

Harbor Seal Recovery: Application of New Technologies for Monitoring Health

Project Number: 01558

Restoration Category: Research

Proposer: University of Alaska Fairbanks

Lead Trustee Agency: ADFG

Alaska SeaLife Center: YES

Duration: 1st of a 3-year project

Cost FY 01: \$172.1

Cost FY 02: \$175,000

Cost FY 02: \$168,100

Geographic Area: Alaska SeaLife Center, Gulf of Alaska

Injured Resource/Service: Harbor seals

ABSTRACT

This study will investigate the potential for new technologies to assess and monitor the endocrine and immune systems as diagnostic measures of the health of harbor seals. Analysis of thyroxine (T₄), triiodothyronine (T₃), and cortisol (primary metabolic and gluconeogenic hormones), and measurement of immunoglobulins (IgG, IgM and IgA) and the body burden of organochlorine contaminants will provide an assessment of both permanently captive seals as well as seals that are brought into the Alaska SeaLife Center (ASLC) for rehabilitation. The work will also employ community involvement through the Alaskan Native Harbor Seal Commission. Once the profiles of healthy seals and those 'failing to thrive' in their natural environment are assessed, these techniques will be evaluated for routine monitoring of free-ranging seals in an effort to restore this species.

INTRODUCTION

The potential exists for several environmental factors to impact the biology of harbor seals (*Phoca vitulina*), resulting in poor survival, recruitment and reproductive rates. While the leading hypothesis is that changes in the availability of high quality prey have reduced the carrying capacity of the Gulf of Alaska, a contributing factor to poor survival and reproduction may include exposure to organochlorine contaminants (OCs), with associated endocrine and immune system impairment (Addison, 1989; De Swart *et al.*, 1994, 1996; Ross *et al.*, 1995; Reijnders, 1986). OCs and their by-products are bioaccumulated, biomagnified and transferred through lactation from mother to pup (Beckmen *et al.*, 1999; Gallenberg and Vodcicnik, 1989; Vreel *et al.*, 1996; Wagemann and Muir, 1984). These contaminants and by-products may continually affect a population of animals even though no major polluting event has occurred. The adverse effects on the physiology of the animal may be subtle or subclinical, or may manifest themselves with symptoms such as, 'failure to thrive' or 'failure to reproduce'. The systems that typically respond to environmental changes, including contamination or suitable prey, are the endocrine and immune systems. This proposed study will develop technologies to examine these two systems to be used to monitor the health of individuals and the well being of subpopulations.

The endocrine system is a complex system that integrates the environment in which an animal lives, with the physiology of that animal. As seasons, nutrition and other environmental parameters change, the neuroendocrine system is the first to work toward ensuring that the body can adapt to the changes. Many compounds in the environment are known to interfere with the endocrine systems of mammals and are often referred to as 'endocrine disrupting compounds' (EDCs). The most commonly known EDCs are the organochlorines, including polychlorinated biphenyls (PCBs), DDT and its metabolites, as well as the phthalates. Some EDCs are known to bind with estrogen receptors (Katzenellenbogen, 1995), either mimicking or blocking the effects of estrogens. Extreme examples of the effects of OCs on reproductive function are the neoplastic occlusions of the uterus resulting in infertility and the development of hermaphroditic offspring (Helle *et al.*, 1976; Baker, 1989; Reijnders, 1998). PCBs can also compete for binding sites on the transport proteins for the thyroid hormones, resulting in hypothyroid conditions that can affect early development or later reproductive performance (Brouwer, 1989). The results from these endocrine disruptions can be varied and also include suppression of the immune system (De Swart *et al.*, 1996; Ross *et al.*, 1995). Atkinson and Oki (2000) used thyroxine and cortisol concentrations along with several morphometric measurements to assess the well being of yearling Hawaiian monk seals that appeared to be malnourished. Their results suggest that a suite of measurements, including these hormones, provides a good indication of the physiology of a seal and its ability to adapt to suboptimal environments.

The immune system of marine vertebrates is a rapidly advancing area of interest, both in the basic components of the immune system as well as the development of immunodiagnostic reagents. Baseline information on the immune system of pinniped species is critical to any future field assessment of immunocompetence. The lack of baseline information on the immune system of the harbor seal population in Europe hindered assessment of the role of pollution-induced immunosuppression in the phocid distemper virus outbreak of 1988 (Dietz *et al.*, 1989a; Vos and Luster, 1989). Studies of levels of immunoglobulins and of isotypes of those immunoglobulins have been reported for a few species of pinnipeds. Cavagnolo and Vedros (1979) evaluated IgG, IgM and IgA levels in sera and colostrums of adult and immature northern fur seals (*Callorhinus ursinus*), finding low immunoglobulin levels in the sera of pups during the

first four months of life. Baker (1984) found similar results for overall gamma globulin levels in grey seal (*Halichoreus grypus*) pups. Carter *et al.* (1990) measured specific immunoglobulin isotype levels in sera and colostrums of the grey seal. Ross *et al.* (1993) evaluated IgG levels in the harbor seal, and also evaluated lymphocyte function in this species by measuring responsiveness to a T-cell mitogen. A number of reports have appeared describing ELISA's or other immunoassays measuring pinniped antibody levels against canine distemper virus (e.g. Dietz, *et al.*, 1989b; Carter, *et al.*, 1990; Bengston, *et al.*, 1991; King, *et al.*, 1993). It is of note that some of the latter studies utilized antibodies specific for canine immunoglobulins to measure pinniped immunoglobulins, with which they cross-react. In assays such as the ELISA's mentioned above that require the use of anti-immunoglobulin indicator antibodies it is generally preferable to utilize species-specific antisera when available, but such antisera are not readily available for most species of pinnipeds.

