I. Application to Amend Permit No. 782-1719 for Scientific Research Under the Marine Mammal Protection Act and for Scientific Purposes Under the Endangered Species Act

II. Date of Application: November 7, 2005

III. Applicant and Personnel

A. Applicant/Permit Holder, Principal Investigator, Co-investigator(s), and other Personnel Directly Involved in Taking

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Co-Investigators – See Appendix I for a list of co-investigators and their areas of responsibility.

B. Qualifications and Experience

C*urriculum vitae* of PI and CI's are on file at F/PR1. See Appendix II for a list of publications, reports and presentations resulting from MMPA Permit No. 782-1438 and MMPA Permit No. 782-1510-02 from 1997 through 2003. See Appendix III for a cruise report on NMML's killer whale studies.

IV. Proposal

A. Summary

This permit application is specifically to cover opportunistic encounters with Southern Resident killer whales. Photo-identification will be conducted to document individual identity, and biopsy samples will be obtained to contribute to ongoing studies of killer whale feeding ecology. Information on the distribution and diet of southern residents has relevance for their conservation status, in particular whether this population is threatened by changes in prey availability (National Marine Fisheries Service, 2005). However, there are key data gaps that currently constrain an understanding of the feeding ecology of southern resident killer whales, and prevent us from determining the extent and magnitude of this threat. Notably, there is a lack of data on the year-round diet and nutritional status of these whales, particularly in the winter months when the whales extend their movements beyond their core range (Killer Whale Recovery Team, 2005). Samples collected opportunistically by NMML surveys could help to fill this data gap. This is not intended to constitute a dedicated sampling regime, but rather to enable the collection of important samples from these whales that may help to fill key data gaps on year-round diet. These samples will be contributed to chemical analyses of diet and nutrition (e.g. Herman et al. 2005), which will be integrated with other sampling efforts from this population. Plans for biopsy sampling would be coordinated with the NWFSC and SWFSC, in support of the Tissue Sampling Plan for Southern Resident Killer Whales, which was approved by NMFS in 2005.

Most of the projected encounters with Southern Resident killer whales will occur during ongoing research projects, and will allow us to determine distribution and food habits of Southern Resident killer whales. This research is as directed to NMFS through the statutes of the Marine Mammal Protection Act (MMPA) and as guided by the Marine Mammal Commission (MMC), the Alaska Scientific Review Group and Pacific Scientific Review Group (SRG), and the International Whaling Commission (IWC), and in collaboration with the National Marine Sanctuary Program.

B. Introduction

1. Status of the Species

(a) Species description

The southern resident killer whale population is small, with recent declines of 17% between 1995 and 2001, and currently contains 88 members (Center for Whale Research (WA), Unpublished data). During the summer and fall, southern residents are primarily found in the U.S. / Canada trans-boundary waters of British Columbia and Washington State. Some members of the population typically remain in the same general area in winter and spring, but others appear to range over much greater distances, and have been reported as far south as Monterey Bay, California, and as far north as Haida Gwaii (the Queen Charlotte Islands). During the summer and fall, the principal prey of southern residents appears to be chinook and chum salmon, (*Onorchynchus tshawytscha* and *O. keta*); little is known of their diet in the winter and spring. The lack of information about winter diet and distribution of the southern residents is a major knowledge gap that impedes our understanding of the principal threats facing the population.

(b) Life History and Population Status:

The size of the southern resident population has been known since the first complete photo-identification census in 1976, and was estimated for the years prior to that (CWR, unpublished data). Although the southern resident population was likely increasing in size in the early 1960s, the number of whales in the community dropped dramatically in the late 1960s and early 1970s due to live capture for aquariums. A total of 47 individuals that are known or likely to have been southern residents were captured and removed from the population. The population increased 19% (3.1% per year) from a low of 70 after the live-captures ended in 1973 to 83 whales in 1980. From 1981-1984 the population declined 11% (-2.7% per year) to 74 whales as a result of lower birth rates, higher mortality for adult females and juveniles, and lower numbers of mature animals, especially males, which was caused by selective cropping in previous years. From 1985 to 1995, the number of southern residents increased by 34% (2.9% per year) to 99 animals. A surge in the number of mature individuals, an increase in births, and a decrease in deaths contributed to the population growth. The latest decline began in 1996, with an extended period of poor survival and low fecundity resulting in a decline of 17% (-2.9% per year) to 81 whales in 2001. Since 2001, the number of southern residents has increased slightly to 88 in 2005 (unpublished data CWR).

In the U.S., the southern residents were listed as ‘depleted’ under the Marine Mammal Protection Act (MMPA) in 2003. The National Marine Fisheries Service (NMFS) is currently proposing designating the southern residents as ‘threatened’ under the Endangered Species Act (ESA). In June 2004, the Washington State Department of Fish and Wildlife added southern resident killer

whales to their endangered species list. In Canada, the southern resident population was designated as endangered under the Species at Risk Act in November 2001.

