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HAWAIIAN MONK SEAL HEALTH ASSESSMENT AND DISEASE STATUS STUDIES: A PROGRESS REPORT

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INTRODUCTION

The endangered Hawaiian monk seal (*Monachus schauinslandi*) population has declined approximately 60% since the first range-wide surveys in the late 1950s and will likely continue to decline as a result of high juvenile mortality and low reproductive recruitment occurring at French Frigate Shoals, the largest subpopulation (Gilmartin, 1983). If this trend continues, it is conceivable that the Hawaiian monk seal could be on the verge of extinction. Understanding the potential role of disease and toxins in this recent decline is a high priority. Several natural sources of mortality have been identified or suggested (e.g., ciguatera poisoning, starvation, shark predation, trauma/mobbing, and disease), but the relative significance of these factors and their effect on population trends are poorly understood (Gilmartin, 1993). Efforts to enhance the recovery of the Hawaiian monk seal will require a better understanding of the health and disease status of the wild population.

Since 1981, captive care and release programs have been an integral part of management efforts to enhance the recovery of the Hawaiian monk seal. Three strategies have been used including 1) on-site protection and release, 2) direct translocation from one site to another, and 3) transportation to Oahu for rehabilitation, followed by release into the wild population. Initial captive care and release efforts were intended to enhance the depleted subpopulation at Kure Atoll. These efforts were notably successful, and this subpopulation appears to be in the process of recovery. In the late 1980s and early 1990s, juvenile survival at French Frigate Shoals declined severely, and the objective of captive care and release efforts was expanded to include salvaging the reproductive potential being lost at French Frigate Shoals (Ragen and Lavigne, 1998).

In 1995, 12 females pups were captured and brought to Oahu for rehabilitation and eventual release into the wild population. These seals were not released as anticipated due to a persistent eye condition of unknown etiology. This unexpected event resulted in a hiatus of the monk seal captive care and release program, and provided the impetus for a review of program activities by a panel of independent wildlife experts (*Captive Care Review Panel Report*, 1997). The panel concluded that translocation is a potentially valuable management tool. Any translocation activity must achieve the goal of supplementing a depleted subpopulation without compromising the potential for growth of the source subpopulation, thereby improving recovery of the total population. The management strategy most likely to achieve the goal of recovery is the direct translocation of larger sized pups from subpopulations with low pup survival (e.g., French Frigate Shoals) to subpopulations with high pup survival (e.g., Midway Atoll). It was recommended that the strategy for the immediate future be translocation with or without conditioning on the beach at the capture or release sites. It is important to determine if these efforts pose significant risks of introducing infectious agents into native sub-populations if they minimize the threat of any potentially catastrophic disease outbreaks resulting from these recovery efforts.

Minimizing the risk of introducing diseases to a population can be accomplished by using the best available diagnostics to test seals for evidence of infection or disease; conducting epidemiological surveys; and taking a proactive approach to disease management (i.e., treatment or immunization as technology becomes available). The current biomedical impact on declining survival and the role of disease in the success or failure of translocation experiments in the Hawaiian monk seal population are unknown. Epidemiological research is needed to provide recommendations for future translocation/reintroduction efforts.

The present pilot study was aimed at obtaining baseline information on selected health and disease parameters from three Hawaiian monk seal subpopulations. The objectives of the present study were to 1) assess baseline biomedical information of wild Hawaiian monk seals from three reproductive subpopulations at French Frigate Shoals, Midway Atoll, and Pearl and Hermes Reef; 2) assess evidence of exposure to specific disease agents in three sampled subpopulations using standard biomedical diagnostics for serology, bacteriology, virology, parasitology, and toxicology; and, 3) evaluate results obtained and provide recommendations regarding translocation strategies between surveyed subpopulations.

MATERIALS AND METHODS

Study Sites and Sample Size

This baseline epidemiological study was designed to assess the health and disease status of three breeding subpopulations (donor, recipient, and control) currently under consideration for enhancement activities. Because of the low survival of juveniles at French Frigate Shoals (FFS; lat. 23˚45'N, long. 166˚10'W), it has been considered as the possible donor subpopulation, and Midway Atoll (MID; lat. 28˚15'N, long, 177˚23' W) represents the potential recipient subpopulation because of its severely depleted status (11 pups born in 1997). Pearl and Hermes Reef (P&H; lat. 27˚50'N, 175˚50'W) has been selected as a control subpopulation for this pilot study which is currently growing at a rate of about 5% annually.