This project will utilize our ability to monitor several hormones and immunoglobulins, and relate their function to the body burden of contaminants and the overall health of individual seals. We propose to provide critical reagents and methodologies necessary for the assessment of several aspects of immunocompetence levels in the harbor seal, and to establish baseline data on these levels for the duration of the project in selected populations of harbor seals. The project will also result in the production of species-specific antisera for use in assays of immunoglobulin class specific antibody levels in the harbor seal population against pathogens, toxins, or other antigens of potential health importance. This project will also determine critical baseline concentrations of the thyroid hormones and cortisol of captive seals, housed in a stable environment with regular and balanced diets, to compare with free-ranging seals. In doing so, we can assess whether the seals in the Gulf of Alaska are being exposed to endocrine disrupting and/or immunosuppressive agents at level that are impacting their ability to survive, grow and reproduce. If contaminants are affecting the physiology of harbor seals, then we need to incorporate this into the working hypothesis under which this species is being managed. In addition, assessing the effects of environmental contaminants should be incorporated into any long-term plans for monitoring harbor seals. Monitoring endocrine and immune levels can also be used as indicators upon which parameters needed to model the population dynamics of harbor seals can be developed. This will become increasingly important if this species continues its population decline in the Gulf of Alaska.

NEED FOR PROJECT

A. Statement of Problem

Harbor seals were one of the resources that were injured by the 1989 *Exxon Valdez* oil spill (EVOS). To date this species is listed as 'not recovering'. Several studies have focused on the general health and metabolism of these seals as it relates to their diet, body condition and habitat (Projects 001, 341, 371, and 441). The proposed study will compliment these investigations as it will utilize new techniques to enhance our understanding of the health and physiology of the species and incorporate the possible affects of environmental organochlorine contaminants. If the techniques can be combined to develop a concise indicator of a given animal's health, then these techniques should be incorporated into the routine assessment and monitoring of harbor seals in the Gulf of Alaska.

B. Rationale/Link to Restoration

In order to recover any species whose population has experienced a major decline, it is necessary to fully understand the biology of the species. A few species of marine mammals have failed to recover with the enactment of the Marine Mammal Protection Act (e.g. Hawaiian monk seals and Steller sea lions). Other species have declined precipitously since the Marine Mammal Protection Act, with some subpopulations more affected than others (e.g. Alaskan harbor seals). The problems that these species face are multifaceted and complex. Many times a combination of factors will synergize to produce a devastating effect (such as the 1988 harbor seal epizootic in the North Sea), while either factor alone may not have had clinical effects. In understanding what the Alaskan harbor seals are experiencing, it is essential to know the degree to which they are being subjected to immunosuppressive or endocrine disrupting agents. Restoration of the species can only be successfully accomplished if the species is thoroughly understood. With this knowledge we can begin to predict the devastating effects of environmental changes and model the long-term population dynamics. In addition to predicting the impact of a given environment, we can also begin to manipulate animals and their environments to assist in their recovery.

The information gained from this study will enable us to assess two groups of animals, those that live in a stable, consistent environment (captivity), with those that experience the natural environment (rehab seals). Seals brought in for rehabilitation are generally young animals that are failing to thrive in their environment. They may not be able to naturally survive the weaning process due to a variety of factors, including immuno-incompetance or inadequate maternal investment (ie, poor milk quality or shorten lactation period). Through morphometric measurements, assessment of immune and endocrine function, and measurement of body contaminant levels, we can evaluate the degree to which these animals are adapting to a changing environment. Once these techniques have been perfected at the ASLC, we plan to test their application to a long-term, field-monitoring program. The ability of harbor seals to adapt to a changing environment is essential to the recovery of this species. Knowing what the animals are dealing with and their ability to adapt will enable resource managers to predict the recovery or mitigate the future decline of this species.

C. Location

Years 1 and 2 of this project will be undertaken at the ASLC using harbor seals that are currently resident and permitted for research under the Marine Mammal Protection Act for research. It will also utilize animals that will be brought in for rehabilitation under the terms of an existing letter of authorization, and through our collaboration with the Alaska Native Harbor Seal Commission. Year 3 of this work is proposed for free-ranging seals in Prince William Sound and areas near South Central Alaska.

COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE

This project will involve a growing collaboration with the Alaska Native Harbor Seal Commission. In addition to the native communities, we propose working with coastal fishing communities to increase the awareness of the plight of this species. In working with community facilitators, we will request that nearby communities inform us of harbor seals needing rehabilitation, including orphaned pups. These animals provide a wealth of information as they

have incorporated any environmental constraints into their physiology. As coastal communities come into contact with these animals more often than we know about, we propose working with these communities to increase our sample sizes. Through the development of brochures and speaking with local community groups, we will collaborate to ensure that seals requiring rehabilitation are brought to ASLC. Partnerships with the Civil Air Patrol and US Coast Guard will be sought to provide transportation of seals to ASLC from neighboring communities. During the rehabilitation process, these animals will be monitored for biochemical changes that indicate their ability to adapt.