A "population stock" as defined in the MMPA, or a "vertebrate species" as defined in the ESA is a group of animals that share a common space and interbreed. The NMFS has the responsibility of determining the status of cetacean stocks. The National Marine Mammal Laboratory has ongoing research projects, both independently and in collaboration with researchers at other NMFS, NOAA and USFWS offices, that are directly tasked with determining the status and structure of cetacean stocks. The most up-to-date information about the status of each species and/or stock and factors affecting the status of these stocks can be found in the stock assessment reports listed below, including appropriate references. These reports include the best data available on stock definition and geographic range, population size, including a minimum population estimate and current population trend, current and maximum net productivity rates, potential biological removal, annual human-caused mortality and serious injury, and status of stock. The pertinent and most current stock assessment reports for the region studied are Carretta et al. 2005 and Angliss and Lodge 2003.

2. Background/Literature Review

Information on the diet of southern residents has relevance for their conservation status, in particular whether this population is threatened by changes in prey availability (National Marine Fisheries Service, 2005). Southern resident killer whales are dietary specialists, feeding exclusively on fish, and primarily on salmon (Ford et al. 1998). Heimlich-Boran (1986) presented evidence based on sport fishing catch statistics that southern resident killer whale occurrence was positively correlated with salmon abundance in the San Juan Islands and portions of Puget Sound (Heimlich-Boran 1986). Fall movements of southern resident pods into Puget Sound were roughly correlated with chum and chinook salmon runs (Osborne 1999). Recent sightings of southern resident killer whales in California were coincident with large runs of chinook salmon (unpublished data, CWR). During dedicated studies during the months of May through October, 135 surface observations of feeding resident killer whales and the collection of prey fragments have revealed that their preferred prey is salmon, particularly chinook salmon (65% *Oncorhynchus tshawytscha*) (Ford et al. 1998). Approximately 25% of these observations were from southern resident killer whales. Preliminary analysis of prey remains collected recently from southern residents support a pattern of preference for Chinook salmon in the summer, with additional feeding on chum salmon in the fall (Fisheries and Oceans Canada, CWR unpublished data).

Despite these data from summer and fall, little is known of the diet of southern residents in winter and spring. The lack of information about winter diet and distribution of the southern residents is a major knowledge gap that impedes our understanding of the principal threats facing the population.

3. Hypothesis/Objectives and Justification

Conservation plans for southern resident killer whales highlight the possible threat of reduced prey availability (NMFS, 2005; Killer Whale Recovery Team, 2005). However, there are key data gaps that currently constrain an understanding of the feeding ecology of southern resident killer whales, and prevent us from determining the extent and magnitude of this threat. Notably, there is a lack of data on the year-round distribution, diet and nutritional status of these whales,

particularly when the whales extend their movements beyond their core range (Killer Whale Recovery Team, 2005).

The objective of our proposal is to collect photo-identification and biopsy samples opportunistically from southern resident killer whales to help fill the key data gap of year round diet and distribution. Plans for biopsy sampling would be coordinated with the NWFSC and SWFSC, in support of the Tissue Sampling Plan for Southern Resident Killer Whales, which was approved by NMFS in 2005.

This is not intended to constitute a dedicated sampling regime, but rather to enable the collection of important samples during NMML research cruises that focus on other populations. We do not expect to encounter southern residents annually on more than five occasions. However, given that the full range of the southern resident population is poorly known, we cannot exclude the possibility of more encounters. Our surveys are staffed by experienced killer whale researchers, who will attempt to identify southern residents in the field by comparison to published photo-identification catalogues (e.g. Ford et al. 2000).

To be relevant to southern residents, samples must come from the southern resident population, and samples from a non-listed killer whale population cannot be used as a proxy. Photo-identification techniques will be used to document the identity of southern resident killer whales by comparison to existing photo-identification catalogues of these whales (Ford et al. 2000; CWR unpublished data). Although the collection of prey remains (e.g. scale samples) could provide "snapshot" data on prey composition at the time of the encounter, biopsy samples can provide more informative data on diet that has been integrated into the whales' tissues over preceding months. Specifically, proven chemical analyses of killer whale biopsy samples, with stable isotope, fatty acid and contaminant analyses, has been shown to provide valuable inference about diet and nutrition (e.g. Herman et al. 2005). It has not been possible to follow, or regularly encounter, southern residents when they are not within their core range. In contrast, biopsy samples can provide data on the diet of southern residents during a period of weeks/months prior to sampling. Therefore, biopsy samples are the only way to collect data with relatively long temporal coverage on the diet of southern residents when they are outside of their core range.

C. Methods

1. Duration of the Project and Locations of Taking

Duration: Projects will be conducted mostly during the late spring and summer, when weather is optimal or to maximize comparisons to previous surveys, but permission is requested for takes in any season as needed to meet research objectives. The dates of the takes will be potentially on any day.

Location: The known range of Southern Resident killer whales extends from Monterey Bay, California to the northern Queen Charlotte Islands, Canada, but we would like to have the authority to take them wherever we may encounter them opportunistically. It is likely that all of the takes will potentially occur in the North Pacific Ocean, as well as the Gulf of Alaska, Gulf of California, and Canadian territorial waters.

Killer whale vessel surveys

Long-term research in southeastern Alaska waters has been conducted since 1989 to characterize the natural history of killer whales. Over the course of the last decade, southeastern Alaskan killer whale population levels and trends have been established. In addition, NMML has identified killer whale stock structure, dive profiles, short- and long-range movements, habitat use, contaminant levels, and dietary preferences. All cetacean sightings are logged thus providing valuable information on southeastern Alaska cetacean abundance and distribution.

