cannot be obtained by non-destructive means. Skin samples can undergo genetic analyses that can subsequently provide information on social organization, kinship, mating, individual gender, and identification and variability within and among populations. Blubber, muscle, and other tissue samples are often used to determine contaminant levels and for obtaining information on feeding ecology and nutritive condition. The recovery actions outlined in the SSL Recovery Plan provide the following recommendations:

- Continue to collect information on food habits using SSL tissue samples.
- Analyze reproductive hormone levels in tissue samples to better estimate birth rates.
- Examine blood and tissue samples for evidence of contaminant-linked endocrine effects.
- Use tissue samples collected from pups to indicate the pregnancy status of nursing mothers.
- Further develop indirect methods, such as the analysis of stable isotopes and fatty acid (FA) signatures in tissues, to determine the diet of SSLs at both the individual and population levels. The isotopic measurement of several tissues from the same individual can provide short-, intermediate-, and long-term dietary information depending upon rates of metabolic activity.

2.12.4 Effects of Research Method

Each capture and restraint event will have some effect on the behavior, life or activities of the animal and can create a variety of somatic, physiological and/or behavioral stressors (Section 2.5). The effects of using standard or remote methods of biopsy retrieval (*not* including the effects of capture, restraint and possibly anesthesia) on SSLs, other pinnipeds, and cetaceans are similar across the groups and minor overall. Biopsy punches for skin and blubber samples produce a small wound that has the potential for infection, especially when considering the unsanitary conditions of the environment. However, an otherwise healthy animal would be able to heal and recover from a properly performed procedure. Muscle biopsies produce a small-diameter deep wound that would tend to close on the surface prior to deep tissue healing, thereby increasing the chances of an abscess forming. This is more likely if the dart or punch is not disposable or is not sanitized properly between uses.

Specific to remote sampling, no damage other than the small biopsy puncture (and associated chance for infection) was detected in South American fur seals regardless of where the point of impact was on the animal's body (Gemmel and Majluf 1997). There was no other tissue damage, bone fracture or bone chipping from the impact of the dart. The animals themselves showed no adverse effects of the sampling. In most cases, male fur seals paid little or no attention to being struck by the biopsy dart and did not move off of their territory and were not at additional risk of attack from neighboring males. The immediate typical response was for the seal to recoil from the impact and search briefly for the "assailant." Similarly bottlenose whales (*Hyperoodon planifrons*) showed no or low-level reactions to biopsy attempts (Hooker et al. 2001).

Other hazards of remote biopsy sampling include missed shots, stuck darts or broken tips remaining attached to the animals causing irritation and possibly abscess and infection, snagging of the retrieval line on flukes or other body parts, and the repeated sampling of one individual thereby compounding the effects on that animal. One study (Best et al. 2005) found that thinner darts were not as likely to become stuck in the animal and were more easily retrieved. A single humpback whale (*Megaptera novaeangliaen*) that was biopsied three times showed progressively increasing reactions (Weinrich et al. 1992). However, Brown et al. (1994), recorded 16 occasions of duplicate sampling of humpbacks and the response to biopsy sampling remained the same or decreased in 14 cases and increased in only two cases. Other researchers found that when the whales were sampled two to four times but in different months or years, the intensity of the behavioral response appeared the same as the first and subsequent biopsies (Gauthier and Sears 1999).

2.12.5 Mitigation

Mitigation techniques specifically related to the collection of biopsy samples include the use of sterile, disposable punches. Where disposable equipment is not available, liquid chemical sterilizers should be used with adequate contact times and the punch should be rinsed with sterile water to remove any chemical agents that might irritate the animal's skin. When taking muscle biopsies from captured sedated animals, leaving the wound open to drain, rather than suturing it closed, may promote healing and reduce abscess formation. Disinfection of the surgical site is paramount to the promotion of healing. If the animal continues to struggle or shows signs of stress, it is released immediately. In order to minimize the risk of infection or cross-contamination, sterile, disposable biopsy punches should be used to obtain the skin, blubber, or muscle sample.

For remote biopsy sampling, the researcher should practice the efficiency of the shot on standard archery targets prior to attempting use in the field. Repeated sampling of the same animal within a single study period of a month or less should be avoided.

2.13 Digestive Tract Sampling

2.13.1 Description of Methods

Endoscopy, enema, stomach intubation, and fecal loops are all used to sample the digestive tracts of SSLs, NFSs, and other marine mammals. Often the use of these methods involves the capture, restraint, and possibly anesthesia of the animal prior to collecting the sample (Sections 2.5 and 2.6). Each capture and restraint event will have some effect on the behavior, life or activities of the animal and can create a variety of somatic, physiological and/or behavioral stressors (Section 2.5). Therefore, capture and restraint, and the use of anesthesia, adds to the potential effects of the endoscopy, enema, intubation, or fecal loop procedure alone.

Once captured and sedated, the animal is restrained on as smooth a surface as possible. An attending veterinarian or other qualified experienced personnel monitors the respiration, heart rate, and temperature of the animal. If the animal continues to struggle or shows signs of stress, it is released immediately. Stomach intubation, endoscopy, enemas, and fecal loops are used to allow analysis of stomach contents and other digestive tract samples without destruction of the animal. In the past, animals have been sacrificed in order to obtain this type of information.

Intubation is used by researchers to conduct lavage for stomach content analyses on live, sedated, animals. The procedure entails introducing an intubation tube into the animal's stomach and using fluids to flush out the contents, which are then collected and analyzed. First the length of the stomach tube needed for a given animal must be estimated by measuring the distance to the stomach along the outside of the animal's body. The tube is then smoothly inserted into the mouth, down the left side of the throat and into the stomach. If the animal cannot vocalize, then it is assumed that the tube has been inserted into the trachea and must be removed. To further determine the proper location of the tube, a small amount of air is blown into the tube while listening for gurgling either through the tube or by using a stethoscope placed on the left abdominal wall (Dierauf 1990).

After the stomach tube is properly in place, it is connected to a manually operated suction pump and sea water is pumped into the animal's stomach (Antonelis et al. 1987). The suction fitting of the pump is then connected to one of two hose fittings on an airtight collecting bottle while the other fitting is attached to the lavage tube. A vacuum is created in the collecting bottle and the slurry of water and undigested food parts are suctioned from the stomach.