This project will also coordinate with the existing volunteer and intern programs at ASLC to make opportunities available for spill-area residents who would like to spend time volunteering at ASLC. To a large extent this will increase our awareness of traditional and local knowledge of harbor seals as well as incorporate local expertise into the project. This project is budgeted for one graduate student and one research associate who will receive training to increase their level of expertise in marine mammal physiology as well as provide the necessary time to ensure that our community involvement is successful.

PROJECT DESIGN

A. Objectives

The overall goal of this project is to develop and test new methods of monitoring the physiology of harbor seals. In doing so the project has the following five objectives:

1. Determine seasonal and circadian patterns of total and free triiodothyronine (T₃), thyroxine (T₄), and cortisol in healthy captive harbor seals (Yr 1).
2. Develop new antibodies specific to harbor seal immunoglobulin classes IgG, IgM and IgA (Yr 1).
3. Determine seasonal patterns of IgG, IgM, and IgA, in healthy captive harbor seals (Yrs 1 and 2).
4. Determine endocrine and immunoglobulin profiles and measure organochlorine concentrations for rehabilitation seals periodically throughout the rehabilitation process (Yrs 1 and 2).
5. Assess endocrine and immunoglobulin profiles for free-ranging seals in Prince William Sound and in South Central Alaska (Yr 3).

B. Methods

Objective 1. Eight harbor seals (4 males, 4 females) housed at the ASLC will have monthly blood samples collected to assay for total and free T₄, T₃, and cortisol. In addition, circadian patterns of these hormones will be assessed from the eight seals during the seasonal extremes of the summer and winter solstices, with samples collected at 2 to 3 hourly intervals over a 24-hour period. A single blood and blubber sample will be collected for organochlorine analysis.

The analyses for these hormones have previously been validated for other pinniped species (Atkinson and Oki, 2000) and will be validated for harbor seals. Concentrations of cortisol will

be measured in unextracted plasma using a single-antibody radioimmunoassay (Atkinson and Oki, 2000; Atkinson and Adams, 1988). The plasma will be heated at 60°C for 30 minutes to denature cortisol-binding proteins before assaying directly. Samples will be analyzed in batches to reduce inter-assay variation. Concentrations of total and free T₄ and T₃ will be measured in unextracted plasma using solid phase radioimmunoassays (Diagnostic Products Corporation, Los Angeles, CA) that are specific to either total or free, T₄ or T₃ (Atkinson and Oki, 2000). The standard curves of each assay will be log-logit transformed, enabling extrapolation of sample concentration (Robard, 1974). A profile of the variation in total and free T₄ and T₃ will be generated and analyzed.

Objective 2. The prerequisite for development of heavy chain specific antisera for the major immunoglobulin classes of the harbor seal is the production of purified preparations of each of these immunoglobulin classes. These purified immunoglobulin classes will be obtained from pooled sera from captive animals at ASLC and will be used as the source of the immunoglobulins to be purified. The first step toward purification of individual immunoglobulin isotypes (IgG, IgM, and IgA) from serum will be to remove non-immunoglobulin proteins, leaving a mixture of all immunoglobulin isotypes present. Serum samples will be centrifuged (five minutes at 10,000 rpm) to remove any large particulate matter present. The supernatant will then be filtered through a 0.45 µm and then a 0.2 µm filter to further remove any remaining particulates and/or aggregates. The next step involves separating serum proteins in the filtrate based on molecular weight. The serum will be placed in a Millipore UltraFree®-15 centrifugal filter device with a molecular weight cutoff of 100,000 daltons. During a thirty-minute centrifugation step (2000 x g) proteins less than 100,000 daltons pass through the filter, while those greater than 100,000 daltons are retained above the filter. Since the immunoglobulin isotypes being studied have molecular weights greater than 100,000 daltons, they will be retained in the fluid retained in the UltraFree®-15, and can be removed and kept available for use in further purification steps. This filtration technique has proven more satisfactory than techniques involving differential precipitation of serum proteins in saturated ammonium sulfate.

Aliquots of such partially purified and concentrated samples will then be applied to one of the types of chromatography columns for purification of a particular immunoglobulin isotype. Antiserum will be produced in rabbits against the precipitated immunoglobulins to permit preliminary analysis of the IgG, IgM, and IgA immunoglobulins in harbor seal serum. Grabar-Williams immunoelectrophoresis will be used in initial examination of harbor seal whole and precipitated serum for immunoglobulins.