Killer whale research in the Gulf of Alaska, Aleutian Islands, and Bering Sea includes a vessel survey designed to estimate the abundance of killer whales throughout a large portion of the range of the Steller sea lion western stock (in the eastern Aleutian Islands through the western Gulf of Alaska), from shore to approximately 30 nmi off shore. The survey would be conducted as a regular line-transect survey for all cetaceans. Methods include photo-identification and biopsy sampling. This multi-year project began in 2001 and is currently scheduled to continue into the period of the new permit.

Cetacean vessel surveys

A vessel line-transect survey for cetaceans will be conducted in the eastern Bering Sea every year as possible in association with a groundfish stock assessment survey aboard the NOAA ship *Miller Freeman.* The survey design usually consists of 29 north-south transect lines spaced 20 nmi apart and proceeding from east to west/northwest along the shelf, starting at longitude W171:26.00 and ending at longitude W178:55.00. This research will include photo-identification and biopsy sampling when possible. Similar studies will be conducted on other research vessels as the opportunity arises to take advantage of available platforms in areas where directed surveys cannot reach due to budget constraints.

2. Types of Taking, Methodology Involved and Numbers of Animals that would be Taken

We request the authority to harass Southern Resident killer whales (*Orcinus orca*) for the purpose of collecting photo-identification and biopsy samples. We are requesting a potential of 500 photo-identification takes and 10 biopsy takes per "species" (i.e. Southern Resident killer whales) per year to sample on an opportunistic, rather than targeted, basis should these whales be encountered during surveys. We will confirm that the whales taken are Southern Resident killer whales using existing (and updated) photo-ID catalogs we will bring along while in the field. We will also ensure that only experienced researchers conduct the biopsy sampling.

Plans for biopsy sampling would be coordinated with the NWFSC and SWFSC, in support of the Tissue Sampling Plan for Southern Resident Killer Whales, which was approved by NMFS in 2005. This is not intended to constitute a dedicated sampling regime, but rather to enable the collection of important samples from these whales that may help to fill key data gaps on year-round diet. These samples will be contributed to chemical analyses of diet and nutrition (e.g. Herman et al. 2005), which will be integrated with other sampling efforts from this population. The takes we've requested are also listed in Table 1

The types of taking involve (1) Level B harassment (i.e., no potential to injure) and (2) Level A harassment (biopsy). Individuals may be subject to take by harassment more than one time.

Biopsy sampling will be conducted in conjunction with photo-identification surveys and tagging projects and during dedicated biopsy projects. Biopsies may be collected from both sexes and all ages, *except* calves and their accompanying mothers.

Aerial and boat surveys:

Large Vessel Line Transect Survey Methods

The study areas will include the Gulf of Alaska, Bering Sea, southeastern Alaska, and may extend to other areas of the North Pacific Ocean and will be surveyed from NOAA Ship John N. Cobb (28.3 m in length), NOAA Ship Miller Freeman (65.5 m in length), or similar vessels. For most surveys, transects are designed to systematically cover the study area usually with a sawtooth pattern from shore to a particular depth contour. Cetacean surveys on the NOAA ship Miller Freeman are a piggybacked on an AFSC acoustic pollock survey where north-south lines are surveyed over the Bering Sea shelf. Line-transect sampling methods (Buckland et al. 1993) will be used to collect data for estimating abundance of cetaceans, as have been used on previous surveys (Barlow 1995, Moore et al. 2002). Three to eight observers rotate through at least 3 positions (port and starboard observers, and a data recorder) during daylight hours and weather permitting (sea state is Beaufort 0-5 and minimal rain). Observers rotate every 30 or 40 minutes between the three positions and a rest period. Either 25X "bigeye" or 7X handheld binoculars are used. The port observer will survey from 10º right to 90º left of the trackline and the starboard observer from 10º left to 90º right of the trackline. The recorder will scan the entire 180º area forward of the ship, focusing primarily on the trackline, using 7X reticled binoculars to confirm sightings.

The ship's global positioning system (GPS) unit or a handheld GPS will interface with a portable computer at the recorder's station. A standardized survey software program such as WinCruz will be used to collect standard line-transect information. The date, time, and position of the vessel are automatically entered into the survey program every 5 min and whenever data are entered by the recorder. At the start of each trackline, observer positions, and environmental conditions will be entered. Environmental conditions include sea state (Beaufort scale), swell height and direction, weather (rain, fog, no rain or fog, both rain and fog), horizontal and vertical positions of the sun, wind speed and visibility. Sighting information include sighting cue (blow, splash, animal), method (binocular type or naked eye), vertical distance (taken from reticles in the binoculars), angle relative to the ship's heading (from an angle ring on the binocular mount or an angle board), species, and group size (best, high, and low count). In some cases, the line-transect effort will be suspended to approach a group to facilitate species identification or group-size estimation, or to conduct photo-identification or biopsy sampling. Methods will be similar to those used in small vessel methods below. Line-transect data for the estimation of abundance will be analyzed using DISTANCE sampling methods (Buckland et al. 1993; Thomas et al. 1998).

