APPENDIX B

SUPPLEMENTAL INFORMATION TO APPENDIX A ON DISEASE TRANSMISSION PREVENTION AND COLD STRESS AVOIDANCE

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LIST OF ACRONYMS AND ABBREVIATIONS

°F	degrees Fahrenheit
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
APHIS	Animal Plant and Health Inspection Service
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CDV	canine distemper virus
CEQ	Council on Environmental Quality
CFR	Code of Federal Regulations
DMV	dolphin morbillivirus
EIS	environmental impact statement
ELISA	Enzyme-Linked Immunosorbent Assays
ESR	Erythrocyte sedimentation rate
LCT	lower critical temperature
MMP	Marine Mammal Program
NBK–Bangor	Naval Base Kitsap at Bangor
NEPA	National Environmental Policy Act
NMFS	National Marine Fisheries Service
PDV	phocine distemper virus
PMV	porpoise morbillivirus
SECNAVINST	Secretary of the Navy Instruction
SISS	Swimmer Interdiction Security System
SSC San Diego	Space and Naval Warfare Systems Center, San Diego, CA
USDA	United States Department of Agriculture

1.0 INTRODUCTION

The potential long-term deployment to NBK–Bangor has raised public concern regarding the potential for these marine mammals to come into contact with or transfer new diseases, as well as the ability of Navy Marine Mammal Program (MMP) marine mammals to remain healthy in the cooler water and air conditions at NBK–Bangor. This Appendix provides additional details on how the MMP manages disease transmission prevention and cold stress avoidance.

1.1 ANIMAL MANAGEMENT AND NBK-BANGOR WORKING GROUP

The MMP Animal Management Program is described in Appendix A Section 5. An NBK-Bangor Working Group would be established to oversee the health monitoring program and ensure consistency in animal health and welfare decisions. The NBK-Bangor Working Group would include senior personnel with experience in animal training, veterinary service, and region-specific expertise. The NBK-Bangor Working Group would be represented in all the standing meetings of the committees listed in Appendix A Section 5, Animal Management Program. The Working Group would provide recommendations to the Senior Scientist for Animal Care and the Head of the Biosciences Division, who would be responsible for approving changes to indicators, thresholds, monitoring programs, and management actions. This management approach is consistent with current processes in place throughout the MMP.

1.2 SCOPE

The following sections describe: (1) the indicators and thresholds developed for monitoring communicable disease and cold stress, (2) the monitoring programs that would be used to assess if the MMP marine mammal indicators are outside of thresholds, and (3) management actions that would be taken if thresholds are exceeded.

2.0 INDICATORS AND THRESHOLDS

2.1 DISEASE TRANSMISSION PREVENTION

Like all natural animal populations, communicable diseases (including bacterial and viral infections) can occur in marine mammal populations. Morbillivirus is perhaps the most widely known marine mammal communicable disease due to its ability to cause illness, increased susceptibility to other diseases, and/or mortality. Morbilliviruses, including dolphin morbillivirus (DMV), porpoise morbillivirus (PMV), phocine distemper virus (PDV), and canine distemper virus (CDV), can be highly communicable among marine mammal populations and have been shown to cross over species (Osterhaus et al. 1995; Cowan 2002). Antibodies to morbilliviruses and morbilliviral ribonucleic acid (commonly referred to as RNA) have been detected in many wild marine mammals throughout the world, including common dolphins (*Delphinus delphis*) from southern California (Reidarson et al. 1998). There is a validated blood test for antibodies to all four morbilliviruses of interest (DMV, PMV, PDV, and CDV), and active morbillivirus infections can be detected using this test (see discussion of serology below). There is no indication that the current MMP marine mammal population has been exposed to and actively infected with morbilliviruses.

Because there are validated blood tests for only two marine mammal communicable diseases, serology is not a comprehensive health screening tool. As such, the MMP relies on a health surveillance system that constantly evaluates many factors (Figure B-1). Similar surveillance systems (called syndromic surveillance systems) have been recommended and established by the Centers for Disease Control and Prevention (CDC) to monitor human populations for existing and emerging communicable diseases (CDC 2004). This Appendix incorporates the MMP's long-standing health surveillance program to avoid, detect, and respond to potential communicable diseases, including morbilliviruses.