In order to obtain immunogens suitable for production of heavy chain specific antisera for immunoglobulins of the harbor seal, purified immunoglobulins will first be enzymatically partially digested with papain to obtain the equivalent of Fab and Fc fragments for each isotype. Use of whole heavy chains as the immunogen produces antisera which include antibodies against the variable region of the heavy chain, which may cross-react with immunoglobulins of various isotypes. The Fc fragment contains only heavy chain constant regions and is more likely to induce isotype specific antisera if used as the immunogen. Purified "Fc" fragments of each isotype will be reduced with 2-mercaptoethanol and alkylated with iodoacetamide to break the disulfide bonds between the linked heavy chains. Chromatography using a Sephacryl S-400HR column will then be used to separate the heavy chain fragments from the other peptides which may be present (e.g. the J-chain of IgA or IgM). Once the purity of heavy chain preparations has been determined, they will be used to produce isotype-specific antisera that can be used to

determine specific IgG, IgM, and IgA levels within a sample. Rabbits will be used to produce these antisera. The animals will be immunized by standard approved protocols. The titer and specificity of the antisera will be determined by (1) standard indirect ELISA (wells coated with purified harbor seal immunoglobulin heavy chain), followed by the rabbit anti-heavy chain antibody being tested, followed by enzyme-labeled anti-rabbit immunoglobulin, and finally by the indicator substrate) and (2) immunoelectrophoresis (IEP) methods including Grabar-Williams, Rocket IEP, Crossed IEP, and Tandem Crossed IEP. The antisera will be partially purified by use of the Millipore UltraFree®-15 centrifugal filter device followed by purification by Protein G Sepharose^R affinity chromatography to obtain the IgG fraction of this rabbit antisera. The purified antisera will be labeled with biotin or an enzyme (e.g. alkaline phosphatase or horseradish peroxidase) using standard labeling linkers (Pierce). The resulting antisera will be analyzed for specificity by several methods, including application of the antisera to Western blots of whole heavy chain preparations obtained by reduction/alkylation of the respective whole immunoglobulin isotype preparations.

Once the antisera for each immunoglobulin's heavy chain isotype has been made, it will be possible to regularly monitor immunoglobulin levels as an indicator of immune status of a population of harbor seals. It will also be possible to determine the level of each isotype present in, for example, samples obtained during a vaccination trial, at particular points in time of interest to a veterinarian or researcher (e.g. during pregnancy, drug therapy, maturation stage, etc.).

Objective 3. An ELISA protocol similar to that described by Suer *et al.* (1988) has been used to evaluate serum antibody levels in several species of marine mammals against several antigens (e.g. Patterson *et al.*, 1994). A “sandwich” ELISA protocol will be employed in an effort to determine general immunoglobulin levels in these samples. In the sandwich ELISA, a plastic solid phase matrix (polystyrene microwells) is coated with unlabeled antibodies against the antigen in question, i.e. in this case against one of the heavy chain isotypes (gamma, alpha, or mu for IgG, IgA, and IgM respectively) of immunoglobulins from the harbor seal (prepared via completion of Objective 2 above). The sandwich ELISA conducted in this manner will allow quantitation of general immunoglobulin levels in samples by comparison with a standard curve generated using preparations made with known concentrations of immunoglobulins purified from the harbor seal.

Blood samples will be collected on at least a monthly basis by project personnel based at the ASLC. Aliquots of each sample (and aliquots of other samples of harbor seal sera which become available) will be quantified for isotype levels using the ELISA described above in completion of Objective 2.

Objective 4. Using the previously described techniques, we will measure total and free T₃, T₄, cortisol, and IgG, IgM and IgA in harbor seals that are brought in for rehabilitation. ASLC has the ability to hold 10 seals for rehabilitation. An assessment of the level of contamination by organochlorines will also be performed from either blood or blubber samples. As these measurements will be diagnostic, the frequency of sampling will be based on the overall condition of the seals and not all of these animals will have the same numbers of samples collected. It is envisioned that samples will be collected upon entrance and before release of all seals. In addition, samples may be collected periodically to assess any effects of different milk formulæ that are fed to very young seals as well as upon weaning when the diet and digestive

efficiency of the animals is maturing. Experimental protocols are based on models using 10-12 harbor seals admitted for rehabilitation. These protocols will comply with the ASLC Institutional Animal Care and Use Committee (IACUC) and Marine Mammal Protection Act (MMPA) guidelines.

Seals admitted for rehabilitation at the SeaLife Center are held in quarantine and placed in individual holding tanks. Currently, the EVOS funded project Harbor Seal Recovery, Phase II, conducted by Dr. Mike Castellini, collects health data such as blood chemistry and morphometrics weekly from each harbor seal admitted for rehabilitation. Blood chemistry and hematology values are used in conjunction with body composition to detect significant changes in health status that might alter water balance, cause anemia, or compromise basic metabolic status (Castellini et al., 2000, 1993). Blood urea, nitrogen (BUN) ketone bodies, and free fatty acids, as well as hematocrit, hemoglobin, and erythrocyte sedimentation rate are measured.

Assimilation efficiencies will be determined for harbor seals prior to and during the weaning process, as well as once the animals are on a stable fish diet. Meal size and feeding frequency will be kept constant during the experimental period. Food digestibility in these seals will be determined using manganese (Mn^{++}) as an inassimilable dietary marker. Concentrations of Mn^{++} from subsamples of the food items fed to individual seals during the acclimation and collection periods, will be analyzed using atomic absorption spectrophotometry (Fadely et al. 1990). Feces will be collected during the course of the feeding trail to determine the clearance rate of food items and fecal Mn^{++} concentrations. Differences in the Mn^{++} concentrations between diet and feces will be used to calculate AE. In addition, diet and fecal samples will be freeze-dried and analyzed for energy (cal/g), nitrogen, total lipid, and ash as reported in Keiver et al (1984). To quantify the passage of digesta (mean retention time) and fecal Mn^{++} concentrations, carmine red will be used as a marker to estimate emptying time of the stomach (Ashwell-Erikson and Elsner 1981).