Small Vessel Survey Methods – Photo-ID and biopsy

The study areas will include the entire range authorized by this permit, and will involve approaches in small craft or larger vessels for photo-identification and biopsy studies. All individual whales will be photographed for identification, if possible. Some of these approaches may also be used to collect biopsy samples.

Whales will be approached and then photographed and biopsied from small boats or from larger vessels. The approaches will be gradual and will be designed to minimize or avoid any startle response. All sex and age groups will be approached for photo-identification studies, but biopsy samples will not be obtained from calves or their accompanying mothers. If there is evidence of avoidance, a maximum of three approaches will be attempted before the encounter is terminated.

Southeastern Alaska killer whale surveys

Killer whales have been known to occur throughout Alaskan waters (Braham and Dahlheim 1982); however, there were few systematic survey data to estimate population levels. Leatherwood et al. (1983) reported the results of eight aerial surveys conducted off Kodiak Island, from Unimak Pass westward along the Aleutian Islands, and between Unalaska Island and the Pribilof Islands. A total of 36 groups (236 individuals) were sighted. Abundance estimates were not attempted because repeat sightings from the same pods could not be distinguished from single sightings. Brueggeman (1987) reported the results of over 38,000 nm of aerial survey effort conducted from April to December 1985 in the Shumagin Islands, the North Aleutian Basin, and St. George Basin. A total of 25 groups (67 killer whales) were observed. Duplicate sightings were not considered thus the actual number of killer whales seen could be less. Prior to 1989, with the exception of Prince William Sound (photo-identification studies began in 1984; Leatherwood et al. 1990), reliable population estimates of Alaskan killer whales were not available.

In 1992 and 1993, NMML conducted surveys throughout Alaska to obtain minimum population estimates for killer whales. Dahlheim and Waite (1993) summarized the results of 1992 photo-identification investigations. In southeastern Alaska, 183 whales were identified. In Prince William Sound, 352 whales were identified. From Kodiak Island west to Atka Island (eastern Aleutian Islands) and north to the Pribilof Islands, 182 whales were identified. The results of NMML's 1992 research yielded a minimum population estimate of 717 individual killer whales throughout Alaska. In 1993 photographic research effort was continued. At least 90 new whales were added to the Bering Sea population of killer whales through NMML's 1993 dedicated research project (Dahlheim 1994). Each year, killer whale photographs collected through the Fisheries Observer Program and other sources of data (e.g., Steller sea lion project, etc.) are reviewed and individual whales are identified and compared to NMML's photographic database. An additional 100 individual killer whales were added to the 1992/1993 Alaskan database.

Dedicated, long-term investigations (1991 to present) in southeastern Alaska have resulted in the recognition of at least 276 individual killer whales. Abundance and trends of this species has been established through photo-identification methodology. Comparison of photographic matches among geographical areas has allowed us to determine short and long-range movements and habitat use. Through biopsy sampling, killer whale stock structure has been determined through genetics research. Three eco-types are known to inhabit the study area: resident, transient and offshore types. Blubber obtained during the biopsy sampling has been analyzed for contaminant levels by our colleagues at the Northwest Fisheries Science Center. Contaminant levels of southeastern Alaskan killer whales will be compared to killer whale samples collected throughout the North Pacific.

By following whales for extended periods of time, we have also collected valuable data on dietary preferences by different whale pods. Recently we calculated kill rates by transient whales on various species of marine mammals occupying the southeastern Alaska study area. This is the first attempt to quantify predation rates of any marine mammal through continuous and direct observation of predatory behavior. Information from southeastern Alaska transient killer whales will hopefully contribute to assessing the impact that killer whales may be having on marine mammal populations in western Alaska where several marine mammal populations are in serious declines.

The NOAA Research Vessel JOHN N. COBB has provided logistic support for past surveys in southeastern Alaska, and will continue to do so if available. All inland waterways of southeastern Alaska have been surveyed each year ranging from Juneau to Ketchikan. Two to three, 12-14 day trips are scheduled each year; one trip each in spring, summer, and fall. Line transect methodology is used during these surveys; transiting at a speed of 10 kts. In southeastern Alaska, the target species is killer whales, and the intent is to find and photograph as many as possible. In addition to photographs, biopsy samples and underwater acoustic recordings have been collected whenever possible. When killer whales are found and weather permits, a small skiff (17-22 foot) is launched to obtain photographic, biopsy, and underwater recordings. In general, there are multiple approaches for each whale/pod to maximize the probability of obtaining a quality photograph and biopsy sample. Species other than killer whales may also be photographed and biopsy darted if time permits.

Western Alaska killer whale surveys

The documented decline of Steller sea lions, harbor seals, and sea otters in the western Gulf of Alaska and Bering Sea has raised questions about the potential impact that killer whale predation may have on these populations. Although killer whale population size and stock structure is well documented for the waters of southeastern Alaska and Prince William Sound (Dahlheim et. al., 1997; Matkin et. al. 1999, respectively), this is not so for killer whales inhabiting Alaskan waters west of Kodiak Island. The only dedicated surveys for killer whales in central Alaska occurred in 1992 and 1993 (Dahlheim and Waite 1993; Dahlheim 1994). Minimum counts of killer whales, ranging from the western Gulf of Alaska to the central Aleutian Islands were obtained through photo-identification studies (Dahlheim 1997). In addition, fisheries observers working with NMFS/Alaska Fisheries Science Center/RACE Division also photograph killer whales. Analyses of photographic data from both the dedicated surveys and fishery observer program have resulted in the identification of approximately 400 individual killer whales.