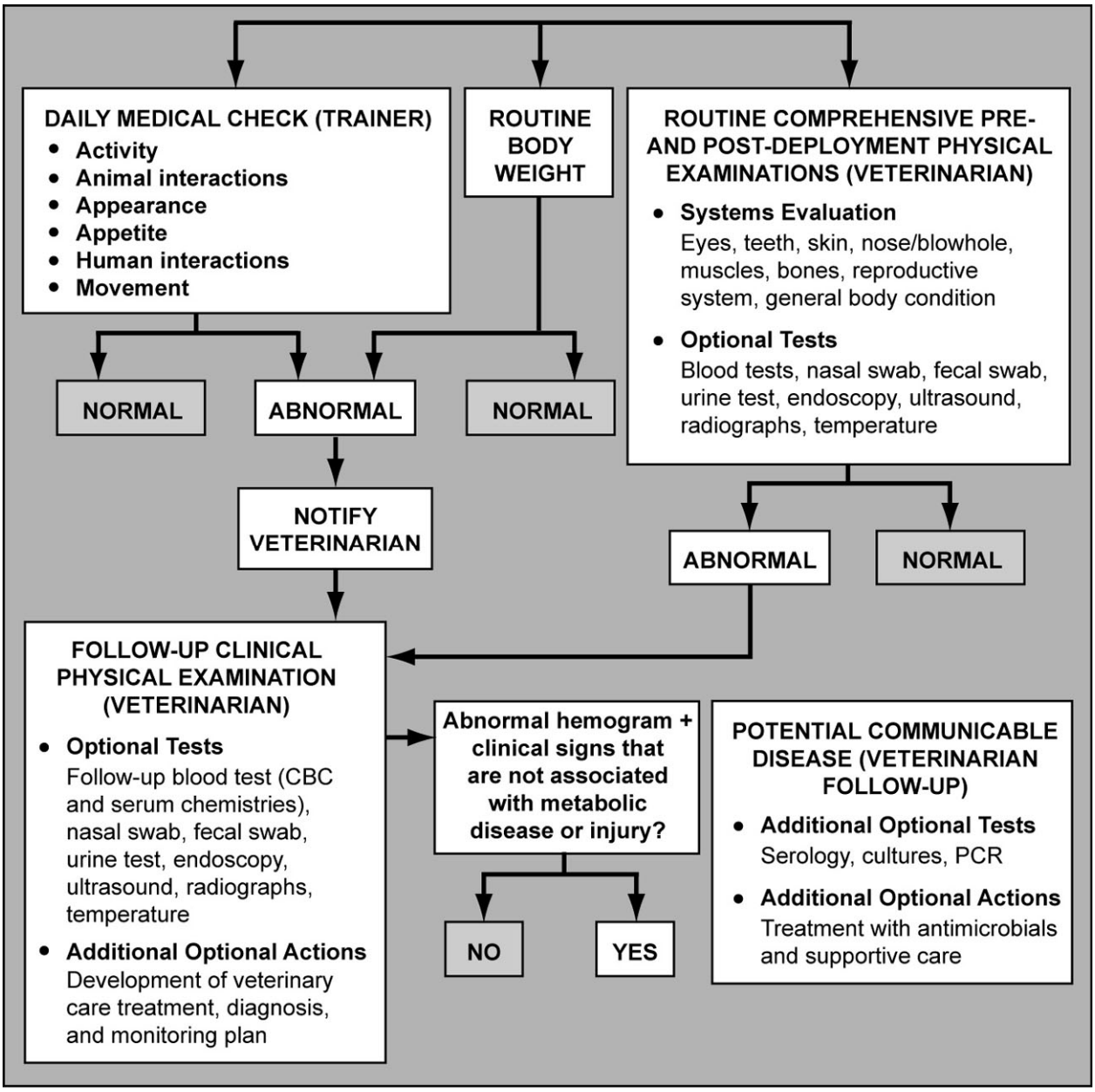

Figure B–1. Navy Marine Mammal Program Health Surveillance System: Communicable Disease Detection

Communicable disease in the marine mammals may include the following indicators. Thresholds for these indicators are defined in Table B-1.

- **Positive Serology Test.** Detection of rising antibodies to specific communicable pathogens in paired blood samples using a validated enzyme linked immunosorbent assay (ELISA).

- **Viral, Bacterial, and Fungal Isolation.** Clinically relevant, cultured (grown) viruses, bacteria, or fungi from animal samples submitted to reference laboratories.

- **Weight Loss.** Unexplained rapid weight loss that is not attributable to changes in diet prescribed by the veterinarian.

- **Behavioral Changes.** Abnormal behaviors such as not eating, listlessness, failure to respond to trainer commands, which are indicative of poor health.

- **Abnormal Hemogram.** Abnormal complete blood counts (CBCs) and erythrocyte sedimentation rates (ESR) consistent with potential communicable disease processes (infection and inflammation) in marine mammals.

- **Abnormal Physical Examination Finding.** Abnormalities may include laboratory results (e.g., urine, feces, gastric fluid), and physiological parameters (e.g., heart rates, respiratory rates, body temperature).

Paired serology is used to assess whether an animal has been exposed to a given infectious agent. These tests are used to detect exposure to bacterial, viral, and fungal infections by measuring the animal's antibody response, usually in serum or blood. Antibodies are produced by the immune system in response to the presence of a foreign agent (e.g., a virus) and would only be detected if the animal had previously been exposed to the virus (Guyton and Hall 2006). Previous exposure, however, does not indicate whether an animal is actively infected with an agent. Therefore, paired serology is required to detect active infections in animals. Two blood samples from the same animal, taken approximately 4 to 6 weeks apart, are tested for antibodies. If the second blood sample has at least four times the amount of antibodies than the first sample, this result strongly suggests active infection by the agent. Currently, only five serological tests have been validated for marine mammal species at the MMP (DMV, PMV, PDV, CDV – bottlenose dolphins and California sea lions; *Brucella* species – dolphins only). While research studies may involve unvalidated serological tests in marine mammals, the MMP limits routine, clinical evaluations to species-specific, validated serologic tests.