Objective 5. The methodology of this objective will be developed over the first 1 to 2 years of the project. The feasibility of sampling as well as the necessities of sample processing will continually be evaluated with the goal of developing techniques that are feasible for field collections. It is hoped that Year 3 will be a collaborative effort with harbor seal researchers that regularly collect samples from free-ranging seals. The sites of collection, numbers of animals and the permits to cover the sampling of wild seals will be negotiated with other researchers who may be collecting samples concurrently. Discussions will also be held with the Alaska Native Harbor Seal Commission to assist with the planning of the field testing.

C. Cooperating Agencies, Contracts and Other Agency Assistance

This project will primarily be based at ASLC, with the National Marine Fisheries Service permits for the captive seals being held by ASLC with Dr. S. Atkinson serving as the Principal Investigator of that permit. Seals needing rehabilitation will be sought with the guidance of the Alaska Native Harbor Seal Commission. The letter of authorization for these seals is also held by ASLC, with Susan Inglis, Director of Research and Rehabilitation Operations serving as the PI.

The samples collected for endocrine evaluation will be analyzed in the Marine Mammal Endocrinology Lab of Dr. S. Atkinson, housed at ASLC. The samples for immune assessment will be analyzed by Dr. Bobby Middlebrooks, University of Southern Mississippi. A subcontract

within this proposal has been negotiated. The samples for contaminant measurements will be analyzed by the Northwest Fisheries Science Center of the National Marine Fisheries Service. A subcontract for these samples has been negotiated with Dr. Peggy Khran.

SCHEDULE

A. Measurable Project Tasks for FY 01 (October 1, 2000 – September 30, 2001)

- October 2000: Blood sampling commences on a monthly basis. In addition, single samples will be taken to initiate the hormone validations and immunoglobulin development.
- November 2000: Blood and blubber samples from the captive seals will be sent for contaminant analysis.
- December 2000: Blood samples will be collected to assess circadian pattern of T₃, T₄ and cortisol.
- January 2001: Endocrine assays will be undertaken with batches of samples to assist with quality control.
- May-June 2001: Seals collected for rehabilitation arrive at ASLC.
- June 2001: Circadian sampling will be performed.
- June- September: Endocrine and immunology samples analyzed.
- September-October: Rehabilitation seals released.

Depending on the age and health of these seals, they are typically kept until Most of the analyses will be accomplished by Sept 2001, although there will be some analyses to complete during FY 02.

B. Project Milestones and Endpoints for Year 1

1. Establishment of baseline levels of total and free T₃, T₄ and cortisol levels in the serum: The hormone concentrations from captive animals will serve as our baseline for both the rehabilitation seals and the free-ranging seals. Both circadian and seasonal baselines will be established. This will be completed during Year 1.
2. Development of species-specific antisera against immunoglobulins of the harbor seal: An important outcome of the proposed project will be the production of such antisera against immunoglobulin isotypes of the harbor seal. These antisera will be available for and of use in future studies involving immunoglobulins of this species. The antisera will also be available for use in ELISA's to measure immune response and immune levels against specific antigens of interest in the harbor seal population. For example, the antisera can be used in future studies involving use of ELISA's to measure antibody levels against selected viruses, toxins, or bacterial antigens of interest and importance in wild populations of the harbor seal. This will be completed during Year 1.
3. Establishment of baseline levels of immunoglobulin isotypes in serum and saliva: The sandwich ELISA (using the respective anti-isotype antisera as capture

antibody) will provide data on general immunoglobulin levels in the harbor seal. This will be completed during Years 1 and 2.

C. Completion Date

The anticipated completion date of the captive portion of this project is October 2002. At this point we will hope to be able to recommend that these techniques be applied to a field-monitoring program. If this is accomplished the feasibility of field sampling will be determined by October 2003.

PUBLICATION AND REPORTS

It is anticipated that all of the work conducted under this proposal be published in peer-reviewed international journals. Potential journals include, General and Comparative Endocrinology, Comparative Biochemistry and Physiology, Marine Mammal Science, and Journal of Developmental and Comparative Immunology. In addition, any student projects will be presented in thesis or dissertation format as well as submitted for journal publication. The presentation of work at conferences and workshops will be encouraged. Such conferences may include, Society for Marine Mammalogy, International Association of Aquatic Animal Medicine, or any EVOS workshops.

PROFESSIONAL CONFERENCES

No professional conferences are requested for FY01. The PI will request to attend the Biennial Conference of the Biology of Marine Mammals in Vancouver, Canada in FY02.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The PI of this proposal also serves as the Science Director of the ASLC. Through this avenue, the PI holds regular discussions on the projects that are currently taking place at ASLC, and is already collaborating on the technical aspects of this study. This project will be using the same animals as have been used for projects 341, 371, and 441, and it is anticipated that discussions will be held whenever scientist are in Seward for sample collections. It is also anticipated that Year 3 samples will be collected from a shared field site, integrating existing field projects with our sample collections.