Sample size at each site was determined by assuming that the detection of a disease process is a binomial process yielding positive (present) or negative (absent) results, by setting desired power of the laboratory analyses at 80% and minimum number of successful trials at five, and by assuming that an endemic disease found in a subpopulation would be present in at least in 20% of the seals. The small sample size at MID reflects 20% of the entire subpopulation which is estimated at 45 to 50 seals.

Field Techniques

Physical examinations were performed by the attending veterinarian and were limited to visual identification of abnormalities, determination of body condition, sex, size group, and evidence of trauma. Prior to capture, seals were observed for several minutes to measure respiratory and cardiac rates from a distance. Seals were then captured while hauled out on the beach. Mature seals (subadults and adults) were captured with a hoop net, immature seals (juveniles) were captured with a stretcher, and weaned pups were captured by hand. Diazepam (Steris Laboratories Inc., Phoenix, AZ) was given intravenously and following induction, cardiac rate, respiratory rate, and rectal temperature (digital thermometer, Fisher Scientific, Pittsburgh, PA) were recorded at least once during the handling procedure. Seals were flipper-tagged and PIT-tagged, and scars/marks were recorded for identification purposes. Dorsal standard length (L) and axillary girth (G) were measured, and weight was calculated using a simple mass-dependent formula (G^2 x L x 0.00005) applied in previous pinniped studies (Pitcher, 1986; Arnould, 1995). Age, sex, and size groups were determined by reviewing previous identification cards on file or by visual examination at the time of capture. Following all biomedical and morphometric procedures, seals were released and monitored post-capture for 10 to 60 minutes or until normal behavior was observed.

Blood (35 to 60 ml) was collected from the bilaterally divided extradural veins by inserting an 18 ga 3½-in spinal needle between the dorsal spinous processes of the 3rd, 4th, or 5th lumbar vertebrae (Geraci and Lounsbury, 1993). Blood specimens were immediately transferred into EDTA (3 mL), heparinized (7 mL) and SST (25 mL) Vacutainer® tubes (Becton, Dickinson & Co., Rutherford, NJ, USA). EDTA tubes were placed in blue ice and SSTs were kept in the shade at ambient temperature for 60 minutes to allow normal coagulation process and then transferred in a cooler with blue ice.

Plasma and serum specimens were separated by centrifugation at 2,000 rpm for 10 minutes, pipetted off in one-mL aliquots in cryogenic vials (Nalgene) and placed in liquid nitrogen. Specimens collected for biochemistry and serology were ultrafrozen on liquid nitrogen in the field and transferred to a -86 ultrafreezer upon arrival to Honolulu prior to analysis. Buffy coats were collected from heparinized and EDTA tubes for virus isolation. Rectal swabs for bacteriologic analysis from all seals were collected and immediately placed in Modified Cary Blair Transport Medium (Para-Fix, Medical Chemical Corp., Santa Monica, CA, USA). All swabs were preserved on blue ice and then transferred to a refrigerator (4˚C) until testing. Fecal specimens from seals at all sites were collected with a fecal loop. Samples were placed in a vial containing 5 mL of polyvinyl alcohol fixative solution (PVA) for parasitoscopic screening.

Blubber biopsies and blood from adult males and blood from seals of other size categories were collected for contaminant analysis including organochlorines, heavy metals and POPs. Blubber biopsies were collected following a modification of the technique

described by Iverson et al. (1997). Briefly, the area was locally blocked with 4-6 mL of Xylocaine 1% (Astra USA Inc., Westborough, MA), then surgically scrubbed with 99% isopropyl alcohol and povidone iodine solution. A 2-cm incision including skin and subcutaneous tissue was performed to expose the blubber layer. Blubber core samples were collected from the pelvic region using 6 mm biopsy punches. Biopsy cores (3-4 cm) were taken through the full depth of the blubber layer. Blubber cores were removed with forceps and scissors, placed into 4-mL amber vials and then frozen at -20˚C until shipping to the laboratory. The wound was then cleansed and not sutured to allow healing by second intention. A sample of whole blood (6-10 mL) from all seals was placed in methylene-chloride-rinsed glass containers and then frozen at -20˚ C or colder as soon as possible following collection.