Enemas are used to collect fecal samples from live, sedated animals (Staniland et al. 2003). In order to obtain the sample, a plastic bottle is filled with warm water. A soft polyethylene hose (12 mm diameter) is connected to the bottle via a one-way valve; the hose is then inserted into the animal's colon via the anus. The warm water is then introduced via the hose by gently squeezing the bottle. Once the bottle is empty or the resistance becomes too great, the hose is removed and the animal's rear flippers are held over a plastic tray in which the material naturally

expelled by the animal is collected. Fecal loops are also used to collect fecal samples from live animals. A flexible plastic loop is inserted into the anus and a sample of the material is obtained.

Endoscopy is a minimally-invasive procedure used to evaluate the interior surfaces of an organ by inserting a small tube into the body, often, but not necessarily, through a natural body opening such as the mouth or anus. Through the scope the researcher is able to see lesions and other surface conditions.

After the endoscopy, intubation, fecal loop, or enema, and any other experiments are completed, the animal is monitored until it can be released.

2.13.2 Objectives of Research Methods

The objectives of conducting endoscopy, stomach intubation, enemas and fecal loops are to collect digestive tract samples that can be used to determine animal diet and condition. This information can then be used to determine the current status of the SSL population for evaluation relative to recovery criteria.

2.13.3 Use of Data

Endoscopies, stomach lavage, enemas, and fecal loops can provide data that cannot be obtained by non-destructive means. The recovery actions outlined in the SSL Recovery Plan provide the following recommendations related to digestive tract samples:

- Collect and analyze stomach contents to determine prey consumption in SSLs.
- Monitor health, body condition, and reproductive status.
- Collect samples of feces and other bodily fluids from live animals for assessment of the intensity and effects of infestations.

2.13.4 Effects of Research Methods

The effects of conducting stomach intubation, enemas, endoscopy, and fecal loops (*not* including the effects of capture, restraint and possibly anesthesia that are discussed elsewhere in this document) on SSLs, pinnipeds, cetaceans, and other marine mammals are similar across the groups and minor overall.

Anytime a foreign object is inserted into the rectum, as in the case of endoscopy, enemas, and fecal loops, there is the possibility of perforation which can lead to peritonitis resulting in death. However, when the procedure is performed by qualified experienced personnel, following accepted standards, the risk is negligible. Disturbance due to the procedure, even when properly performed, can also occur, but is likely to be minor and short-term. For example, in conducting enemas on Antarctic fur seals (*Arctocephalus gazella*), Staniland et al. (2003) found that of the animals sampled, all were observed suckling their pups within the same season and no aberrant behavior was visually observed. Eleven animals received a series of enemas (7-16) after successive foraging trips. Upon each recapture and close visual inspection, no obvious external signs of damage were recorded.

Stomach intubation involves the risk of introduction of liquid into the trachea, initiating aspiration pneumonia or death. Therefore, procedures and checks to ensure that the tube is properly inserted into the stomach must be carefully completed before the introduction of fluids. There is also a risk of introducing infection or cross-contamination among animals if the intubation equipment is not properly sterilized between animals. This also applies for enemas, endoscopy, and fecal loops.

2.13.5 Mitigation

Mitigation techniques specifically related to conducting studies using endoscopy, stomach intubation, enemas, and fecal loops include the use of sterile, disposable stomach, endoscopy, and enema tubes and loops. Where

disposable equipment is not available, liquid chemical sterilizers should be used with adequate contact times and the equipment should be rinsed with sterile water to remove any chemical agents that might irritate the animal's skin. Because cold sterilization techniques take time, researchers should bring an adequate number of tubes or loops to ensure that all are properly sterilized between animals or that there is one tube or loop available per animal.

For stomach intubation, only qualified personnel (veterinarians or biologists) who know how to properly pass a stomach tube to avoid introduction of liquid into the trachea should attempt the procedure. Rounding the edges of the end of the tube and coating it with surgical lubricant facilitates passage of the tube into the stomach (Antonelis et al. 1987).

For enemas, there is a concern that if the water pressure inside the animal became too high (i.e., through too vigorous pumping) it could cause internal damage. However, it was found in all cases that with the diameter of hose used, any build-up of pressure was dissipated through leakage via the animal's anus.

2.14 X-Ray

2.14.1 Description of Methods

In most cases bones and teeth of marine mammals that are stranded or perish due to other means are chosen for x-ray studies (e.g., Arkowitz and Rommel 1985; Cranford 1999; Dalebout, et al. 2003). However, in some studies animals are sacrificed and their teeth or bones are removed and x-rayed (Stewart et al. 1996; Stewart et al. 1998). In these studies, the jaws, teeth, or other bones are removed from the animal, frozen, and returned to the laboratory for x-ray analyses.

If done, x-ray studies of living, non-captive, SSLs, NFSs, and other marine mammals would involve the capture, restraint, anesthesia, and transport of the animal (Sections 2.5 and 2.6). Once captured, sedated, and transported to the laboratory, the animal is restrained on a smooth surface while the x-ray is completed. An attending veterinarian or other qualified personnel monitors the respiration, heart rate, and temperature of the animal. After the x-ray is completed, and other experiments are done, the animal is monitored until it can be transported and released.

2.14.2 Objectives of Research Method and Use of Data

X-raying marine mammals is most often used to determine age. In the absence of known-age specimens, age is interpreted from growth layers in hard tissues, such as teeth. X-rays of these hard tissues show the growth layers. For example, Stewart et al. (1998) used X-rays of mandibles from ringed seal fetuses, newborns, and young-of-the-year to determine the presence, location, and eruption patterns of deciduous and permanent teeth. A detailed knowledge of the growth and development of those tissues enhances researchers' ability to interpret annual markers used for age estimation. This information can then be used in conjunction with other studies to determine the current status of the sea lion population for evaluation relative to recovery criteria.

2.14.3 Effects of Research Method

If x-raying of live marine mammals is done, the effects (*not* including the effects of capture, restraint and possibly anesthesia) on SSLs, other pinnipeds, and cetaceans, are similar across the groups and minor overall.

2.14.4 Mitigation

Mitigation techniques include proper care during capture and restraint, and ensuring that the same animal is not x-rayed repeatedly.

2.15 Urinalysis

2.15.1 Description of Methods

Collection of urine for urinalysis in SSLs, NFSs, and other marine mammals involves the capture, restraint, and anesthesia of the animal prior to collecting the sample (Sections 2.5 and 2.6). Once captured and sedated, the animal is restrained on as smooth a surface as possible. An attending veterinarian or other qualified personnel monitors the respiration, heart rate, and temperature of the animal. If the animal continues to struggle or shows signs of stress, it is released immediately. Urine samples are collected by catheterizing the animal while it is restrained and anesthetized. After the urine is collected, along with any other experiments, the animal is monitored until it can be released.