Infectious agents may be isolated (cultured) from marine mammal samples by reference laboratories. If the microbe is determined to be neither an environmental contaminant nor a commensal (a naturally occurring microbe co-existing in a healthy animal), then it may be a clinically relevant infectious agent; this likelihood increases if the microbe was isolated from a clinically relevant sample from an animal with relevant clinical signs (e.g., isolation of a known gastrointestinal pathogen from the feces of an animal with diarrhea).

Table B–1. Thresholds for Indicators of Communicable Disease in MMP Marine Mammals

PARAMETER	QUALIFIERS	STANDARD	THRESHOLD	INDICATOR TYPE
Paired Serology	Validated, species-specific test	No indication of pathogen or significant rise (four-fold or greater) in antibodies to the pathogen	Four-fold rise in antibodies to the pathogen	Primary[1]
Viral, bacterial and fungal isolation	1. Isolate has been ruled out as a contaminant or commensal 2. Microbe was isolated from clinically relevant samples 3. (+/-) Animal has clinical signs consistent with infection by the microbe	No indication of clinically relevant viral, bacterial, or fungal infection	Isolation of virus, bacterium, or fungus that is clinically relevant	Primary
Weight- dolphins	Weight loss is unexpected (i.e., not due to natural, healthy seasonal changes) and not due to non-infectious causes	Minimum weights for individuals would be consistent with Ridgway and Fenner 1982; goal weights would be established by the attending veterinarian in consultation with the program manager or cognizant technical representative	Weight below the minimum weight or unexpected weight loss as determined by veterinarian	Secondary[2]
Weight- sea lions	Weight loss is unexpected (i.e., not due to natural, healthy seasonal changes) and not due to non-infectious causes	Minimum weights for individuals would be established by the attending veterinarian; goal weights would be established by the attending veterinarian with approval of the Senior Scientist for Animal Care	Weight below the minimum weight or unexpected weight loss as determined by veterinarian	Secondary
Behavior	Behavior changes are unexpected and are not due to non-infectious causes (e.g., reproductive status)	Normal behavior for each individual animal would assessed and determined by the trainers	Animal exhibits abnormal behavior indicative of illness such as listlessness, non-responsiveness to trainers, or loss of appetite	Secondary
Abnormal Hemogram (blood values)	Abnormal CBC and ESR are not due to non-infectious causes, such as metabolic disease or injury	Normal CBC count and ESR	CBC is abnormal by in-house reference ranges for age and sex of animal. CBC and ESR are abnormal for individual animal	Secondary
Abnormal physical examination findings	Not due to non-infectious causes, such as metabolic disease or injury	Physiological and laboratory findings within normal limits	Physical examination findings are outside normal for population and for individual animal.	Secondary

[1] Primary indicators- exceedance of threshold for a primary indicator would elicit an immediate management action or response.
[2] Secondary indicators- elicitation of a management action for a secondary indicator may require exceedance of thresholds for more than one secondary indicator.

Each MMP marine mammal's individual goal weight is set by the attending veterinarian based on that animal's age, length, gender, activity level, and seasonal temperatures. Goal weights are maintained by feeding each animal a specific diet determined to provide sufficient energy for the animal's activity level. Weight loss in an animal that cannot be explained by changes in diet

ordered by the veterinarian or by increases in energy expenditure by that animal could indicate a change in health status.

Changes in normal marine mammal behaviors can be indicative of health status or potential affliction with disease. Animal trainers assess each animal's activity, animal interactions, appearance, appetite, human interactions, and movement on a daily basis.

CBC and serum chemistry profiles provide the veterinarian with information regarding the health of the animal. In general, the most common blood profile abnormalities in animals with infectious diseases are an abnormal white blood cell count and an increased ESR. Normal, in-house reference ranges for bottlenose dolphins have been determined and published by the MMP (Venn-Watson et al. 2007). The same methodology used to determine the dolphin reference ranges has been applied to determine in-house California sea lion reference ranges; publication of these reference ranges are pending.

Abnormal findings during physical examinations that are not due to non-infectious causes (e.g., metabolic disease or injury) could indicate presence of a communicable disease. Such abnormalities may include laboratory results from tests on urine or feces and changes in physiological parameters such as heart rate, respiratory rate, and body temperature.

2.2 COLD STRESS AVOIDANCE

The goal of cold stress avoidance is to minimize the impacts of cold and maintain the health and well-being of the marine mammals in the program. Marine mammals deployed to NBK–Bangor would experience water and air temperatures that are cooler than conditions at SSC San Diego. Potential indicators of cold stress in bottlenose dolphins and California sea lions are listed below. Thresholds for these indicators are defined in Table B-2.

- **Body Temperature.** Decreases in body temperature below the normal range.
- **Respiration Rate.** Increases in respiration rate might indicate the animal has increased its metabolism to generate heat.
- **Shivering.** Marine mammals can shiver to generate heat.
- **Skin Conditions.** Discolorations of the skin can occur during prolonged exposure to cold water and air.
- **Behavioral Changes.** Changes in some normal behaviors can indicate an animal is stressed by exposure to the cold.
- **Weight Loss.** Rapid weight loss can indicate increased metabolism of fat stores to generate heat.