Laboratory Techniques

Hematology

In the laboratory, EDTA tubes were warmed to room temperature and gently mixed. Pack cell volume (PCV) was determined using a microhematocrit centrifuge and reader (StatSpin, Norwood, MA). Absolute white blood cell counts were performed using the Unopette System (Test 5804/5853) within 1 to 6 hours of blood collection. Blood smears were prepared in duplicate and air-dried for differential white blood cell counts and hemoparasites that were performed by D. Borjesson, Department of Clinical Pathology, University of California-Davis. Nucleated cell estimates were performed and a lower and upper range number given (1,500 cell range). These values correlated reasonably well with the absolute leucocyte counts performed with the Unopette system. Following plasma centrifugation, total solids were measured with a refractometer; glucose with a glucometer and blood urea nitrogen (BUN) with BUN reagent strips (Quickchek, Centaur, Overland Park, KS).

Serum Biochemistry

Biochemistry analysis of serum was performed at Consolidated Veterinary Diagnostics, Inc. (Sacramento, California). Serum biochemistries were determined using an automated random access analyzer. Hemolyzed samples were not included in this study. The following serum determinants were measured: protein electrophoresis, total protein, albumin, globulin, total bilirubin, direct bilirubin, alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), alkaline phosphatase (AP), creatinine kinase (CK), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), BUN, creatinine, calcium, phosphorus, bicarbonate, cholesterol, triglycerides, glucose, iron, sodium, potassium, chloride, A/G ratio, B/C ratio, Na/K ratio, and anion gap.

Atrial Natriuretic Factor

Atrial natriuretic factor (ANF) is a hormone that is synthesized by and secreted from the heart. The most effective and best documented stimulus for ANF release is atrial distension, and it appears that this stimulus is universal among the vertebrate groups. If present in the serum of monk seals, it is most likely that ANF has a key role in the regulation of blood pressure and could serve as another indicator of individual health. Immunoreactive-ANF in the monk seal samples was measured using a radioimmunoassay (RIA) specific for human-ANF-(99-126). Radioimmunoassays to measure peptides from the N-terminus of the ANF 126 a.a. prohormone were developed to amino acids 1-30 (proANF 1-30), 31-67 (proANF 31-67), and 79-98 (proANF 79-98) of this prohormone as previously described (Vesely et al.,1989, 1994; Winters et al., 1989).

Serology

One-mL aliquots of serum were sent to NMFS-certified diagnostic laboratories to determine previous exposure or levels of antibodies to selected infectious agents known to occur in other pinniped populations (Table 3). The Laboratory for Calicivirus Studies at Oregon State University examined sera for group antibody and neutralizing antibodies to 31 different viruses using immunoblot ELISA and cell culture virus neutralization techniques to provide an initial estimate of virus activity and then each was tested against each individual calicivirus listed using cell culture and microtiter plates. One hundred infective virus units were challenged with sera at the 1:10 dilution and if virus replication was blocked, the sera was titered to endpoint dilution. Each test was accomplished using replicates of 4 in the microtiter system.

Virology

Rectal swabs and buffy coat specimens were examined for the presence of virus. This was accomplished using monoclonal antibodies, cDNA probe and isolation techniques. Virus isolation was accomplished by vortexing and clarifying fecal swab material. Supernatants were absorbed to cell culture monolayers in two distinctly different cell lines and incubated and examined daily for cytopathology. Positive samples were further processed for virus isolation, and characterization and negative samples were passed at least 3 times. Each sample processed for virus isolation was also screened for caliciviral presence using a calicivirus specific group epitope binding monoclonal antibody (4AD8D8) as a probe. The buffy coats were layered over monolayers to examine them for cell-associated virus. Then freeze-lyzed and processed in the same manner as the swab specimens.

Bacteriology

Rectal swabs were sent for isolation and identification of *Salmonella* and other enterobacteria to the Veterinary Medical Teaching Hospital, University of California-Davis.