2.15.2 Objectives of Research Method

The objective of urinalysis is to collect samples that can be used to determine animal health and condition. This information can then be used to determine the current status of the sea lion population for evaluation relative to recovery criteria.

2.15.3 Use of Data

The recovery actions outlined in the SSL Recovery Plan provide the following recommendations related to urine samples:

- Examine urine samples for evidence of contaminant-linked endocrine effects.
- Monitor health, body condition, and reproductive status.

Urinalysis can also be used to study the role hormones play in water conservation in marine mammals. For example, Ortiz et al. (1996) analyzed urine from elephant seal pups serially throughout the postweaning period to quantify changes in urine concentrating ability and electrolyte homeostasis at various stages of fast.

2.15.4 Effects of Research Method

The effects of catheterization to collect urine for urinalysis (*not* including the effects of capture, restraint and possibly anesthesia that are in Section 2.5) on SSLs, other pinnipeds, and cetaceans are similar across the groups. Anytime a foreign object is inserted into the urethra and bladder, there is the possibility of perforation which can lead to infection and death. In addition, cross-contamination among animals can occur if disposable catheters are not used and are not adequately sterilized between uses. However, when the procedure is performed by qualified experienced personnel, on healthy animals, following accepted standards, the risk is negligible.

2.15.5 Mitigation

Mitigation techniques include proper care during capture and restraint and the use of sterile, disposable catheters. Where disposable equipment is not available, liquid chemical sterilizers should be used with adequate contact times and the catheter should be rinsed with sterile water to remove any chemical agents that might irritate the animal's skin. Because cold sterilization techniques take time, researchers should bring an adequate number of tubes to ensure that all are properly sterilized between animals or that there is one tube available per animal. To ensure proper placement of the catheter, qualified personnel (veterinarians or biologists) who know how to properly collect the urine sample should conduct the tests.

2.16 Ultrasound

2.16.1 Description of Methods

Ultrasound of SSLs, NFSs, and other marine mammals is generally done on captive animals (e.g., Brook et al. 2002) using portable ultrasound transducers in the field (Sections 2.5 and 2.6). Once captured and sedated, the animal is restrained on as smooth a surface as possible. An attending veterinarian or other qualified personnel monitors the respiration, heart rate, and temperature of the animal. If the animal continues to struggle or shows signs of stress, it is released immediately. After the ultrasound is done, and other experiments are completed, the animal is monitored until it can be released.

2.16.2 Objectives of Research Method

The objectives of conducting ultrasound on SSLs and NFSs are to determine general body condition and reproductive state and health of the animals. This information can then be used to determine the current status of the sea lion population for evaluation relative to recovery criteria. The SSL Recovery Plan recommends that health, body conditions, and reproductive status be monitored in SSLs. Ultrasound can be used to further those objectives.

2.16.3 Use of Data

Ultrasound can provide data that cannot be obtained by non-destructive means. It has now been proven to be a very useful and effective method by which to monitor and document reproductive events in captive dolphins (Brook et al. 2002). In addition, researchers have used portable ultrasound transducers to determine blubber thickness in SSL pups on Marmot Island (Gemmel and Maljuf, 1997).

2.16.4 Effects of Research Method

There are no known effects of conducting ultrasound procedures (*not* including the effects of capture, restraint and possibly anesthesia that are discussed elsewhere in this document) on SSLs, other pinnipeds, and cetaceans.

2.16.5 Mitigation

Only qualified personnel (veterinarians or biologists) who know how to properly care for the animal during capture and restrain and conduct an ultrasound test should attempt the procedure. There are no other mitigation measures necessary.

2.17 Skin and Mucosal Swabs

2.17.1 Description of Methods

Swabbing of the skin and/or mucosa of SSLs, NFSs and other marine mammals, is done on captured, restrained, and possibly anesthetized animals. Once captured and sedated, the animal is restrained on as smooth a surface as possible. An attending veterinarian, or other qualified personnel, monitors the respiration, heart rate, and temperature of the animal. If the animal continues to struggle or shows signs of stress, it is released immediately. Skin samples are collected by using a sterilized nylon scrub pad. Harlin et al. (1999) used this technique on dusky dolphins (*Lagenorhynchus obscurus)* and found that it did not puncture the skin and minimized the time spent in physical contact with the dolphins. Cetacean researchers have used skin that sloughs naturally from large whales for genetic analysis. However, such non-invasive collection of tissue is not possible with small cetaceans who do not shed a sufficient amount of skin.

Mucosal swabs can be collected of the nasal passages, eyes (ocular swabs), vagina, and rectum. Clean, cotton-tipped swabs are used to collect the mucosa. After the swabbing is done, and other experiments are completed, the animal is monitored until it can be released.

2.17.2 Objectives of Research Method

The objectives of collecting skin and mucosal swabs of SSLs and NFSs is to collect deoxyribonucleic acid (DNA) samples that can be used to determine general body condition and health of the animals. This information can then be used to determine the current status of the sea lion population for evaluation relative to recovery criteria. The SSL Recovery Plan recommends that health, body conditions, and reproductive status be monitored in SSLs. Data from skin and mucosa samples can be used to further those objectives.

2.17.3 Use of Data

Analysis of skin and mucosal swabs can provide information on disease and overall health of the animal. For example, Goldstein et al. (2006) collected nasal swabs from captive and free-ranging Hawaiian monk seals. Samples were collected by swabbing the nasal cavity of each animal with a clean cotton-tipped swab. The swabs were then placed into a sterile cryovial and frozen at −70◦C until analyzed. DNA was extracted from the samples. Information from sequencing the DNA was used to determine the role that viral diseases may play in the decline of these seals. Harlin et al. (1999) collected skin samples from free-ranging dusky dolphins, and found that a sufficient amount of skin was collected in this manner to provide for DNA sequencing analyses.

2.17.4 Effects of Research Method

Effects of collecting skin and mucosal samples from SSLs, NFSs and other marine mammals (*not* including the effects of capture, restraint and possibly anesthesia that are discussed elsewhere in this document) would be similar across the group. There may be some remaining skin irritation from the scraping action of the pad, or irritation in the mucosal linings from the cotton swab. There is the possibility for damage to the cornea of the eye if ocular swabbing is done incorrectly. When performed by a qualified, experienced person using commonly accepted standards of good practice, these risks are likely to be negligible.