This project will benefit from new equipment that has recently been purchased by UAF and UAF Foundation in an effort to establish an endocrinology laboratory at ASLC. The lab will be regulated under the Nuclear Regulatory Commission License to UAF. It is in this lab that the students and research associate on this project will work.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR (qualifications)

The PI of this project is a new professor at UAF, with half time duties to serve as the Science Director at ASLC. She has 18 years experience in analyzing body fluids for hormone concentrations. She has established and worked in two other endocrinology laboratories, one at Hawaii Institute of Marine Biology, University of Hawaii, and the other at Murdoch University in Western Australia. The PI also has extensive experience working with a variety of marine mammals, including the endangered Hawaiian monk seal, California harbor seals, northern elephant seals, Risso's, rough-toothed, white sided, and bottlenose dolphins, and, humpback, beluga, and false killer whales. The PI will be responsible for the completion of all project objectives. Her curriculum vitae is attached.

OTHER KEY PERSONNEL

Ms. Susan Inglis is the Director of Research and Rehabilitation Operations at ASLC. She has extensive experience in the rehabilitation of seals and birds. She has 15-year experience managing research projects, including numerous species of fish, sea birds and marine mammals. Her organizational and technical skills will be invaluable to this project. Her curriculum vitae is attached.

Dr. Bobby Middlebrooks is a Professor at the University of Southern Mississippi. He has an immunology laboratory that focuses on the basic components and functioning of the immune systems of marine vertebrates. He has developed immunodiagnostic assays for pinnipeds and is highly qualified to undertake the immunological aspects of this study. He will be responsible for the developing any specific reagents necessary to assay for immunoglobulins in harbor seals, as well as for performing and analyzing the results from those assays. His curriculum vitae is attached.

Salaries have been included for a research associate and a graduate student. The research associate will assist with the overall coordination of the sample collection from the captive seals as well as organize and coordinate sample collections from the rehabilitation seals. The research associate will also work with the community facilitators to increase the sample size of rehab seals entering ASLC. This will include collaborations with the Civil Air Patrol or Coast Guard to assist with transport of seals from nearby communities. In addition, the research associate will work in the endocrinology lab at ASLC and help to maintain quality control and assurance standards for the assays performed there.

The graduate student will be responsible for drafting the experimental designs and sampling protocols. They will assist with the sample collections and perform the laboratory work. With assistance from the PI, they will analyze the data and present them in graphical and tabular form. They will be responsible for the first draft of any manuscripts that arise from the work included in their thesis or dissertation.

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- Patterson, R. A., M. Al-Kurd, M. A. Solangi and B. L. Middlebrooks. 1994. Development of methods for evaluation of the humoral immune response of bottlenose dolphin (*Tursiops truncatus*) against protein antigen and a bacterial antigen. p 623 *In* Abstracts of the 94th General Meeting of the American Society for Microbiology.
- Reijnder, P.J.H. 1998 Reproductive developmental effects of endocrine-disrupting chemicals on marine mammals. Marine mammals and persistent ocean contaminants: Proceedings of the marine mammal commission workshop, Keystone, Colorado, October.
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- Ross, P.S., R.L. De Swart, P.J.H. Reijnders, H. van Loveren, J.G. Vosand and A.D.M.E. Osterhaus 1995 Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. *Environ. Health Perspect.* 103:162-167.
- Suer, L. D., N. A. Vedros, J. P. Schroeder, and J. L. Dunn. 1988. *Erysipelothrix rhusiopathiae*. II. enzyme immunoassay of sera from wild and captive marine mammals. *Dis. Aquat. Org.* 5:7-13.
- U.S. Fish and Wildlife Service. 1988. Black-footed ferret recovery plan. U.S. Fish and Wildlife Service, Denver, Colorado. 154pp.
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Wagemann, R. and D.C.G. Muir. 1984 Concentrations of heavy metals and organochlorines in marine mammals of northern waters: Overview and evaluation. Can. Tech. Rep. Fish. Aquat. Sci. 1279:1-97.

Curriculum Vitae (abbreviated)

Name and Title: Shannon Atkinson, Ph.D., Professor of Marine Science

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Education: Ph.D. Murdoch University, School of Veterinary Studies, 1985
M.Sc. University of Hawaii, Department of Animal Science, 1981
B.Sc. University of Hawaii, Department of Animal Science, 1978

Professional Experience

Professor, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks 2000-present
Science Director, Alaska Sealife Center, Seward, AK 2000-present
Associate Researcher, Hawaii Institute of Marine Biology, University of Hawaii 1991- present
Affiliate Researcher, Hawaii Institute of Marine Biology, University of Hawaii 1989-1991
Experimental Scientist, Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Animal Production, Western Australia

Recent Research Projects:

- Reproduction and development of rough-touther and bottlenose dolphins 1998-2001, NOAA/Seagrant
- Metabolism and reproduction in Hawaiian monk seals, 1998-2001
- Distribution and abundance of odontocetes around Oahu, Hawaii 1999-2001, NOAA/private foundation
- Feeding ecology of Hawaiian monk seals, 1994 to 1996, Waikoloa Marine Life Fund

Selected Relevant Publications:

West, K.L., ***Atkinson, S.***, Carmichael, M.J., Sweeney, J.C., Krames, B. and J. Krames 2000. Concentrations of progesterone in milk from bottlenose dolphins during different reproductive states. *General and Comparative Endocrinology* 117(2):218-224.