Parasitology

PVA-fixed fecal specimens were brought to the NMFS Kewalo Research Facility Diamond Head Laboratory and analyzed for the presence of helminth ova and adults by M. Kliks, CST Foundation, Honolulu, HI. Fecal samples were analyzed by preparing a standard 2-mg fecal smear from the undiluted sample and microscopically examined for parasite ova and cysts or spores. The remainder of the specimen was commutated in formol saline, passed through three graded sieves, the smallest of which had an opening of 750 u. All retained materials were examined grossly and with the aid of a dissecting microscope for any parasite stages. All candidate objects were stored in glass screw top vials in 70% alcohol plus 5% glycerin for further study; all other solid residues were returned to their original containers in their original fixative solutions. For further study of adult nematode specimens, these were processed into pure glycerin, mounted on labeled microscope slides under a coverslip and studied under low power using a compound microscope. Cestodes and acanthocephalan worms were stained in aqueous carmine solution, de-stained in acid alcohol, dehydrate through absolute alcohol, cleared in xylene and mounted in Canadian balsam on slides under coverglasses. All unmounted and mounted specimens have been processed and stored for further taxonomic studies.

Toxicology

Blubber and blood specimens will be analyzed for chlorinated hydrocarbons (Chs) by a screening method using high performance liquid chromatography coupled with photodiode array detection by NMFS Environmental Conservation Division (ECD), Seattle, WA (Khran et al., 1994). The CH analyses included are selected polychlorinated biphenyls (PCBs) congeners, DDTs and their metabolites (DDEs, DDDs) and hexachlorobenzene (HCB). Selected samples were analyzed for the detection of heavy metals (mercury, selenium, aluminum, copper, zinc, arsenic, cadmium, lead, nickel) using the more time-and-cost-intensive gas chromatography/mass spectrophotometry (Khran et al., 1997).

Statistical Analysis

Statistical analyses included descriptive statistics reported as the mean $\pm$ 1 standard error for the hematologic and serum biochemistry data by location. Statistical differences among the means were detected by multiple comparison procedures including a multifactorial ANOVA with unequal sample sizes using the regression approach. The ANOVA model was used to compare serologic results and interactions of sex, size, and season. All tests were considered significant at $P \leq 0.05$.

RESULTS

Seals

A total of 107 monk seals from both sexes (37 females, 64 males, 6 unknown) and different age groups including weaned pups (12), non-adults (37), adults (52), and 6 of unknown size were sampled. Sample size distribution by site includes 51 seals at FFS, 46 seals at P&H and 10 seals at MID. Monk seals were captured for several reasons and in some cases opportunistic sampling was used. A total of 71 seals were specifically sampled for this epidemiological survey, 26 seals were captured at FFS (8) and P&H (18) for satellite radio deployments and retrievals and 10 seals at FFS were captured for crittercam attachments and retrievals.

Clinical Exams

Following physical restraint with a hoop net, monk seals (n = 106) were tranquilized with diazepam at a mean dose of 0.2 $\pm$ 0.007 (range 0.05 to 0.46) mg/kg. All monk seals included in this study were considered healthy, with a normal body condition except for one seal which was very thin in MID and which recently died of a shark bite (Table 1). All clinical parameters were similar between adult and non-adult seals. No significant abnormalities were noted on any animal except for old scars and marks that were used to identify individuals. One adult seal at P&H had recently been wounded by a cookie-cutter shark (*Isistius brasiliensis*) but no clinical problems were observed. External parasites were not found.

Clinical Pathology

Hematologic and biochemistry data were summarized for each subpopulation (Table 2). The total WBC counts of monk seals were within the standard reference range of other phocid species. Estimates provided counts of neutrophils 1200-5000; bands 0-250; lymphocytes 800-3400; monocytes 50-850; eosinophils 300-2000 and basophils 0-150. Seals at MID tended to have higher absolute white blood cell counts than those at other subpopulations. Seals at FFS had higher neutrophil and lower lymphocyte counts than seals at MID and P&H. Reactive lymphocytes were present in all subpopulations including 1/21 (5%) at FFS, 6/10 (60%) at MID and 26/51 (51%) at P&H. Macroplatelets (2-5 times greater than normal platelets) were present in 7 of 21 (33%) seals at FFS, 8 of 10 (80%) at MID and 15 of 51 (29%) at P&H. Erythrocyte morphology was remarkably uniform excepting for poikilocytosis and anisocytosis (10%) and polychromasia and crenation (12%) that were observed in seals at P&H. No hemoparasites were noted.

Enzymatic values for alkaline phosphatase, aspartate aminotransferase and creatine kinase (CK) were higher for MID seals when compared to other subpopulations. Lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and BUN/creatinine ratios were lower at

FFS, and seals at P&H had the lowest glucose levels of the three subpopulations. It is important to note that the wide range of values for CK and LDH at all subpopulations may be related to skeletal muscle cell damage from restraint or number of venipuncture attempts. TCO_2 (bicarbonate) values were consistent with captive seal samples considering that it took as many as four hours in blue ice prior to processing and placing in liquid nitrogen. In general, most biochemistry parameters were similar for the three subpopulations.