Skin samples can also be collected from free-ranging (not restrained or captive) animals as demonstrated by Harlin et al. in the dusky dolphin research mentioned above. Their research showed that eleven percent of 128 contacts resulted in no visible behavioral response. 89 percent of dolphins responded to contact, with 29 percent and 34 percent responding by making a lateral move to the right or left, respectively. Tail slap and startle occurred only once each in 114 responses. Overall, dolphins showed little or no aversion to the sampling conducted in this study.

2.17.5 Mitigation

Only qualified personnel (veterinarians or biologists) who know how to collect the samples should attempt the procedure. To minimize cross-contamination and infection, clean cotton swabs and sterilized nylon scrub pads should be used. There are no other mitigation measures necessary.

2.18 Tooth Extraction

2.18.1 Description of Methods

Teeth may be extracted from of SSLs and NFSs collected as part of subsistence harvest, from dead stranded animals, or from live free-ranging animals. If collected from non-captive marine mammals, the animal is first captured, restrained, and anesthetized. Once captured and sedated, the animal is restrained on as smooth a surface as possible. An attending veterinarian or other qualified personnel monitors the respiration, heart rate, and

temperature of the animal. If the animal continues to struggle or shows signs of stress, it is released immediately. Arnbom et al. (1992) first immobilized individual Antarctic fur seals and southern elephant seals with a 1: 1 mixture of the anesthetic tiletamine hydrochloride and the tranquilizer zolazepam after capturing them in the wild. Before extraction, teeth and gums were cleaned with antiseptic solution and the mouth of the seal was kept open by placing a soft wood block between the jaws. The extraction area was cleansed with antiseptic disinfectant before, during and after the extraction. These researchers found that it took one to two minutes to remove a tooth from an Antarctic fur seal.

After the tooth is extracted, and other experiments are completed, the animal is monitored until it can be released.

2.18.2 Objectives of Research Method

The objectives of collecting teeth from harvested or living SSLs and NFSs is to collect information regarding age, general body condition, and health of the animals. This information can then be used to determine the current status of the sea lion population for evaluation relative to recovery criteria. The SSL Recovery Plan recommends that demographic modeling of the SSL population be continued. Tooth ring age data can provide information on the age and reproductive status of these animals.

2.18.3 Use of Data

Teeth are often collected from mammals to assist in determining age and population statistics. For example, Baker and Fowler (1998) collected teeth from juvenile (mostly three- and four year-old) male NFSs throughout the annual commercial harvest (five weeks from late June to early August) on St. Paul Island for all but two years between 1948 and 1984. The objectives of this study were to use the tooth weight of harvested seals as a record of growth of individuals over several decades in order to: (a) characterize the relationship between tooth weight and body length; (b) investigate the relationship between growth and population density; and (c) explore evidence for differences in growth of seals associated with different rookeries.

Other researchers have used teeth from SSLs to determine diet. Stable isotope analysis of teeth of marine mammals can provide valuable information on trophic level and source of feeding (Hobson and Sease 1998). Hobson et al. (2004) used stable isotope values of individual tooth annuli of female SSLs collected from the 1960s through the 1980s for retrospective analyses of temporal changes in food webs in the Gulf of Alaska and North Pacific Ocean. Arnbom et al. (1992) extracted post-canine or incisor teeth from live antarctic fur seals and southern elephant seals, respectively, and used the teeth to determine age in a field situation.

2.18.4 Effects of Research Method

Effects of tooth extraction in SSLs, NFSs, and other marine mammals (*not* including the effects of capture, restraint and possibly anesthesia that are discussed elsewhere in this document) would be similar across the group. The potential adverse effects of tooth extractions alone relate to the possibility of infection following extraction. The procedure may result in more than momentary pain, which could temporarily interfere with the animal's ability to forage. However, there are no data on the long-term effects of this procedure. When performed by a qualified, experienced person using commonly accepted standards of good practice, these risks are likely to be negligible. For example, Arnbom et al. (1992) found that no seal recaptured up to one year after tooth extraction showed any signs of infection.

2.18.5 Mitigation

Only qualified personnel (veterinarians or biologists) who know how to safely remove teeth should attempt the procedure. Cleaning and disinfection of the mouth, teeth, and gums as described in Section 2.18.1, and possibly the administration of antibiotics, could serve to reduce the possibility of infection. However, Arnbom et al. (1992)

found that it was important to keep the animal's head pointing down during extraction to prevent disinfectant fluids from being swallowed.

2.19 Vibrissae, Hair, and/or Nail Collection

2.19.1 Description of Methods

Collection of vibrissae, hair, and nails from SSLs, NFSs, and other marine mammals is done on captured, restrained, and possibly anesthetized animals. Once captured and sedated, the animal is restrained on as smooth a surface as possible. An attending veterinarian, or other qualified personnel, monitors the respiration, heart rate, and temperature of the animal. If the animal continues to struggle or shows signs of stress, it is released immediately. Aurioles et al. (2006) collected hair samples from pups without the need for restraint or anesthesia. Hair samples were collected with scissors at the base of the hair without removing the follicle and then rinsed with distilled water to eliminate salt and sand residues. Caudron et al. (2006) adapted an existing method (crossbow skin biopsy) to remotely sample seal hair without causing skin puncture,

Researchers have also used photography of vibrissae, in lieu of collection of the whiskers, as an even less invasive method. Greaves et al. (2004) photographed the vibrissae of female grey seals biweekly over a 5 month period. Seals were captured and immobilized on a spinal board outfitted with Velcro™ straps, and the nose and mystacial vibrissae were inserted through a hole in a photography board while the jaws were held closed manually. After the collection or photography, is done, and other experiments are completed, the animal is monitored until it can be released.

2.19.2 Objectives of Research Method

The objectives of collecting vibrissae, hair, and nails from SSLs and NFSs are to collect tissue samples and subsequently DNA that can then be used to determine general body condition and health of the animals. This information can then be used to determine the current status of the sea lion population for evaluation relative to recovery criteria. The SSL Recovery Plan recommends that health, body conditions, and reproductive status be monitored in SSLs. Data from vibrissae, hair, and nail samples can be used to further those objectives.

2.19.3 Use of Data

Analysis of vibrissae, hair, and nails can provide information on disease and overall health of the animal. For example, Caudron et al. (2006) extracted DNA from seal hair samples. DNA from hair, vibrissae, and nails can be used for population studies. Aurioles et al. (2006) sought to gain insight into the foraging behavior of elephant seals in Mexican waters through study of natural variation in stable carbon and nitrogen isotope values in hair samples from the animals. Stable isotopes can provide information on migration and foraging location and trophic level of prey consumed by marine mammals. As described above, Greaves et al. (2004) used photographs of seal vibrissae in an attempt to investigate their applicability for stable isotope diet analysis. However, they found that because the growth of vibrissae is neither continuous nor synchronous, it is a challenge to accurately identify the dates when the isotopes were incorporated into the tissue.