Table B–2. Thresholds for Indicators of Thermal Stress in MMP Marine Mammals

PARAMETER	STANDARD	THRESHOLD	INDICATOR TYPE
Body temperature	96.8-98.6 degrees Fahrenheit (°F) is the range of core body temperature for dolphins (Hampton et al. 1971; Pabst et al. 2002; MMP unpublished data), 97.7-99.5°F has been reported for California sea lions (South et al. 1976; Whittow et al. 1975)	Hypothermia (decrease in internal temperature by 2°F) for more than 30 minutes-94.8°F for dolphins; 95.7°F for sea lions	Primary[1]
Respiration rate	Maximal resting respiratory rate will vary by individual and taken into account when calculating the threshold	Increase the maximal resting respiratory rate to a level twice the maximal respiratory rate for a period greater than 2 hours (not attributable to exercise or other factors)	Secondary[2]
Shivering	Not shivering	Shivering for greater than 30 minutes	Secondary
Skin Discoloration	Skin appears uniform with no discoloration	Changes in skin appearance to include discoloration	Secondary
Weight-dolphins	Minimum weights for individuals would be consistent with Ridgway and Fenner 1982; goal weights would be established by the attending veterinarian in consultation with the program manager or cognizant technical representative	Weight below the minimum weight or unexpected weight loss as determined by veterinarian	Secondary
Weight- sea lions	Minimum weights for individuals would be established by the attending veterinarian; goal weights would be established by the attending veterinarian with approval of the Senior Scientist for Animal Care	Weight below the minimum weight or unexpected weight loss as determined by veterinarian	Secondary
Behavior	Normal behavior for each individual animal would assessed and determined by the trainers	Animal exhibits abnormal behavior such as listlessness, refusal to perform, and/or poor appetite, indicative of cold stress	Secondary

Footnotes: [1] Primary indicators- exceedance of threshold for a primary indicator would elicit an immediate management action or response.
[2] Secondary indicators- elicitation of a management action for a secondary indicator may require exceedance of thresholds for more than one secondary indicator.

The maximum cold water temperature that causes an animal's metabolic rate to increase is known as the Lower Critical Temperature (LCT). This temperature defines the lower limit of the thermal neutral zone, which is the range of environmental temperatures over which a mammal's metabolic rate remains stable. Below the LCT, an animal compensates for heat loss by increasing its metabolic rate. At some temperature below the LCT, heat loss is greater than can be compensated for by increases in metabolism. At this point, the body temperature begins to fall and a condition that is commonly termed hypothermia will eventually occur. The LCT (water) calculated for bottlenose dolphins is between 41.9° and 51.1°F. The LCT (water) derived for California sea lions is 42.6°F. See SISS EIS Section 6.2.1.2.2, Water and Air Temperature, for a complete discussion of the derivation of these values.

Normal range of body temperatures for bottlenose dolphins is approximately 96.8° to 98.6°F in thermally neutral environments (Hampton et al. 1971; Pabst et al. 2002). A core body temperature of 94.8°F would be used to delineate the onset of hypothermia in bottlenose

dolphins. Normal range of body temperatures for California sea lions is approximately 97.7° to 99.5°F in thermally neutral environments (Whittow et al. 1975; South et al. 1976). For California sea lions, a core body temperature of 95.7°F would be used to delineate the onset of hypothermia. Core body temperatures would be monitored through the use of a rectal thermometer. Measurements would be taken based on veterinary assessment during work periods where the water temperature is equal to or less than the LCT for the animal.

Respiration rate, how often an animal takes a breath within a given period of time, is often used as an indicator of increased metabolism in mammals (see SISS EIS Section 6.2.1.2.2., Water and Air Temperature). Observance of increased respiration rates of the MMP marine mammals might be a useful indicator that the animals are increasing their metabolism to compensate for colder temperatures. However, respiration rates vary by individual depending upon that individual's activity and metabolic level, and there is a large amount of individual variability in respiration rates across bottlenose dolphins and California sea lions. Utilizing respiration rate as a potential indicator of increased metabolism would require determining the maximal resting respiratory rate of the individual animal for baseline comparisons.

Shivering is an unconscious response of the body to cold and can increase the internal heat production of an animal for short time periods. Cold signals from the skin and spinal cord initiate the process of increased heat production as a means to prevent a fall in internal body temperature (Guyton and Hall 2006). Therefore, observation of shivering in the marine mammals is potentially a good indicator for cold stress because it will begin before an animal's core body temperature decreases, and it generally occurs at temperatures colder than that at which metabolism increases.

There are multiple reasons for skin discoloration in marine mammals, including disease, injury, and aging. Various skin conditions have been observed in marine mammals when exposed to water temperatures near or below their LCT. Such conditions include discoloration, lesions, and bumps (Chun and Harris 1978; Scronce and Bowers 1985). Shunting of the blood supply, where blood is directed away from appendages and in toward the body core as a way to conserve heat, can cause darkening of the skin (Chun and Harris 1978). However, in the studies by Chun and Harris (1978) and Scronce and Bowers (1985), skin discoloration did not appear until 1 to 4 days after the onset of increased respiration. Skin lesions are indicators of cold stress in marine animals because they can occur as a direct result of prolonged exposure to cold, although other indicators (such as increased respiration rate) are likely to be observed before onset of skin discoloration occurs. Thus, skin discoloration would be considered as a secondary indicator of cold water stress, and more than one secondary indicator would be needed to ensure that skin discoloration is representative of cold water stress for any specific individual.