Atrial Natriuretic Factors

Preliminary results of sera tested from 41 seals from P&H for circulating ANP (proANF 31-67) yielded mean values of 91.8 $\pm$ 9.6 (10-235) pg/ml. Five of the seals presented values above 200 pg/ml for proANF 31-67 (i.e., vessel dilator). The circulating ANP detected in monk seals was one-third to one-half lower than those found in humans. The majority of the ANP values were in the low end of the radioimmunoassay curve, and further testing is pending.

Serology

Laboratory results for 35 seals recently collected at French Frigate Shoals (July 7-15, 1998), including 12 weaned pups, were in the process of being analyzed at the time this report was written. In addition, serologic results for exposure to *Chlamydia psittaci*, *Dirofilaria immitis, Leptospira* spp., seal influenza virus, sea lion adenovirus, *Sarcocystis* spp. and *Toxoplasma gondii* are pending for all seals.

To date, serum specimens from 82 seals (21 at FFS, 10 at MD and 51 at P&H) have been tested to determine serologic exposure to *Brucella abortus*, *B. canis*, caliciviruses 1 through 17, Dahl porpoise calicivirus, F-9 calicivirus, grey whale calicivirus, Hawaiian calf calicivirus, McAllista calicivirus, mink calicivirus, rabbit calicivirus, primate calicivirus, Tillamook calicivirus, walrus calicivirus, California sea lion rotavirus-like agent (prototype A111R), fur seal herpesvirus (prototype no. 206), canine distemper virus (CDV), dolphin morbillivirus (DMV), phocine distemper virus (PDV), porpoise morbillivirus (PMD), walrus adenovirus (prototype W77R), walrus retrovirus-like agent (prototype no. T_2/19), and walrus enterovirus-like virus (prototype no. 7-19).

Four seal sera from P&H were positive to walrus adenovirus and two from P&H were positive to the walrus retrovirus-like agent. Two of 21 (10%) from FFS, 3 of 10 (30%) from MID and 8 of 51 (16%) seal sera were positive to the *Brucella abortus* card test. A juvenile male captured in P&H during October 1997 and February 1998 tested positive on both occasions. All 82 specimens were negative to *B. canis*, CDV, DMV, PDV and PMV (Table 3).

Virology

A total of 36 buffy coats and 34 rectal swabs were processed for virus isolation and all were passaged 3 times (1 week per pass) on Vero cells and PK15 cells. No cytopathology was observed, and no virus isolations occurred during that time. Nine of the rectal swabs from seals at P&H and one from MID that were further processed for caliciviral presence were positive ranging from 1+ to 4+ reaction intensity. Two seal specimens from P&H (BP03 and BX04) were further processed for direct electron microscopy to confirm the specificity of the dot-blot reactions and virus-like particles morphologically identified as caliciviruses (electron photomicrographs to be included in final report). Testing of serum, buffy coats, and rectal swabs recently collected at FFS from 35 seals is pending.

Bacteriology

Fecal swabs collected for bacterial isolation from 10 seals at MID and 26 seals at P&H have yielded *Edwardsiella tarda* in most specimens but no other enteric pathogens except for *Salmonella* group B isolated from seal BA70, an adult female from P&H. Specimens collected at FFS are currently being tested.

Parasitology

PVA-fixed fecal specimens from 10 seals at MID and 10 seals at P&H have been analyzed to date. The results of direct fecal unstained smear readings indicated that three types of helminth eggs including diphylobothrid, ascaridoid and trichuroid ova were present in both subpopulations. Tapeworm egg densities were higher at P&H averaging 2469 ± 1027 (65-10,980) eggs/slide than in MID at 912 ± 478 (0-4760) eggs per slide. Ascaridoid ova were also more common in P&H at 7 ± 3 (0 - 24) eggs/slide when compared to MID at 3 ± 2 (0-12) eggs/slide. An incidental finding in P&H seals regarding trichuroid eggs in one of the 10 specimens which contained 12 eggs/slide is reported. Specimens collected at FFS in July 1998 have not been analyzed; results are pending.

Toxicology

Toxicology results of blood and blubber specimens for all subpopulations are pending.