Based on the preliminary analyses of photographic and behavioral data, both resident (i.e., primarily fish-eaters) and transient (i.e., primarily mammal-eating) forms of killer whales inhabit central Alaskan waters. Another killer whale eco-type (the offshores) has also been observed in Alaska. In Alaska, offshore killer whales have only been seen as far north as the inland waterways of southeastern Alaska (Dahlheim et al. 1997). Most of the killer whale information available from central Alaska pertains to the resident form. Large aggregations of resident whales are frequently reported off the eastern side of Kodiak Island, waters surrounding the Trinity Islands, Unimak Pass, and the north side of Unalaska Island (Dahlheim 1997; NMML, unpublished data). Transient whales, typically occurring in small groups of less than 10 whales, have been observed throughout this area. Out of the 400 photo-identified whales, approximately 40 whales have been provisionally classified as transients. In addition to killer whale photo-identification and biopsy sampling, other species encountered are also sampled as time allows, including right whales, humpback whales, fin whales and sperm whales, providing a valuable addition to the genetic and photographic data for these species.

Cetacean Surveys in the Eastern Bering Sea

Until recently, cetacean distribution and abundance in the Bering Sea has been poorly described, with even recent reviews of cetaceans' role in the ecosystem reliant on data from the commercial whaling era (e.g. Springer et al. 1996; Springer et al. 1999). Commercial harvests of baleen whales were extensive in the North Pacific and Bering Sea (Miyashita et al. 1995), especially

between 1835 and 1850 for North Pacific right whales (Webb 1988) and between 1965 and 1979 for fin and humpback whales (Wada 1981). The effect of these large-scale removals on the marine ecosystem is largely unknown. Similarly, some species of odontocetes are sometimes killed in the course of commercial fishing operations. Pelagic dolphins and Dall's porpoise were especially vulnerable during high seas driftnet fishing in the North Pacific in the 1980s (*e.g.*, Hobbs and Jones 1993), with the long-term ramifications of these removals also unknown. One reason for this uncertainty is the lack of data on current cetacean distribution and estimates of abundance in pelagic environments. Surveys to determine distribution and abundance are costly and therefore often confined to coastal waters where the logistics are most practical (e.g., Barlow 1995), or to areas of the ocean where marine mammal mortality associated with commercial fishing is particularly high (*e.g.*, Hobbs and Jones 1993). The pelagic waters of the Bering Sea have not met either criteria and so are comparatively under-sampled for cetaceans.

In 1997, the first of a series of cruises was undertaken in association with the Alaska Fisheries Science Center/Resource Assessment and Conservation Engineering (AFSC/RACE) division to conduct visual surveys for cetaceans during the semi-annual acoustic trawl surveys for walleye pollock (*Theragra chalcogramma*) on the Bering Sea shelf (Tynan 1998), and to conduct photo-identification and biopsy sampling when time allows on species such as right whales, humpback whales, fin whales, and killer whales. Biologists from the AFSC/National Marine Mammal Laboratory (NMML) were able to join subsequent surveys in the central-eastern Bering Sea (CEBS) in 1999 and 2002 and in the southeastern Bering Sea (SEBS) in 2000 and 2002, providing an opportunity to describe cetacean distribution and calculate abundance over a broad area of the Bering Sea shelf. Moore et al. (2002) present the first estimates of cetacean abundance that can be compared between two broad pelagic zones, as well as cetacean distribution and sighting rates relative to hydrographic domains and fronts commonly referenced in papers describing oceanographic processes in the eastern Bering Sea (*e.g.*, Springer et al. 1996; Hunt 1997; Napp et al. 2000; Stabeno et al. 2001).

Capture:

No animals will be captured**.**

Handling/Restraint:

No animals will be restrained.

***Sample collection and analysis*:**

Biopsy samples will be collected using pneumatic rifles projecting small, light-weight darts (e.g. Barrett-Lennard et al. 1996) or simple-type light-weight (not compound) crossbows. These systems have been used extensively on killer whales in Canadian and Alaskan waters for more than a decade, and have been shown to elicit only minor behavioral reactions. Photographs will be obtained from each sampled whale to document individual identity.

All biopsy tissues will be conserved for fatty acid, stable isotope and contaminant analyses, as well as for genetic confirmation of stock. Skin samples will generally be conserved in a DMSO-based solution for genetics, and both skin and blubber will be conserved for chemical analyses other tissues by freezing. Tissues from biopsy samples have been used to measure contaminants (Krahn et al. 2001), determine trophic levels (Gendron et al. 2001) and stable isotopes (Todd et al. 1997). These methods have proven to be satisfactory for chemical analyses of diet for killer whales (e.g. Herman et al. 2005).