Blubber layer thickness varies seasonally and with age, nutritional status, and reproductive status (Iverson 2002). During times of cold stress, marine mammals can use the energy stored in the blubber layer to produce internal body heat. Without an increase in daily caloric intake, the increased mobilization of blubber lipid will result in a reduced blubber layer and a decrease in the insulative value of the blubber (Iverson 2002). Weight loss, which can correspond to decreases in the blubber layer, might indicate that an animal is utilizing energy stores at a higher than normal rate in order to produce more body heat. However, weight loss can occur for many different reasons, thus requiring increased monitoring and investigation by veterinary staff.

Captive bottlenose dolphins have been observed to alter their normal behavior when exposed to extreme cold water and air temperatures. For example, when air temperatures fell below freezing and surface water temperatures fell below those of deeper water, bottlenose dolphins were observed spending more of their time in deeper waters (Scronce and Bowers 1985). Trained marine mammals may also fail to obey simple commands from their trainers when exposed to colder temperatures, particularly with respect to behaviors that require exposing skin to air temperatures below freezing (Scronce and Bowers 1985). Deviations from normal behavior, such as altered swimming behavior and refusal to beach, might be good indicators of cold stress because they can indicate that the animal is trying to minimize exposure to cold temperatures. However, to date, variation in behavior has not been observed in MMP California sea lions as a function of cold water or air exposure when deployed in areas with colder water temperatures than those that occur at SSC San Diego.

3.0 MONITORING

3.1 DISEASE TRANSMISSION PREVENTION

3.1.1 Health Monitoring Program

A schedule for monitoring of health status of the MMP marine mammals is provided in Table B-3. The table also provides the procedures that would be performed during pre- and post-transport processes (which are the same as those undertaken during an annual or semiannual comprehensive physical exam), the measures recorded daily, and the minimum intervals for the recording of other health and disease screenings.

The transport health screen, daily observation, and routine comprehensive exams and their components relevant to disease transmission are provided in Table B-3 and are described in more detail below.

Marine mammal transportation procedures are in alignment with and exceed applicable Federal regulations. The MMP has a program in place for screening the health of animals before and after they are deployed. This includes physical examinations performed by a veterinarian no less than 2 days and no more than 10 days before departure for any animal planned to be transported for more than 2 hours. Health screen procedures that would be used before and after the transport of marine mammals from SSC San Diego to NBK–Bangor are described below.

MMP animal trainers observe each marine mammal daily. Each animal's appearance, activity, appetite, movement, and interactions with humans and animals are assessed as normal or abnormal, and observations are recorded. Abnormal findings are reported to an attending veterinarian who responds as appropriate.

3.1.2 Daily Observation

As described above in Section 3.1.1, Health Monitoring Program, MMP marine animals deployed to NBK–Bangor would be observed daily by their trainers. Each animal's appearance, activity, appetite, movement, and interactions with humans and animals would be recorded daily.

Table B–3. Schedule for Health and Disease Monitoring for MMP Marine Mammals

PARAMETER	DAILY	MONTHLY	SEMIANNUAL OR ANNUAL	PRE-TRANSPORT	POST-TRANSPORT
Paired Serology			X	X	X
Weight[1]		X	X	X	X
Blood Counts		X	X	X	X
Serum Chemistry		X	X	X	X
Medical Check (activity, animal interactions, appearance, appetite, human interactions, and movement)	X	X	X	X	X
Routine comprehensive physical examination			X	X	X

[1] Weights would be taken monthly or more often at the direction of the attending veterinarian when ambient water temperatures fall below 50°F.

3.1.3 Monthly Health Screening

Monthly health screening would include, at a minimum, animal weights and examination of physical appearance.

3.1.4 Routine Comprehensive Physical Exams

As described in Section 2.1, Disease Transmission Prevention, changes in animal health can be useful secondary indicators of communicable disease. The marine mammals at the MMP receive comprehensive semiannual or annual physical examinations and also when an animal's behavior or physical appearance warrants a health review by a veterinarian or a secondary threshold is surpassed during daily monitoring. The physical involves an assessment of the animal's general physical appearance (including eyes, abdomen, genitalia, musculoskeletal system, head and neck, skin, extremities) by a qualified marine mammal veterinarian and may also include assessment of vital signs (temperature, pulse, and respiratory rate), length, and weight. Diagnostic testing may include laboratory examination of feces, urine, and blood, as well as endoscopy, ultrasound, or radiographs. A clinical laboratory is used to process, archive, and ship animal samples to reference laboratories. Mobile clinical laboratories are used during animal deployments.