DISCUSSION

The results of this study provided for the first time standardized health and disease baseline information for three subpopulations of wild Hawaiian monk seals. This information will be incorporated into the Epidemiology Plan (Aguirre and Reif, 1998) and

will form the basis of the biomedical decision process for future translocation of individual animals among subpopulations. Seals at all subpopulations were considered normal and healthy. Hematologic and biochemistry values were similar to previous studies (Banish and Gilmartin, 1988; Castellini et al., 1993; Rea et al., 1998). Further testing and pending results will provide insights regarding the infectious agents shared among these subpopulations. Preliminary serologic results in Hawaiian monk seals are encouraging when comparing the exposure of other pinnipeds to infectious agents (Lowry et al., 1996).

Absolute leucocyte numbers for each cell type (reference ranges) appear wider than those of captive pinnipeds. The eosinophil count in some seals tested during this study was high and may be related to chronic exposure to parasites in the wild. Atypical or reactive lymphocytes were a common finding in blood smears at all sites.

Preliminary results from this study indicated that 10-30% of the subpopulations have suspect titers to a *Brucella abortus*-like organism. Future *Brucella* screening of sera from all seals using BAPA, Rivanol and complement fixation techniques are necessary to confirm the presence or absence of these antibodies. Brucellosis is an important infectious disease of many mammalian species including humans. Infection is typically followed by abortion or stillbirth and by epididymitis and infertility in males. The disease is spread horizontally by contact with infective discharges from aborting females, by ingestion and by other routes. The genus *Brucella* has been recently identified in marine mammals isolated from common seals (*Phoca vitulina*), a grey seal (*Halichoerus grypus*), a hooded seal (*Cstophora cristata*), and several species of cetaceans (Ross, 1996). A similar *Brucella* species was isolated from the aborted fetus of a bottlenose dolphin along the California coast. The strains do not appear to be members of known species of the organism, and a new species has been proposed (Jahans, 1997). Recently, *Brucella* titers were detected in 18 of 102 Pacific harbor seals and 4 of 50 California sea lions from Puget Sound, Washington indicating relatively widespread infection among Pacific coast pinnipeds. The organism was recently isolated from *Parafilaroides* lungworms in a Pacific harbor seal, suggesting a potential role for the parasite as a secondary mechanism of transmission (Garner, 1997). Further studies should include the collection of tissues from necropsied seals to isolate, culture, and further describe the nature of brucellosis in monk seals. It is recommended that Hawaiian monk seals with antibody to *Brucella* should not be considered candidates for translocation (Aguirre & Reif, 1998).

The lack of group antibodies to calicivirus in the specimens tested is inconsistent with previous studies (Poet et al., 1993). Caliciviruses were detected in this study using a monoclonal antibody probe and direct electron microscopy but could not be cultured in vitro from rectal swabs. A report previously published (Poet et al., 1993) confirmed the presence of caliciviruses in both avian and fish specimens collected at FFS. It is interesting to note that the seal (YZ03) positive to calicivirus recently sampled at MID was a seal born and collected at FFS in 1991 and brought to Oahu for rehabilitation. She was released at MID in 1992 and has been sighted there consistently ever since. The continuous

development of cell lines, immunoglobulins and re-agents specifically for monk seal testing (Lu et al., 1998) will provide a better understanding of the immunologic and virologic status of the species.

The endoparasites identified in this preliminary study have been previously reported in Hawaiian monk seals (Golvan, 1959; Rausch, 1969; Dailey et al., 1988). All parasites were present at all subpopulations; therefore, concerns should be focused on the intensity of parasitism of individual seals prior to their translocation.

Little is known about the direct effects of massive infection with *Diphyllobothrium* species common in this host. Similar *Diphyllobothrium* species in humans and other animals are known to cause considerable morbidity. The gastric ascarioid, *Contracaecum* is a large and robust anisakid nematode worm. Infection with even one larval form of this and related species causes severe acute gastrointestinal distress (Whittow and Balazs, 1979). The present study indicates a high prevalence and intensity of infection. Further pathologic studies are necessary to determine if *Contracaecum* infections cause serious morbidity in both adult and immature monk seals. The finding of ova of an unknown trichuroid nematode in two rectal swabs and more recently in seven scats (Kliks, unpubl. data, 1998) should be evaluated. Several species of trichuroids have been reported from seabirds, although none are known from these islands or from the most common avian species found there. An overall interpretation of the impact of helminth infections on the HMS includes the evaluation of fresh and preserved specimens of organ systems and tissues that is currently being performed.