2.19.4 Effects of Research Method

Effects of collecting vibrissae, hair, and nails from SSLs, NFSs and other marine mammals (*not* including the effects of capture, restraint and possibly anesthesia that are discussed elsewhere in this document) would be similar across the group. Clipping whiskers, hair and nails is not likely to result in any pain. The effects on the animal of clipping a whisker, toenail, or patch of hair or pulling a whisker are probably largely incidental to the effects of capture and restraint. When performed by a qualified, experienced person using commonly accepted standards of good practice, these risks are likely to be negligible.

2.19.5 Mitigation

Only qualified personnel (veterinarians, biologists) who know how to collect the samples should attempt the procedure. To minimize cross contamination and infection during nail clipping, sterilized nail clippers should be used and care should be taken such that the "quick," or attached portion, of the nail is not cut. There are no other mitigation measures necessary.

2.20 Bioelectric Impedance Analysis

2.20.1 Description of Methods

Bioelectrical impedance analysis (BIA) measures resistance and reactance of a current as it passes through an organism (Gales et al. 1994; Arnould 1995). BIA is conducted on animals that have been captured, restrained, and anesthetized. Once captured and sedated, the animal is restrained on as smooth a surface as possible. An attending veterinarian or other qualified personnel monitors the respiration, heart rate, and temperature of the animal. If the animal continues to struggle or shows signs of stress, it is released immediately. For the BIA, vacutainer needle electrodes are placed on and in the sedated animal (Bowen et al. 1998). Resistance (Rs) and reactance (Xc) are measured using a tetrapolar impedance plethysmograph. This unit comprises a localized current injection system that provides a measure of total body resistivity via two pairs of electrodes placed on and in the animal (Gales et al. 1994; Arnould 1995). The voltage drop between the inner and outer electrodes is measured with a high input impedance amplifier. Electrodes remain in place on the animal until readings of Rs and Xc stabilize, usually <30 seconds. Biological impedance (Z), Rs, and Xc vary inversely with the volume and composition of the body. After completion of the test, and collection of any other samples, the animal is monitored until it can be released.

2.20.2 Objectives of Research Method

The objective of conducting BIA on SSLs and NFSs is to estimate body composition. This information can then be used to determine the current status of the sea lion population for evaluation relative to recovery criteria. The SSL Recovery Plan recommends that health and body condition be monitored in SSLs. BIA can be used to further this objective.

2.20.3 Use of Data

Measures of body composition in seals have been used as an index of the animals' response to variation in environmental quality (Gales et al. 1994; Arnould 1995). Accurate evaluation of body composition of living animals is critical for understanding their energy and material flux rates. This is true for many marine mammals which show dramatic seasonal shifts in body mass, primarily due to changes in the extent of their subdermal blubber layer. This fat layer functions in part as an energy store and, as such, is essentially a measure of body condition. Because fat-free mass, including the protein matrices of fat, contains most of the body water and electrolytes, conductivity is greater in fat-free tissues than in fat. Therefore, the conductance of an electrical current through an organism is dependent on body composition. BIA measures this conductance. Determination of body composition allows the estimation of body condition, which is essential for examining population health and, in some cases, availability of prey.

2.20.4 Effects of Research Method

The effects of BIA on SSLs, NFSs and other marine mammals are likely to be incidental to those effects associated with capture, restraint, and anesthesia (Section 2.5 and 2.6). Pain would not be expected to be associated with placement of the needles because the animals are sedated. However, Bowen et al. (1998) found that although sedated, most seals reacted to the placement of the electrodes and exhibited some movement during the period of measurement. In most cases the reaction appeared to be transient and animals seemed relaxed while

the measurements were taken. However, some seals continued to react to gentle restraint and the electrodes while measurements were taken. Gales et al. (1994) observed that initially it was necessary to restrain seals in a net, but this later proved unnecessary as the animals became accustomed to the procedure. At that time, only four people were required to hold the seal and insert the needle electrodes. Needles were in for less than 10 seconds and the entire procedure was completed in about 2 minutes.

Subsequent to disturbance effects, the insertion of the needles also poses a risk of infection. Infectious agents may be present on the animal's skin or hair that can then be introduced under the skin. In addition, cross-contamination among animals can occur if disposable needles are not used and equipment is not adequately sterilized between uses. Repeated use of BIA on the same animal could cause skin and subcutaneous lesions. For example, an instance of a subcutaneous abscess on a captive adult female SSL was attributed to apparent tissue necrosis induced by the focal electrical current at the site of the a BIA electrode (Annual Report for Permit No. 881-1443, Alaska Sea Life Center).

2.20.5 Mitigation

Only qualified personnel (veterinarians or biologists) who know how to place the needles and conduct a BIA test should attempt the procedure. In general, if an experienced person uses commonly accepted standards of good practice, risks of the procedure can be greatly minimized. Mitigation techniques specifically related to BIA include the use of disposable needles. Where disposable needles are not available, liquid chemical sterilizers should be used with adequate contact times and the needles should be rinsed with sterile water to remove any chemical agents that might irritate the animal's skin. Because cold sterilization techniques take time, researchers should bring an adequate number of needles to ensure that all are properly sterilized between animals or that there is one set available per animal.

2.21 Diet Manipulation Studies

2.21.1 Description of Methods

Diet manipulation studies are conducted on captive, living marine mammals that are short or long-term residents at universities, marine laboratories, or aquariums. The animals are either not fed for a period of time (induced fasting), or are fed a diet consisting of specific prey items that may or may not mimic natural conditions. After the fasting or feeding period, blood and digestive tract samples are collected, and other studies such as bioelectrical impedance analyses (as described in Section 2.20) are conducted to determine body composition, health, nutritional stress, and general condition of the animal.

2.21.2 Objectives of Research Method

The objectives of conducting diet manipulation studies on captive marine mammals is to collect dietary and food web information that can be used to assess and predict the health of the animals. This information can then be used to determine the current status of the sea lion population for evaluation relative to recovery criteria. The SSL Recovery Plan recommends the energetic costs to foraging sea lions be determined to assess population status.