3.1.5 Transport Health Screen

For the deployment to NBK–Bangor, a pre-transport health screen would occur 4 weeks prior to transport and would include quarantine of the deployment animals from the MMP marine mammals at SSC San Diego. Procedures would include, but are not limited to:

1. Paired serology for communicable diseases – Serum samples would be collected 4 and 2 weeks prior to transport and within 7 days after transport. With paired serology, a second sample is taken some time after the first sample for comparison. A validated ELISA is used at a reference laboratory to assess increases in antibody response over time and would indicate if the animal had active infection with a specific pathogen. Paired serological tests are limited to ELISAs that have been validated for bottlenose dolphins and/or California sea lions. To date, species-specific validated ELISAs are limited to morbillivirus (bottlenose dolphins and California sea lions) and *Brucella* bacteria (bottlenose dolphins only).

2. CBCs and serum chemistries – 2 to 10 days prior to transport and 2 to 10 days after transport. Blood constituents measured in a CBC and serum chemistry panel include: white blood cell count, hematocrit, platelets, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils; and serum glucose, blood urea nitrogen, creatinine, uric acid, sodium, potassium, chlorine, carbon dioxide, total protein, albumin, globulins, cholesterol, bilirubin, triglycerides, calcium, inorganic phosphate, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotranferase, gamma-glutamyl transpeptidase, iron, and creatine kinase. Actual values are compared with in-house reference ranges (Venn-Watson et al. 2007, bottlenose dolphins; MMP unpublished data, California sea lions) as well as expected ranges for individual animals. Communicable diseases are most often associated with abnormal white blood cell counts and increased ESR, although non-infectious conditions may also be associated with abnormal CBC and serum chemistry values.

3. Fecal/Oral/Blowhole viral, bacterial, and fungal cultures and examination – 4 weeks prior to transport and within 7 days after transport. Fecal/oral/blowhole microbial (viral, bacterial, parasitic, and fungal) screening would test the marine mammals for exposure to viruses, bacteria, parasites, and fungi that may be associated with disease in bottlenose dolphins or California sea lions. A microbe is most likely to be considered disease-associated if it has been ruled out as a contaminant or commensal, was isolated from a clinically relevant sample, and the animal has clinical signs consistent with infection by the microbe (e.g., isolation of a known gastrointestinal pathogen from feces of an animal with diarrhea).

3.2 COLD STRESS AVOIDANCE

3.2.1 Health Monitoring Program

As described in Section 3.1.1, Health Monitoring Program, the MMP has a program in place for the screening of animal health before and after they are deployed (for the schedule, see Table B-3). Water temperatures would be maintained above the dolphin LCT within the enclosures. Although trainers and handlers monitor marine mammals constantly during open-water work, special care would be taken for cold-stress indicators when animals are working in water temperatures approaching the LCT of the animal. The animals would be continuously monitored for increased respiration rate, behavior change, and shivering. Other secondary indicators (skin discoloration, weight loss) would not be evident during individual sessions because these conditions occur over a longer period of time, and thus would be considered as part of the longer term monitoring program. When during a session the thresholds for the secondary indicators of increased respiration rate, shivering, and behavior changes are exceeded, the core body temperature of the animal would be measured. Measurements would be repeated at 30-minute increments until the end of the session. The method of core body temperature measurement would be one of several approved for use in MMP animals by the veterinary staff (e.g., rectal temperature probe, stomach temperature pill, implantable temperature sensor, etc.).

3.2.2 Daily Observation

As described in Section 3.1.2, Daily Observation, MMP marine mammals deployed to NBK–Bangor would be observed daily by their trainers. Each animal's appearance, activity, appetite,

movement, and interactions with humans and animals would be recorded. Any abnormalities that could indicate stress due to cold, such as shivering, altered behavior, or abnormal skin conditions, would be reported to the veterinary staff.

3.2.3 Monthly Health Screening

As described in Section 3.1.3, Monthly Health Screening, monthly health screening for MMP marine mammals deployed to NBK–Bangor would include, at a minimum, animal weights and an examination of physical appearance.

3.2.4 Routine Comprehensive Physical Exams

As described in Section 3.1.4, Routine Comprehensive Physical Exams, marine mammals at the MMP receive annual or semiannual comprehensive physical examinations. Elements of the physical that are relevant to monitoring temperature acclimation in marine mammals include:

- vital signs (temperature, pulse, and respiratory rate);
- general physical appearance;
- weight;
- blubber thickness; and
- blood counts and serum chemistries.

3.2.5 Transport Health Screen

The transport health screening for the transport from SSC San Diego to NBK–Bangor is described in Section 3.1.5, Transport Health Screen. The following screening aspects relevant to monitoring cold stress for MMP marine mammals include:

- vital signs (temperature, pulse, respiratory rate, and general physical appearance);
- weight;
- behavior; and
- blood counts and serum chemistries.

While stationed at NBK–Bangor, MMP marine mammals would be transported from their enclosures to the NBK–Bangor waterfront area when working. Typically, they would be in the water accompanying the boat, or in an appropriate carrier on the boat. Whenever any marine mammal is out of the water for transport during cold temperatures, the animal would be transported in a closed, tri-fold mat to minimize exposure to the cold air.