Biopsy dart tips will be thoroughly cleaned between sampling events and sterilized by immersion in 70% ethanol or equivalent sterilizing technique. Some researchers heat the tip with propane burner, but we prefer to use ethanol, as it is far safer in an inflatable vessel. Cleaning before sterilization will be a done by a thorough wash and immersion overnight in 8% peroxide/bleach or equivalent solution (removes any DNA from the past sample - and thus reduces the risk of contamination).

Feces, and sloughed skin are useful sources of DNA and will be collected opportunistically. Additionally, prey remains (e.g. scale samples) will be collected if they are observed. These opportunistic collections will be conducted in conjunction with photo-identification surveys. No additional takes will be associated with these activities, as this involves inspection of the water that has been disturbed by surfacing whales *after* they have moved from the vicinity.

Biopsy tissue samples will be shared with the Marine Mammal Tissue Bank and archived at the Southwest Fisheries Science Center for genetics material or at the Northwest Fisheries Science Center for fatty acid, stable isotope or contaminant analysis. In addition, samples or PCR products will be made available to researchers upon request.

Biopsy samples will be collected using standard protocol, and samples will be divided and distributed to a number of research organizations, including archival storage at the Southwest Fisheries Science Center. Each sample will be labeled with a unique code, as well as location, source, date and location of collection.

Research objectives: All research is designed to meet mandates of the MMPA and ESA. The NMML is tasked with assessing status of stocks, population trends, stock structure, habitat utilization and general health of many cetacean populations, both for US management purposes and for international management purposes (International Whaling Commission). In addition, information we collect is used to update and refine stock assessment reports for both the Alaska and Pacific regions.

***Scientific instruments*:**

No scientific instruments will be placed as part of this research.

Marking:

No animals will be marked during the course of these surveys.

Acoustics

No active acoustic studies will be conducted as a part of this research.

3. Import/Export of Marine Mammals/Marine Mammal Parts

No samples will be imported or exported under this permit. All import or export will be conducted through NMML's permit 782-1694 or subsequent import/export permits.

4. Removing Animals from the Wild into Captivity/ Research or Enhancement on Captive or Rehabilitating Animals s

No animals will be removed from the wild and no research on captive animals will be done.

5. Lethal Take

No marine mammals will be lethally taken as a part of these studies.

D. Resources needed to accomplish objectives

Research proposals are appended in Appendix Table IV.

E. Effects of the Research and Measures to Minimize Stress, Pain, Suffering, and/or Harassment:

1. Effects

A number of studies have been published on the effects of biopsy darting on whales (Weinrich et al. 1992, Clapham and Mattila 1993 and Brown et al. 1994), and specifically killer whales (Barrett-Lennard et al. 1996). Biopsy darting has been shown to have only a short-term effect on the animals studied if care is taken while approaching animals. Short-term impacts include a small <10mm diameter hole in the skin and the stress of injury and approach. We expect no long-term effects on individual animals. Detailed data will be collected at the time of sampling (notes, photographs), so reactions and wound healing can be documented.

2. Alternatives

Although the collection of prey remains (e.g. scale samples) could provide "snapshot" data on prey composition at the time of the encounter, biopsy samples can provide more informative data on diet that has been integrated into the whales' tissues over preceding months. Specifically, proven chemical analyses of killer whale biopsy samples, with stable isotope, fatty acid and contaminant analyses, has been shown to provide valuable inference about diet and nutrition (e.g. Herman et al. 2005). It has not been possible to follow, or regularly encounter, southern resident killer whales when they are not within their core range. In contrast, biopsy samples can provide data on the diet of southern residents during a period of weeks/months prior to sampling. Therefore, biopsy samples are the only way to collect data with relatively long temporal coverage on the diet of southern residents when they are outside of their core range.

3. Incidental effects

Incidental harassment, both from aerial and vessel studies, should have no effect.

(c) Aerial and boat surveys

Some studies have linked vessel noise and traffic to short-term behavioral changes (such as increased swimming speeds, longer dive times) in Southern and/or Northern Resident killer whales (Kruse, 1991; Jelinksi et al. 2002; Williams et al. 2002a; Williams et al 2002b, Foote et al. 2004). Many of these studies were focused on the reactions of whales to whale-watching boats and other types of vessels. These platforms are usually much larger that research approach vessels, and the duration of the encounters can sometimes be lengthy. .

Kruse's (1991) study found that Northern Resident killer whales in Johnstone Strait BC increased swimming speeds when whale-watching vessels were present (which differed from findings by Williams (2002a) on the same population of whales). Jelinski (2002) used GIS methods to measure reactions of whales to whale-watch boats (and vice versa). He did not study effects of research approaches to whales. Williams (2002a) conducted experimental "whale-watching" type vessel approaches (not research approaches) and found some short-term behavioral changes in the presence of one boat (and fewer behavioral changes in the presence of

multiple boats). He documented that there did not appear to be any measurable long-term effects in the Northern Resident population that he was studying, and noted that the "whales continue to return each year" and the "population continues to grow". Williams (2002b) analyzed reactions to whale-watch type approaches known colloquially as "leapfrogging" (placing whale-watch boats in the path of a whale). Our research approaches never include the use of "leapfrogging techniques. Foote et al. (2004) studied behavior of Southern Resident killer whales in the presence and absence of whale watching boats and found that the whales adjusted their behavior to compensate for anthropogenic noise beyond a certain threshold level (that is, in the presence of "intense whale-watching activities", specifically not research approaches).