2.21.3 Use of Data

Dietary manipulations are often combined with blood chemistry and other physiological analyses to characterize the potential for nutritional stress in an animal, especially where nutritional stress has been implicated in population declines (Trumble et al. 2006). These researchers quantified changes in plasma metabolites and hematology values in captive harbor seals fed different diets over two years. However, captive seals are often maintained on a single species of fish and have activity patterns that bear little resemblance to those of free-living

animals. Thus, data obtained from these studies provide little more than a general guide to the range of consumption rates that are likely to occur in wild populations (Harwood and Croxall 1988).

2.21.4 Effects of Research Method

Effects of conducting diet manipulation studies on SSLs, NFSs and other marine mammals (*not* including the effects of short or long-term captivity and the effects of subsequent blood and other tests discussed elsewhere in this document) would be similar across the group. When the test animals are closely monitored during the study and the study is performed by a qualified, experienced person using commonly accepted standards of good practice, any risks are likely to be negligible.

2.21.5 Mitigation

Only qualified personnel (veterinarians or biologists) who know how to safely conduct feeding and fasting studies should attempt them on captive animals. SSLs undergoing fasting should be monitored daily and removed from the trial (i.e., returned to feeding) if there is any indication of illness. The experiment should be terminated for any animal whose rate of mass loss is greater than 3 percent of initial mass per day or whose total mass loss exceeds 15 percent of initial body mass. Finally, any SSLs subjected to the controlled fasting experiments should be allowed time to recover and readjust metabolism prior to being returned to the wild, if applicable.

2.22 Internal Scientific Instruments

2.22.1 Description of Methods

Direct measurements of mortality events can be obtained through the use of implantable, satellite-linked life history transmitters (LHX). LHX transmitters are capable of continuously monitoring five built-in sensors, including pressure, motion, light levels, temperature, and conductivity. The transmitter will establish the death of an animal. When the instrument is exposed to the ambient conditions outside of the carcass, all information stored in to the LXH will be transmitted to the ARGOS system via a NOAA satellite. LHX transmitters have only been in use since 2004. The tags are surgically implanted intraperitoneally while the animal is under anesthesia.

A system designed by Andrews (1998) to monitor foraging behavior of pinnipeds includes a stomach temperature transmitter (STT) and a data logger with a built-in telemetry receiver for recording dive depth, swim speed, and water temperature. The STT is inserted into the stomach of the SSL while the animal is under anesthesia.

2.22.2 Objectives of Research

LHX transmitters are specifically designed to obtain long-term data records from individual animals over a period of up to 10 years and for estimating age-specific survival rates.

STTs are designed to record the precise timing of prey ingestion in marine mammals.

2.22.3 Use of Data

LHX transmitters provide survival and longitudinal cumulative dive effort data from individual animals for up to 10 years. Researchers specifically monitor two major areas: 1) dive effort and dive behavior and 2) body condition and health characteristics.

STTs are used to determine timing of prey ingestion by relying on the drop in stomach temperature that occurs when a relatively warm animal ingests much cooler prey (Mackay 1964).

2.22.4 Effects of Research

In addition to the effects of capture and restraint described previously, the predominant problems of this method are related to excessive tissue reaction, infection, and subsequent rejection of implanted materials.

Subcutaneous and intraperitoneal transmitters have been used successfully in birds (Petersen et al. 1995), polar bears (Mulcahy and Garner 1999), sea otters (Ralls et al. 1989; Siniff and Ralls 1991; Thomas et al. 1987), and harbor seals (Lander et al. 2005) with no deleterious effects. LHX transmitters were implanted into rehabilitated California sea lions with no short- or long-term effects noted (Horning and Hill 2005).

2.22.5 Mitigation

In early applications of implantable telemetry devices, the predominant problems were related to the issues of relative size, packaging, and sterility of instruments and procedures. Subsequently, recommendations were made not to exceed 3 to 5 percent of animal body mass (MacDonald and Amlaner 1980). Modern implantable telemetry tags typically remain under 1 percent of body mass. Using appropriate instrument sterilization and sterile surgery techniques, infections from implant procedures have virtually been absent.

2.23 External Scientific Instruments

2.23.1 Description of Methods

Instruments that externally attach to SSLs and NFSs that record diving depths over time (time-depth recorder or TDR) have existed since the 1970s, and have allowed researchers to track pinniped movements vertically in the water column. Coupled with a separate very high frequency (VHF) radio transmitter and a ship/aircraft, it is possible to obtain specific movement information. For example, Kooyman et al. (1983) and Gentry and Kooyman (1986) measured diving behavior and foraging ecology of pinnipeds using a TDR from which dive data were retrieved after the animals returned from feeding trips. Merrick et al. (1994) and Brandon (2000) presented information on female pup-attendance behavior of SSLs with VHF radio transmitters.

Developments in satellite telemetry allow tracking of marine animals using satellite-linked tags or platform transmitter terminals (PTT). Through the ARGOS system on board the NOAA Tiros-series satellites, it is possible to track and retrieve data from free-ranging animals using uplinked communications between PTT attached to the animals and receivers onboard satellites. Locations at sea are determined from the Doppler shift of the frequencies of a series of signals received by the satellite. Baba et al. (2000) were able to follow a yearling SSL for 5 months using two location-only satellite-linked tags.

By combining a PTT and TDR it is possible to simultaneously determine locations and collect diving information while the animal is at sea. TDR collects dive data, which can be reported by the PTT while the animal is at sea or saved for later while the animal is on land. The satellite-linked time-depth recorder (SLTDR) is now commonly used on SSLs and NFSs. Because the SLTDR transmits dive and transmitter status to orbiting satellites when the animal surfaces, the need to recapture the animal is eliminated (e.g., Merrick et al. 1994).

The method most commonly used to attach SLTDRs is to glue them mid-dorsally either directly to the hair (Loughlin et al. 1993) or to a mesh patch fixed to the hair using fast-setting epoxy (Merrick et al. 1994). The tags currently used weigh between 170 grams (g) and 425 g, depending on the battery configuration.

Modified PTT tags that also measures position and temperature, Smart Position and Temperature (SPOT2) tags, weigh as little as 82 g. Because of their size, these units are commonly attached to the top of the animals' head via epoxy and mesh. Because the transmission time on these instruments is much shorter than that of an SLTDR, the head-mounted units allow for more at-sea positions to be transmitted. Both units fall during the annual molt and are generally not recovered.

Ultrasonic acoustic transmitters may also be used to track sea lions. An ultrasonic tag is approximately 90 mm in length and weighs 14 g in the water. The tag is attached to the animal by a mesh patch fixed to the hair using epoxy at the dorsal mid-line of the animal.