4.0 MANAGEMENT ACTIONS

4.1 DISEASE TRANSMISSION AVOIDANCE

4.1.1 Primary Thresholds

4.1.1.1 Paired Serology

If paired serological monitoring revealed that an MMP marine mammal had evidence of active infection with morbillivirus, MMP staff would quarantine the animal in a separate enclosure. The animal would not be allowed to perform work in the open water environment. The veterinarian would take actions to stabilize the health of the animal and ensure its comfort. A complete health assessment would be performed by the veterinarian. The veterinarian would make recommendations as to the course of action to maintain the health of the animal. Actions may include supportive care and treatment with medication.

Detection of active infections by other pathogens would lead to supportive care and treatment as needed for the individual animal. Decisions to quarantine animals for any communicable disease other than morbillivirus would be dependent upon the severity of the disease; the potential of transmission among animals; the novelty of the pathogen to either the MMP animal population or to local, wild marine mammal populations; and the direction of the attending veterinarian. By analogy to human populations, a person with a dangerous and rare strain of tuberculosis may be quarantined, but a person with a common influenza virus (flu) would not be quarantined.

4.1.1.2 Viral, Bacterial, and Fungal Isolation

Management actions initiated would be the same as described for Section 4.1.1.1, Paired Serology, if fecal, oral, or blowhole viral, and bacterial and fungal screening revealed that any MMP marine mammal had a clinically relevant, active infection with morbillivirus or another communicable virus or bacteria. The attending veterinarian may prescribe appropriate antibiotics in the case of bacterial infection.

4.1.2 Secondary Thresholds

4.1.2.1 Weight

If an animal is determined to be below its minimum weight, the attending veterinarian would be notified, and the animal would not work in open water until it gains weight and is determined to be healthy. The veterinarian would perform a health assessment to determine possible causes of weight loss. Feeding records would be examined for possible trends in the animal's decrease in food consumption. The attending veterinarian may decide to increase the frequency of blood draws to assess the presence of an adverse health condition. Upon completion of the health assessment, the veterinarian would make recommendations for increasing caloric content of the animal's diet. Should a communicable disease (viral or bacteriological) be identified during the health assessment, management actions would continue as described in Section 4.1.1.1, Paired Serology.

If an animal is determined to be below its goal weight, the attending veterinarian would be notified. The animal would only be removed from open water work upon a veterinarian's recommendation. Feeding records may be examined, frequency of blood draws may be increased, and consultation with work group members may occur to determine further actions.

In the event of two or more animals failing to maintain minimum or goal weight, records of all the animals of the same species would be examined to determine if there are any trends indicating a possible need for changes in the MMP diet or feeding regimen for all animals. For example, after review of data for all program animals by the Senior Scientist it may be determined that some animals are having difficulty maintaining their goal weights. This would initiate collaboration of the NBK–Bangor MMP Work Group to determine the best course of action.

4.1.2.2 Behavior

Diagnosis of abnormal behavior is dependent on daily interactions between the MMP marine mammals and their trainers. Any behaviors observed in the MMP marine mammals that are clearly indicative of conditions immediately threatening the animal's health (e.g., refusal to eat for multiple days) or indicating presence of disease would initiate an immediate response as if a disease had been detected in the animal. The management response would follow the same course as for Section 4.1.1.1, Paired Serology, with the exception that notification of disease to the appropriate agency would not occur without further confirmation of the presence of morbillivirus or a disease associated with an unusual mortality event.

Abnormal behaviors not clearly associated with communicable disease or environmental temperature changes may not necessarily be cause for animal health concerns. For example, during part of the reproduction cycle in healthy female bottlenose dolphins, behavioral changes may be seen, including a short-term decrease in appetite. In such cases, the Senior Scientist may meet with the animal's trainers and the attending veterinarian to assess the animal's overall health and determine the most appropriate course of action.

4.1.2.3 Abnormal Hemogram

As described above, CBC and ESR are used in assessment of health, specifically inflammatory and potential communicable disease. Deviations from defined ranges of various blood constituents can indicate an animal is actively infected with a disease. One example is white blood cell count, which may increase or decrease in response to various disease conditions. If monitoring of any MMP marine mammal indicates that animal has an abnormal CBC and elevated ESR, several management actions might follow notification of the attending veterinarian and performance of a health evaluation. Should the health evaluation reveal presence of a communicable disease, the management response would follow the same course as if a positive serology test had been made, as described in Section 4.1.1.1, Paired Serology.

If the health evaluation does not implicate communicable disease as the cause for the variation in CBC and elevated ESR, further testing may be ordered by the attending veterinarian. The attending veterinarian may consult with the Senior Scientist and trainers to determine the most appropriate course of action to return the animal to optimal health.

4.1.2.4 Abnormal Physical Examination Findings

If an abnormality was noted during routine physical examination of any animal, the attending veterinarian would perform a health evaluation for that animal. If the health evaluation reveals presence of a communicable disease, the management response would follow the same course as if a positive serology test had been made, as described in Section 4.1.1.1, Paired Serology.

If the health evaluation does not implicate communicable disease as the cause for the observed abnormality, further testing may be ordered by the attending veterinarian. The attending veterinarian may consult with the Senior Scientist and trainers to determine the most appropriate course of action to return the animal to optimal health.