CONCLUSIONS

This preliminary study provides the first data on the health and disease status of wild Hawaiian monk seals at three breeding subpopulations. Preliminary baseline values for hematology, serum biochemistries, serology, virology, bacteriology and parasitology provide important insight decisions that must be made for translocation efforts of the endangered Hawaiian monk seal. The seal specimens tested in this study indicate that the three subpopulations may share common disease agents and in general present excellent health status and condition. Further studies need to be established to determine the epidemiological consequences of card test antibody titers to *Brucella abortus*. There is no indication provided in these preliminary test results that would preclude translocation of seals between donor and recipient subpopulations.

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Table 1. Life history parameters of Hawaiian monk seals (*Monachus schauinslandi*) sampled at three subpopulations in the North Western Hawaiian Islands.

Morphometric parameter	French Frigate Shoals (N = 51)			Midway (N = 10)			Pearl & Hermes Reef (N = 46)		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Age (years)	9.7	0.5	1-18	6.3	1.4	1-14	8	0.8	1-16
Dorsal Standard Length (cm)	194.2	3.2	142-216.5	195.4	9.9	147-233	187.9	3.9	137-225.5
Axillary Girth (cm)	124.3	2.2	89.5-147	126.6	7.4	85-160	121.6	2.8	88-154
Weight* (kg)	154.9	6.8	65-223	169.4	74.5	57-298	148.8	8.9	55-267

* Weight was calculated based on the formula Axillary girth^2 x Length x 0.00005 (Pitcher, 1986; Arnould, 1995).

Table 2. Comparative hematological and serum biochemistry results obtained from Hawaiian monk seals (*Monachus schauinslandi*) at three subpopulations in the Northwestern Hawaiian Islands.

Hematology & serum chemistry parameter	French Frigate Shoals (n = 21)			Midway (n = 10)			Pearl and Hermes Reef (n = 51)		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
White blood cell count min (X 10^3/uL)	7.0	2.5	5.3-8.0	9.0	1.1	3.3-13.2	7.5	2.6	3.9-14.6
Neutrophils (%)	57	2.0	41-71	52	3.0	38-70	50	1.0	28-68
Neutrophil bands (%)	0.5	0.2	0-3	0.0	0.0	0.0	1.0	0.2	0-6
Lymphocytes (%)	23.8	1.7	11-42	28.8	1.8	19-39	29.0	0.94	13-51
Monocytes (%)	5.4	0.67	2-12	5.7	0.93	0-10	5.3	0.3	1-10
Eosinophils (%)	13	1.2	4-21	13.1	2.0	1-24	14.4	0.7	6-27
Basophils (%)	0.42	0.16	0-2	0.4	0.16	0-1	0.41	0.08	0-2
Alkaline Phosphatase (IU/L)	233.5	42.7	75-1007	375.6	66.2	126-676	267.5	18.2	111-770
Alanine aminotransferase (IU/L)	106.6	13.3	54-281	111.7	11.5	35-164	114.7	7.3	38-295
Aspartate aminotransferase (IU/L)	124.9	22.3	59-498	143.1	18.7	55-241	105.3	4.2	45-198
Creatine kinase (IU/L)	505	53.3	204-1270	899.8	140.0	407-1569	706.6	48.5	305-1522
Gamma glutamyltranspeptidase (IU/L)	9.8	1.0	6-27	7.5	0.78	2-11	7.7	0.35	3-16
Lactate dehydrogenase (LDH)	606.5	42.2	380-1188	1010.5	135	657-2065	1002.8	68.2	486-2227
Albumin (g/dL)	3.1	0.10	2.4-3.6	3.1	0.10	2.7-3.5	2.9	0.03	2.4-3.4
Total Protein (g/dL)	8.4	0.15	7.5-11	8.7	0.23	7.5-9.5	8.1	0.08	7.2-9.5
Globulin (g/dL)	5.3	0.13	4.3-7.4	5.5	0.17	4.6-6.2	5.2	0.08	4.3-6.7