2.23.2 Objectives of Research Method

The SSL Recovery Plan identified the need to identify habitat requirements and areas of biological significance for SSLs and to investigate feeding ecology. Participants in a telemetry workshop convened by the Recovery Team in 1997 reiterated the importance of telemetry studies, especially those targeting feeding ecology and movements of juvenile SSLs.

2.23.3 Use of Data

Data obtained from SLTDR and VHF transmitters will contribute to ongoing investigations into seasonal movements, diving behavior, habitat selection, and foraging ecology of SSLs. They are the only practicable tool for following the movements of SSL and NFSs during foraging trips, and for monitoring diving behavior. It will be particularly important for identifying winter foraging areas and refining the knowledge of the foraging capabilities of young sea lions. It will be crucial in assessing the potential effects of commercial fisheries on the status of SSLs and NFSs.

2.23.4 Effects of Research

2.23.4.1 Steller Sea Lions/Northern Fur Seals

In addition to the effects of capture and restraint described previously, the attachment of an instrument can have both short- and long-term effects. Possible short-term effects can include a reduction in foraging activity or an increase in grooming at the expense of other behaviors (Kenward 1987). These types of effects are likely to be present after most tagging events and may be as much a delayed result of the capture and handling as the tag's presence.

The attachment of instruments to the hair with epoxy should not cause any pain if done properly, but may result in discomfort if the placement of the instrument causes pulling of the hair or skin as the animal moves. In addition, if the ratio of the resin and catalyst is not correctly measured, the resultant exothermic reaction can burn the animal's skin. Both the resin and the catalyst can cause skin irritation (itching, rash, hives) and prolonged or repeated skin contact may cause sensitivity. The low vapor pressure of the resin by itself makes inhalation unlikely. There is the possibility that an instrument would be knocked or torn off, pulling out the hair and/or some of the underlying skin, which would then be open to infection.

It is conceivable that carrying the instrument itself might influence the animal's diving behavior through increased hydrodynamic drag or altered buoyancy.

The first satellite-linked transmitters (PTT) were used to tag SSLs in 1987. Studies showed that if a tag was placed on the shoulder region of the animal, it cleared the water and was able to transmit a signal, but it slowed down the animal and required it to expend more energy. The first (Type 2) SLTDR units were deployed in 1990 and many of these units became detached during the molt or fell off because the fur was damaged by the application of too much glue. The Type 3 SLTDR has been used since 1992, and generally has few problems.

2.23.4.2 Pinnipeds

Baker and Johanos (2002) found that there were no deleterious effects on survival, migration, or condition associated with research handling (including tagging) of Hawaiian monk seals. Henderson and Johanos (1988)

also found no indication that tagging pups resulted in measurable harmful effects. Walker and Boveng (1995) found that attachment of TDRs to foraging Antarctic fur seals lengthened their foraging cycles.

Ultrasonic tags have been used successfully to track several marine mammal species, including ringed seals (Lydersen 1991), Weddell seals (Wartzok et al. 1992), and sea otters (Haverlack et al. 2001). Captive and free-ranging ringed seals showed no response to acoustic transmitters in the 50 to 75 kilohertz (kHZ) range (Wartzok et al. 1992).

2.23.4.3 Cetaceans

A study on a captive harbor porpoise (*Phoecena phoecena*) by Geertsen et al. (2004) found that the attachment of satellite tags had minor long-term effects of the animal's behavior. Changes in behavior were evident in the first hours or days after tagging, but thereafter the animal appeared to behave normally other than a slight increase in the mean dive duration.

2.23.4.4 Other Animals

Froget et al. (1998) found that flipper banding resulted in an adverse effect on both the survival and reproductive cycle of king penguins (*Aptenodytes patagonica*) on the Crozet Islands. They showed that returning birds were laying late the following breeding season, double-banded birds laid significantly later than single-banded birds, and that there was a lower return rate for the double-banded birds. Other researchers have reported that many instruments slow penguin swimming speeds significantly (Culik et al. 1994). Ballard et al. (2001) recommended using the smallest and most streamlined instruments to avoid affecting the animal, as well as for researchers to consider individual variation of timing.

2.23.5 Mitigation

Recent technology has led to miniaturization of instrument packages, which helps minimize effects caused by weight and added drag. Care is also taken to adjust the proportions of resin and catalyst to prevent a "hot" mix, and to use the minimum practical amount of epoxy to prevent burning the sea lion's skin.

3.0 CONCLUSIONS

3.1 Current State of Knowledge on Effects of Research

At the most basic level of analysis, research activities can be divided into two broad categories: non-intrusive and intrusive. Non-intrusive activities are those that do not result in physical contact between researchers or research tools and SSLs or NFSs. Thus, aerial surveys, vessel surveys, and observational activities would be considered non-intrusive. Anything that requires capture and handling (including blood and tissue sampling, marking, attachment of instruments, administering chemicals) or some form of physical contact with the animal (including remote biopsy sampling, remote marking, and remote darting for sedation) would be considered intrusive.

It should be noted that even non-intrusive activities might have adverse effects that cause an animal to be injured or die, particularly if the activities are repeated or cause substantial disturbance during the breeding season. Although studies of the effects of human disturbance in the marine environment are somewhat limited, the literature on effects of human disturbance of wildlife in general, including that from the terrestrial animal world, indicates there is reason to assume that human disturbance, even when it does not result directly in physical injury, can have substantial adverse impacts on marine mammal individuals or populations. Studies of stress in humans suggest that chronic stress can have serious consequences, such as weakened immunity leading to more frequent illness and shortened life span.

In general, the risks of adverse effects (such as stress, pain, injury, or mortality) on individual sea lions are greater from intrusive activities than from non-intrusive activities for the following reasons. First, wild animals are often stressed by the presence or close approach of humans, whether on foot or in some kind of vehicle. For example, studies on terrestrial mammals have shown that bighorn sheep (*Ovis canadensis*), mule deer (*Odocoileus hemionus*), and moose (*Alces alces*) have demonstrated greater fear responses to people than to machinery (MacArthur et al. 1982; Freddy et al. 1986; Andersen et al. 1996). Because non-intrusive activities typically take place at a greater distance from the animals than intrusive activities, the potential for this type of stress is reduced relative to intrusive activities. However, long-term effects of human disturbance that interferes with the activity pattern of hauled-out sea lions could potentially have consequences on life cycles and activities (Kucey 2005). For rare or declining species, displacement may reduce reproductive success, presence in the area, parental care, foraging efficiency and prey intake rates, and increase stress and vigilance levels (Andersen et al. 1996; Riffell et al. 1996; Gill et al. 2001a). At a population level, species with high fitness costs and few habitat choices are the ones most likely to be adversely affected by disturbance (Creel et al. 2002).