4.2 COLD STRESS AVOIDANCE

4.2.1 Primary Thresholds

The bottlenose dolphin enclosures would be operated in closed-circuit mode and maintained at a water temperature within the LCT of bottlenose dolphins. Ambient temperatures in the natural environment are unlikely to be below the LCT of the bottlenose dolphins for extended periods. Therefore, keeping temperatures within the enclosures approximately 6° to 10°F above the LCT would minimize temperature differences between the enclosures and the work environment.

As discussed in SISS EIS Section 6.2.1.2.2, Water and Air Temperature, bottlenose dolphins have been observed spending up to 7 hours at temperatures below their LCT without demonstrating effects from the cold exposure beyond an increase in their metabolic rate (Yeates and Houser 2008). The amount of time bottlenose dolphins and California sea lions are working outside of their enclosures may be limited at the discretion of the attending veterinarian when water temperatures drop below their respective LCTs. In the event that monitoring of an MMP marine mammal indicates the threshold for the primary indicator has been exceeded, that animal would be immediately returned to its enclosure for further observation by the attending veterinarian. If one or more of the secondary thresholds are exceeded, the animal may undergo increased testing and observation by the veterinary staff.

4.2.1.1 Body Temperature

If monitoring of any individual marine mammal indicates a drop in body temperature of more than 2°F for more than 30 minutes, that animal would be immediately removed from the work or training session and returned to its enclosure for further observation by the attending veterinarian. The veterinarian would take actions to ensure the health of the animal and ensure its comfort. The attending veterinarian would perform a health assessment to determine if the animal is exhibiting any other symptoms of cold stress. If the core body temperature of the bottlenose dolphin does not return to normal within 24 hours of being returned to its enclosure, the attending veterinarian would have the water temperature of its enclosure raised or would have the animal moved to a warmer enclosure. California sea lions exhibiting hypothermia would either have pens modified to reduce cold exposure or would be moved to another location where temperature can be controlled or elevated, depending upon an assessment of the attending veterinarian.

4.2.2 Secondary Thresholds

4.2.2.1 Respiration Rate

Increased respiration rate, above that observed at rest, is expected in working animals. Furthermore, an increase in respiration rate as a result of an increase in metabolism is not a sufficient reason for removing an MMP animal from open-water work. However, if respiration rate is observed to increase above twice the maximal resting respiration rate over the duration of a work session, the air and water temperature would be noted, and the attending veterinarian would determine if an increase in caloric intake is warranted. If the animal's respiration does not return to normal levels within 4 hours of being returned to its enclosure, the attending veterinarian would perform a health assessment to determine if the animal is exhibiting any other symptoms of cold stress and whether the animal is hypothermic. Further testing may be implemented, at the discretion of the attending veterinarian, to rule out other potential causes of increased respiration rate.

4.2.2.2 Shivering

If monitoring of any individual marine mammal indicates prolonged shivering (lasting more than 30 minutes), the air and water temperature would be noted, and the attending veterinarian would determine if an increase in caloric intake is warranted. If shivering continues within 30 minutes of being returned to the enclosure, the attending veterinarian would perform a health assessment to determine if the animal is exhibiting any other symptoms of cold stress and whether the animal is hypothermic. Further testing may be implemented, at the discretion of the attending veterinarian, to rule out other potential causes of shivering.

4.2.2.3 Skin Discoloration

If any new skin discoloration is observed on a working MMP marine mammal that cannot be attributed to a cause other than exposure to cold temperatures, the air and water temperature would be noted, and the attending veterinarian would perform a health assessment to determine if the animal is exhibiting any other symptoms of cold stress and whether the animal is hypothermic. Further testing may be implemented, at the discretion of the attending veterinarian, to rule out other potential causes of skin discoloration.

4.2.2.4 Weight

In the event any marine mammal is determined to be losing weight in the absence of seasonally warming temperatures or dietary changes made by the attending veterinarian, management actions would be the same as for the disease portion of the animal management plan, Section 4.1.2.1, Weight.

4.2.2.5 Behavior

As described in Section 4.1.2.2, Behavior, diagnosis of abnormal behavior may be subjective and is highly dependent on the daily interactions between the MMP marine mammals and their trainers. Any behaviors observed in the MMP marine mammals that may indicate conditions immediately threatening the animal's health (e.g., refusal to eat for multiple days) would initiate an immediate assessment of health by the attending veterinarian to determine if the animal is

exhibiting any symptoms of cold stress. The veterinarian would take actions to stabilize the health of the animal and ensure its comfort, and the animal would be removed from duty.

5.0 REGULATORY COMPLIANCE

The Animal and Plant Health Inspection Service (APHIS) of the USDA is responsible for setting standards for care and maintenance of marine animals under the Animal Welfare Act. The MMP maintains a program of animal care that meets or exceeds APHIS regulations but, as a United States government organization, does not require a license from APHIS and is not subject to inspections. NBK–Bangor facility reports would be provided to APHIS. The NBK–Bangor MMP and facilities would be inspected semiannually by the Navy MMP Institutional Animal Care and Use Committee. The MMP is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), which is a nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs. Annual reports on the NBK–Bangor MMP facilities would be provided to AAALAC by the MMP.

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