Research approaches are in a different category than whale-watching approaches. Whales will be approached from small boats. If there is evidence of avoidance, a maximum of three approaches will be attempted before the encounter is terminated. Research approaches for biopsy sampling are likely to be closer than photo-ID approaches, but the encounter is usually brief and the encounter ends when the data have been obtained or the approach rules have been invoked. Because killer whales travel in stable groupings, all individuals encountered will be subject to approaches. These approaches may cause minor behavioral responses, such as subtle changes in direction. However, experience has shown that these reactions are normally short lasting, and approaches will be gradual and will be designed to minimize or avoid any sort of startle response.

(d) Capture

No marine mammals will be captured as a part of these studies.

(e) Handling/Restraint

No marine mammals will be handled or restrained as a part of these studies.

(f) Sample collection

Individuals that are biopsy sampled may exhibit an initial startle reaction as a response to the sound of the biopsy dart splashing into the water after contacting the whale. There may be a startle reaction in response to the dart contacting the whale, but because the dart tips will be less than 40mm long and will not penetrate beyond the blubber layer. Biopsy sampling does not appear to cause appreciable pain. Light-weight darts fired from low power crossbows or pneumatic rifles will be used to minimize impact weight and therefore minimize short term behavioral reactions. We do not anticipate any longer-term behavioral reactions. Completely sterilized equipment will be used for biopsy sampling, and we do not expect any infection or mortality. We will collect detailed data on the location of the biopsy sample site (including photographic documentation) so we can monitor the healing process.

(g) Scientific Instruments

No instruments will be places on marine mammals as a part of these studies.

(h) Marking

No marine mammals will be marked as a part of these studies.

(i) Acoustics

Active acoustic devices will not be used as part of this study.

(j) Incidental Harassment

Because killer whales travel in stable groupings, all individuals encountered will be subject to vessel approaches for photo-identifications. However, only specific target animals will be approached for biopsy sampling, to minimize the number of whales that are closely approached. These target individuals will be carefully chosen to limit the number of whales that are closely approached. Calves and mothers of calves will not be targeted. Biopsy sampling will only be attempted when target animals can be clearly distinguished from their associates, and where there is minimal risk to non-target whales being hit by the dart.

4. Measures to minimize effects

During aerial surveys, if animals appear to respond to the presence of an aircraft, the aircraft will leave the vicinity immediately.

During biopsy studies, experienced researchers will be conducting the studies and providing intensive training and supervision for all less experienced field assistants. Whales will be approached and then photographed and biopsied from small boats or from larger vessels. The approaches will be gradual and will be designed to minimize or avoid any sort of startle response. If there is evidence of avoidance, a maximum of three approaches will be attempted before the encounter is terminated.

5. Monitoring effects of activities

A photograph will be taken of all biopsied individuals, so that these can be identified and matched to the existing photo-identification catalogue for southern resident killer whales. This will allow these individuals to be monitored during subsequent encounters. Additionally, detailed notes and photographs will be taken to document the specific location of the biopsy wound so that the progress of wound healing can be carefully monitored using subsequent photographs.

F. Publication of Results

Within a few months of the completion of each study, a field report will be circulated to specific members of the respective community, native groups, environmentalists, research scientists, and managers as appropriate. For most studies, within one year, an annual report will be finalized and circulated along with other annual reports in a NMFS Processed Report (e.g., Lopez and Angliss 2001). When several years of data have been collected, each study will be published in a scientific journal and may be presented in scientific conferences or workshops. See the Reference section for examples of scientific publications that resulted from previous studies by NMML researchers. This list includes only documents resulting from studies conducted during the past few years under the prior permit No. 782-1438.

V. National Environmental Policy Act (NEPA) Considerations

*(1) Will your research or enhancement activity involve equipment (*e.g., *scientific instruments) or techniques that are new, or may be considered innovative or experimental? If yes, are they likely to be adopted by other researchers in the future?*

No new, innovative or experimental equipment or techniques will be applied. Photo-identification studies have become accepted as the primary means of studying demographics, life history, distribution and abundance since the mid-1980s. Biopsy studies have been conducted on

killer whales since the late-1980s, with a 100% survival rate (Barrett-Lennard et al. 1996; Hoelzel et al. 1998; Ross et al. 2000; Barrett-Lennard, 2000; Ylitalo et al. 2001; Hoelzel et al. 2002; Herman et al. 2005).

The research techniques are already standard and in use by other researchers. NMML researchers have been involved in a number of photo-identification and biopsy studies since the inception of these techniques.

(2) Does your activity involve the collection, handling, or transport of potentially infectious agents or pathogens (e.g., biological specimens such as blood), and/or does your activity involve the use or transport of hazardous substances (e.g., toxic chemicals)? If so, provide a description of protocols to be used for safe specimen and/or chemical handling, storage, and shipment to ensure human safety from injury or zoonotic disease transmission. Does your proposed research involve animal handling or dangerous work conditions? If so, explain and provide protocols that would be followed to ensure human safety.

No. The research does not involve the collection of potentially infectious agents or pathogens.