Second, many intrusive activities have an inherent risk of injury or mortality, either direct or indirect, or injuries result in varying degrees of pain and stress. Some injuries may increase an individual animal's risk of infection. Some injuries or secondary infections can lead to reduced fitness or mortality of individual animals. Even those injuries that do not result in infection or death have physiological costs associated with healing. Whether the cost of wound healing leads to reduced fitness would depend on many factors including the body condition of the animal at the time of the injury, the time of year (as it relates to thermoregulatory and other homeostatic demands), and the availability of adequate nutrients.

3.2 Connection with Recovery and Conservation Plans

3.2.1 Steller Sea Lion Recovery Plan

According to the SSL Recovery Plan, the following recovery actions were identified that are specifically tied into the various research activities discussed in this document:

***Task 1,* Baseline population monitoring.**

Baseline population monitoring is necessary to support all of the recovery actions. They describe the status and trends, vital rates, and health and body conditions of individuals. Research methods that provide data on baseline population include aerial and land-based surveys, branding/re-sighting program, tissue sampling, and live capture/restraint. The SSL Recovery Plan also calls for improvement and/or development of methods with which to establish reproductive rates; provision of indices of health and status using chemical methods; and improvement of live capture methods and non-lethal sampling techniques.

Task 2, Insure adequate habitat and range for recovery.

The SSL Recovery Plan identifies the need to better understand habitat and range for recovery of SSLs. This task is, by far, the most dependent on existing and continued research.

To determine critical habitat, sea lion foraging habitat, seasonal distribution patterns, historical aerial and land-based survey data are used, as well as satellite telemetry. The SSL Recovery Plan identifies the need for improved satellite telemetry data to obtain fine-scale data on foraging habitat, seasonal distribution, and environmental factors that influence foraging and survival. Useful technologies include global positioning system (GPS), STT in conjunction with SLTDR, sonar tracking, and integration of physical/biological oceanographic data that influence prey and SSL distribution.

To estimate prey consumption and determine essential characteristics of the habitat, the research methods typically used are scat collection, analysis of stable isotopes and fatty acid signatures, analysis of whiskers for period of growth. The SSL Recovery Plan identifies the need to improve upon these methods.

The SSL Recovery Plan identifies the need to develop methods to measure energetic costs and physiological diving capabilities of diving pinnipeds.

The SSL Recovery Plan also identifies the need to assess prey resources for SSLs and interactions with fisheries. Integration of data from SSL research and fisheries research will be important in understanding this interaction.

Task 3, Protect from over-utilization for commercial, recreational, scientific, or education purposes.

The SSL Recovery Plan calls for researchers to use new technologies that reduce disturbance, potential mortality, and the need for invasive methods. Approach and handling methods will be reviewed periodically to minimize the potential for injury or mortality, In addition, studies should be undertaken to evaluate the effects of disturbance by particular research activities.

Task 4, Protect from diseases, contaminants, and predation.

The SSL Recovery Plan calls for analysis for agents or diseases with potential to affect the survival, growth, reproductive, etc. effects on SSLs. Research methods that provide these data include blood sampling, fecal samples, tissue sampling, and stomach content analysis. Research on the effects of predation on SSLs will focus on killer whales. Integration of data collected from those studies with SSL data is important.

Task 5, Protect from other natural or man-made factors and administer the recovery program

The SSL Recovery Plan notes that scientific research is essential for understanding and mitigating the threats to SSL recovery. A new, streamlined process should be investigated to reduce the permitting process for SSL-related research to less than 6 months to facilitate research opportunities that would aid in implementation of the SSL Recovery Plan.

3.2.2 Northern Fur Seal Conservation Plan

According to the NFS Conservation Plan, the following conservation actions were identified as specifically tied to the various research activities discussed in this document.

Objective 1, Identify and eliminate or mitigate the cause or causes of human-related mortality of the Eastern Pacific stock of NFSs.

Research would be directed at examining the distribution and abundance of debris onshore and at sea relative to juvenile and female NFSs at various reproductive stages (beginning of reproduction, lactation, departing of females); determine the probable fate of discarded fishing gear and other debris near areas inhabited by NFSs; and monitor and review data collected from fisheries observers related to NFS incidental takes. Research methods would include aerial and land-based surveys, physiological studies, and collection of telemetry data.

Objective 2, Assess and avoid or mitigate adverse effects of human related activities on or near the Pribilof Islands and other habitat essential to the survival and recovery of the Eastern Pacific stock of NFSs.

Research would be directed at evaluating the potential vulnerability of NFSs to vessel traffic, oil spills, offshore oil and gas development, and harbor development. Studies would need to continue to monitor radio and/or satellite tagged animals to determine seasonal distribution, age-class behavior, etc. Aerial and land-based surveys would continue to provide data on pup production, territory structure, and population trends. The analysis of environmental pollutants/contaminants would be conducted via use of tissue sampling and oceanographic sampling.

Objective 3, Continue and, as necessary, expand research or management programs to monitor trends and detect natural or human-related causes of change in the NFS population and habitats essential to its survival and recovery.

The NFS Conservation Plan identified the need to monitor changes in the size, productivity, and vital rates of the NFS stocks. Research methods used to conduct these studies include aerial and land-based surveys, satellite telemetry, scat collection, marking/re-sighting, and biological/physiological sampling. The NFS Conservation Plan also calls for improvements of these methods to reduce disturbance, as well as coordination and integration of intra- and interspecies research.

Objective 4, Coordinate and assess the implementation of the conservation plan, based on implementation of Conservative Actions and completion of high priority studies.

The NFS Conservation Plan notes that scientific research is essential for understanding and mitigating the threats to NFS conservation. In particular, it states:

> "*Data collected through any research outlined in this plan should be analyzed and reported in a timely manner. Reports should be thoroughly referenced, independently reviewed and be organized to facilitate comparison with existing reports. As much as possible, data should be presented in peer-reviewed periodicals and other open publications to ensure that research programs benefit from regular peer commentary. To the maximum extent possible, research efforts should collect data that can be compared with historical data. Studies may need to be conducted to calibrate results from newly developed techniques with those obtained by previous methods. Data analysis should examine trends over time and attempt to correlate observed changes with physical, biological, or human-induced changes in the environment. Analysis should emphasize correlations between regional differences in fur seal population trends with factors such as physical oceanography, food resources, and human activities (e.g., fishing, habitat degradation, harassment). Such correlations can indicate causes of declines which may lead to more effective management.*"

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