Serum chemistry parameter	French Frigate Shoals (n = 21)			Midway (n = 10)			Pearl and Hermes Reef (n = 51)		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Total Bilirubin (mg/dL)	0.24	0.02	0.1-0.4	0.17	0.02	0.1-0.3	0.17	0.01	0.1-0.3
Direct Bilirubin (mg/dL)	0.10	0.01	0-0.2	0.08	0.02	0-0.2	0.06	0.007	0-0.2
Indirect Bilirubin (mg/dL)	0.1	0.01	0-0.3	0.1	0.01	0-0.1	0.1	0.007	0-0.2
Blood Urea Nitrogen (BUN) (mg/dL)	27.7	2.7	11-58	37.2	5.8	12-64	42.4	1.7	17-65
Creatinine (mg/dL)	1.4	0.05	0.9-1.8	1.2	0.11	0.8-1.8	1.03	0.03	0.6-1.6
Cholesterol (mg/dL)	237.9	16.2	155-476	240.5	14.0	169-304	196.6	7.1	91-341
Glucose (mg/dL)	92.8	3.8	48-120	77.5	5.0	54-99	88.8	2.4	49-124
Calcium (mg/dL)	9.4	0.3	8.5-15.3	10.2	0.2	9.2-11.2	9.6	0.08	8.5-11.3
Phosphorus (mg/dL)	7.5	1.3	3.8-33.4	7.2	0.5	4.4-9.8	6.8	0.2	4.1-10.2
TCO_2 (Bicarbonate) (mEq/L)	23.1	1.2	5-32	25.1	1.2	18-31	24.9	0.8	17-37
Chloride (mEq/L)	105.9	0.9	90-110	105.1	1.2	100-111	107.4	0.5	98-117
Potassium (mEq/L)	5.9	1.4	3.9-34.4	5.3	0.3	3.8-6.5	4.9	0.06	4.2-5.9
Sodium (mEq/L)	151.3	1.1	136-159	153.8	1.6	148-165	151.5	0.6	143-159
Albumin/Globulin Ratio	0.6	0.01	0.4-0.7	0.6	0.02	0.5-0.7	0.6	0.01	0.4-0.7
BUN/Creatinine Ratio	21.9	2.8	6.5-58	36.2	7.7	7.5-80	43.5	2.3	9.4-77.5
Sodium/Potassium Ratio	32.9	1.5	4-38	29.6	1.3	25-39	31.1	0.3	26-36
Anion Gap (mEq/L)	28.1	2.5	19-75	29	2.0	23-41	24.1	0.8	9-34

Table 3. Diagnostic laboratory, serologic tests performed, methods used, antibody titer considered positive and number of seropositive seals found in the health and disease status evaluation in wild Hawaiian monk seals (*Monachus schauinslandi*).

Diagnostic laboratory	**Disease agent**	**Test procedure (positive titer)**	**No. positive/ No. tested**
IDDEX CVD, Sacramento, CA	*Chlamydia psittaci*	Elementary body agglutination	pending
	Dirofilaria immitis	ELISA	pending
Oklahoma Animal Disease Diagnostic Lab, Stillwater, OK	*Brucella abortus*	Card	13/82
	Brucella canis	Card	0/82
	Canine distemper virus	Serum neutralization (≥1:8)	0/82
	Dolphin morbillivirus	Serum neutralization (≥1:8)	0/82
	Phocine distemper virus	Serum neutralization (≥1:8)	0/82
	Porpoise morbillivirus	Serum neutralization (≥1:8)	0/82
	Phocine herpes virus 1	Virus neutralization (≥1:8)	pending
Oregon State University Callicivirus Research Lab, Corvallis, OR	*Leptospira* spp. -8 serovars	Microscopic agglutination	pending
	Caliciviruses (39 serotypes)	Serum neutralization (>.200)	0/82
	California sea lion rotavirus	Serum neutralization (>.200)	0/82
	Fur seal herpesvirus	Serum neutralization (>.200)	0/82
	Walrus adenovirus	Serum neutralization (>.200)	4/82
	Walrus enterovirus	Serum neutralization (>.200)	0/82
	Walrus retrovirus	Serum neutralization (>.200)	2/82
USDA Zoonotic Disease Lab Beltsville, MD	*Sarcocystis* spp.	Modified agglutination	pending
	Toxoplasma gondii	Modified agglutination	pending
USDA Foreign Animal Disease Diagnostic Lab, Plum Island NY	Seal influenza virus	Reference antisera H & N	pending
	Sea lion adenovirus	Serum neutralization	pending