(3) Would any of your activities occur in or near unique geographic area such as wetlands, National Marine Sanctuaries, Marine Protected Areas, State National Parks or wilderness areas, wildlife refuges, wild and scenic rivers, designated critical habitat for endangered species, essential fish habitat, etc.? If so, would any aspect of your activities impact the physical environment, such as by direct alteration of substrate (e.g., bottom trawling, net setting, anchoring vessels or buoys, erecting blinds or other structures, disrupting nesting bird habitat, etc.)?

Some opportunistic research encounters may be conducted near or in National Marine Sanctuaries (with prior approval and appropriate permits), or near or in Marine Protected Areas. Although some research may be conducted near National Marine Sanctuaries or Marine Protected areas, the research would not impact the physical environment.

(4) Are you aware if the types of research or enhancement techniques to be employed could be perceived to be controversial by the public in any way? If so, to what degree would it be considered controversial and why?

No.

Photo-identification studies are widely used as the primary means of studying demographics, life history, distribution and abundance since the mid-1980s. Biopsy studies have been conducted on killer whales since the late-1980s, with a 100% survival rate (Barrett-Lennard et al. 1996; Hoelzel et al. 1998; Ross et al. 2000; Barrett-Lennard, 2000; Ylitalo et al. 2001; Hoelzel et al. 2002; Herman et al. 2005). We therefore do not consider these to be controversial techniques.

(5) Could your proposed actions affect entities listed in or eligible for listing in the National Register of Historic Places, or cause loss or destruction of scientific, cultural, or historic resources (e.g., archeological resources, species used for subsistence purposes, etc.)? Explain.

No. The research does not involve any land-based techniques, nor would it affect species used for subsistence purposes.

(6) Would any of your proposed activities include actions (e.g., transport of animals or tissues, ballast water discharge, working in sensitive remote areas, etc.) that could result in the introduction or spread of non-indigenous or invasive species (including plants, animals, microbes, or other biological agents)? If so, explain the types of activities and indicate any measure you would take to prevent or limit such spread or introduction.

No. The research does not involve transport of any tissues that could result in the introduction of microbes or other biological agents. Tissue samples will be preserved immediately, either by freezing or with a chemical preservative.

VI. Previous and Other Permits

A. Previous Permits

The National Marine Mammal Laboratory has held MMPA research permits since the MMPA was implemented. A list of NMML's permits is on file at the Office of Protected Resources.

We have authority to biopsy sample killer whales under our current MMPA Permit No. 782-1719. Other cetacean research was covered under our prior permits. MMPA Permit No. 782-1438 (cetacean research) and Permit No. 782-1510-02 (killer whale studies).

B. Other Permits

Permits will be requested from marine sanctuaries as required.

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VIII. Certification and Signature

I hereby certify that the foregoing information is complete, true, and correct to the best of my knowledge and belief. I understand that this information is submitted for the purpose of obtaining a permit under one or more of the following statutes and the regulations promulgated thereunder, as indicated in Section I of this application:

The Endangered Species Act of 1973 (16 U.S.C. 1531-1543) and regulations (50 CFR 222.23(b)); and/or

The Marine Mammal Protection Act of 1972 (16 U.S.C. 1361-1407) and regulations (50 CFR Part 216); and/or

The Fur Seal Act of 1966 (16 U.S.C. 1151-1175).

I also understand that any false statement may subject me to the criminal penalties of 18 U.S.C. 1001, or to penalties provided under the Endangered Species Act of 1973, the Marine Mammal Protection Act of 1972, or the Fur Seal Act of 1966, whichever are applicable.

Signed and Dated

_____ _____

Dr. John L. Bengtson, Director Date
National Marine Mammal Laboratory
Alaska Fisheries Science Center, NOAA Fisheries
7600 Sand Point Way, NE
Seattle, WA 98115-6349

ATTACHMENTS:

Table 1. Takes of Southern Resident killer whales, only. These takes are to be in addition to existing killer whale takes already covered in Permit No. 782-1719.

Activity	Species	Age	Sex	Location	Takes per animal per year	Takes per species per year
Unintentional harassment from aerial surveys	Southern Resident killer whale, *Orcinus orca*	All ages	Both sexes	North Pacific Ocean, Gulf of Alaska, Gulf of California, Canadian territorial waters, or wherever they are encountered opportunistically	30	500
Unintentional harassment from vessel surveys	Southern Resident killer whale, *Orcinus orca*	All ages	Both sexes	Same as above	30	500
Photo-identification	Southern Resident killer whale, *Orcinus orca*	All ages	Both sexes	Same as above	10	100
Biopsy	Southern Resident killer whale, *Orcinus orca*	All ages excluding calves and accompanying mothers	Both sexes	Same as above	1	10

List of Appendices

Appendix I: List of Co-investigators

Appendix II: List of publications, reports and presentations resulting from MMPA Permit No. 782-1438 and MMPA Permit No. 782-1510-02 from 1997 through the present.

Appendix III: Cruise report: Surveying killer whale abundance and distribution in the Gulf of Alaska and Aleutian Islands. AFSC Quarterly Report, Oct-Dec 2003

Appendix IV: Southeast Alaska Cetacean Vessel Survey and Killer Whale Abundance and Foraging Research proposals