Atkinson S, Combelles C, Vincent D, Nachtigall P, Pawlowski J and Breese M (1999) Monitoring of progesterone in captive female false killer whales, *Pseudorca crassidens*. Gen. Comp. Endo. 115:323-332.

Goodman-Lowe, G., Carpenter, J. and S. Atkinson 1999. Assimilation efficiency of prey in the Hawaiian monk seal (*Monachus schauinslandi*). Can. J. Zool. 77:653-660.

- Mazzuca, L., Atkinson, S., Keating, B. and E. Nitta 1999. Cetacean mass strandings in Hawaiian Archipelago 1957-1998. *Aquatic Mamm.* 25(2):105-114.
- Theodorou J and *Atkinson S* (1998) Monitoring of testosterone in the saliva of captive Hawaiian monk seals. *Mar. Mamm. Sci.* 14(2):304-310.
- Mazzuca L, *Atkinson S* and Nitta E (1998) Deaths and entanglements of humpback whales in the main Hawaiian Islands, 1973-1995. *Pac. Sci.* 52:1-13.
- Silvers LE, *Atkinson S*, Iwasa M, Combelles C and Salden D (1997) A large placenta encountered in the Hawaiian winter grounds of the humpback whale, *Megaptera novaeangliae*. *Mar. Mamm. Sci.* 13(4):711-716.
- Atkinson S* (1997) Reproductive biology of seals: reproductive processes and events of phocid and otariid seals. *Reviews of Reproduction.* 2:175-194.
- Iwasa M, *Atkinson S* and Kamiya S (1997) Lipofuscin granular cells in regressing corpora lutea and corpora albicantia of ovaries from wild Hawaiian monk seals. *Mar. Mamm. Sci.* 13:326-332.
- Goodman-Lowe, G, *Atkinson, S*, and Carpenter, JR. (1997) Initial defecation time and rate of passage of digesta in adult Hawaiian monk seals. *Can. J. Zool.* 75:433-438.
- Iwasa, I and *Atkinson, S*. (1996) Analysis of corpora lutea to estimate reproductive cycles of wild Hawaiian monk seals (*Monachus schauinslandi*). *Mar. Mamm. Sci.* 12: 182-198.
- Atkinson, S.*, Becker, B.L., Johanos, T.C., Pietraszek, J.R. and Kuhn, B.C.S. (1994) Reproductive morphology and status of female Hawaiian monk seals (*Monachus schauinslandi*) fatally injured by adult male seals. *J. Reprod. Fert.* 100:225-230.
- Pietraszek JR & *Atkinson, S*. (1994) Concentration of oestrone sulfate and progesterone in plasma and saliva, vaginal cytology and bioelectric impedance during the oestrous cycle of the Hawaiian monk seal (*Monachus schauinslandi*). *Mar. Mamm. Sci* 10:430-441.
- Atkinson, S*, Gilmartin, WG, & Lasley, BL (1993) Testosterone reduction after injection of gonadotropin releasing hormone in male Hawaiian monk seals. *J Reprod Fert* 97:35-38.
- Atkinson, S* and Atkinson, MJ. (1992) Detection of estradiol-17 β during a mass coral spawning. *Coral Reefs.* 11:33-35.
- Atkinson, S* & Gilmartin, WG (1992) Seasonal testosterone pattern in Hawaiian monk seals. *J Reprod Fert* 96: 35-39.
- Atkinson, S* and Atkinson, MJ. (1992) Detection of estradiol-17B during a mass coral spawning. *Coral Reefs.* 11:33-35.

Adams, NR, *Atkinson, S*, Hoskinson, RM, Abordi, JA, Briegel, JR, Jones, M and Sanders, MR (1990) Immunization of ovariectomized ewes against progesterone, oestrogen or cortisol to detect effects of adrenal steroids on reproduction. *J. Reprod. Fert.*89:477-483.

Curriculum Vitae (abbreviated)

NAME: B. L. Middlebrooks

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E-MAIL: bobby.middlebrooks@usm.edu

DATE AND PLACE OF BIRTH: January 12, 1941, Greenville, Texas

SOCIAL SECURITY NUMBER: 456-68-8457

EDUCATION:

B.A. (1962) in Biology from Rice University, Houston, Texas
M.A. (1964) and
Ph.D. (1966) in Microbiology from the University of Texas
Southwestern Medical School, Dallas, Texas
Postdoctoral (1966-68) in Virology at Army Biological Research
Laboratories, Ft. Detrick, Maryland

EXPERIENCE:

1974-present Professor (1982-present), Associate Professor (1977-1982), Assistant Professor (1974-1977), of Microbiology; Administrative positions held: Associate Provost (1998-1999), Assistant Vice President for Academic Affairs (1997-1998), Chair of Biological Sciences (1991-1997), Interim Dean of the Graduate School (1990-1991), University of Southern Mississippi, Hattiesburg, Mississippi
1972-1974 Assistant Professor of Biology, Plymouth State College of the University of New Hampshire, Plymouth, New Hampshire
1968-1972 Assistant Professor of Microbiology, University of Texas Medical Branch, Galveston, Texas

HONORS AND AWARDS:

Recipient of Outstanding Faculty Research Award at the University of Southern Mississippi (1988)

Co-recipient of Mississippi Innovation Advocate Award, presented by the Small Business Administration (1986)

O. B. Williams Award, Texas Branch, American Society for Microbiology (1964)

PUBLICATIONS AND PRESENTATIONS (Representative):

- Patterson, R.A. and B. L. Middlebrooks. 2000. Methods for purification and study of cetacean immunoglobulins, *in* Cell and Molecular Biology of Marine Mammals (in Press)
- Middlebrooks, B. L., J.C, Jones, and R. A. Patterson. 2000. Application of ELISA methodology for detection of *Erysipelothrix rhusiopathiae* antibody titers in cetaceans, *in* Cell and Molecular Biology of Marine Mammals (in Press)
- Jones, J. C., R. A. Patterson, and B. L. Middlebrooks. 1999. The antigenic components of a wild strain of *Erysipelothrix rhusiopathiae* determined by immunostaining of extracted bacterial surface components with serum from *Tursiops truncatus*, *Lagenorhynchus obliquidens*, and *Delphinapterus leucus*. p103 *In* Proceedings of the 29th Annual International Association of Aquatic Animal Medicine Conference
- Osgood, R, R. A. Patterson, and B. L. Middlebrooks . 1999. Application of biochemical, immunochemical, and molecular analysis to comparison of *Erysipelothrix rhusiopathiae* isolates from two species of cetaceans to each other and to strains obtained from the American Type Culture Collection. p103 *In* Proceedings of the 30th Annual International Association of Aquatic Animal Medicine Conference
- Patterson, R.A. and B.L. Middlebrooks. 1998. The jacalin affinity chromatography column proves to be an effective method for purifying IgA from Atlantic bottlenose dolphin (*Tursiops truncatus*), beluga whale (*Delphinapterus leuca*), and Pacific whitesided dolphin (*Lagenorhynchus obliquidens* serum. p103 *In* Proceedings of the 30th Annual International Association of Aquatic Animal Medicine Conference.
- B. L. Middlebrooks, Yeuk-Mui Lee, Min Li, and R. D. Ellender. 1994. Effects of repeated immunization and wound trauma on changes in hemolymph agglutinin levels in brown shrimp (*Penaeus aztecus*). *Ann. N. Y. Acad. Sci.* 712:358-360.
- A.K. Najafabadi, R.D. Ellender, and B.L. Middlebrooks. 1993. Analysis of shrimp hemolymph and ionic modification of a *Penaeus* cell culture formulation. *J. Aquatic Animal Health* 4:143-148.
- R.D. Ellender, Ali Najafabadi, and B.L. Middlebrooks. 1992. Observations on the primary culture of *Penaeus hemocytes*. *J. Crustacean Biology.* 12:178-185.
- Middlebrooks, B.L., P.G. Voss, W.L. Douglas, and P.M. Toom. 1991. Procedure for selecting monoclonal antibodies for use in a ligand displacement assay of serum antibody levels. *J. Immunoassay.* 12:125-144.
- Middlebrooks, B.L., N.J. Brown-Peterson, P.M. Toom, and W.L. Douglas. 1988. Evaluation of specific binding affinity and biochemical properties of fish eye-lens reagents from seven teleost species. *Comp. Biochem. Physiol.* 90B:721-730.

Curriculum Vitae

NAME AND TITLE: Susan D. Inglis, Director of Research and Operations/Rehabilitation

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Seward, AK 99664

Telephone: (907) 224-6345
Fax: (907) 224-6360

EDUCATION

MSc. University of British Columbia, Vancouver, BC 1993

BSc. University of British Columbia, Vancouver, BC 1984

Diploma: Bamfield Marine Station, Bamfield BC 1985

Diploma: Canadian Junior College, Marine Research Station, Carriacou, West Indies July-Sept. 1977

Certificates: Radionuclide Safety and Methodology, University Hospital, Vancouver, BC 1991
GIS-ARC/INFO Method Level 1, BC Institute of Technology, Vancouver BC
1996

ACADEMIC AWARDS:

Federal Industrial Research Assistance Program Award (IRAP)-1990

BC Student Challenge Awards- 1980, 81, 82, 83

PREVIOUS POSITIONS:

Senior Fisheries Biologist, British Columbia Ministry of Environment, Vancouver BC-1994-96

Principal Investigator/Project Manager, SDI Consulting, Vancouver, BC-1987-94

Supervisor/Biochemist, West Van. Fish Nutrition Laboratory, Dept. of Fisheries and Oceans Vancouver,
BC 1986-87

SELECTED PROJECTS:

2000. Co-investigator- Scoter life history and ecology: linking satellite telemetry with traditional ecological knowledge.

1998-2000. Co-investigator- Biological & behavioral impacts of radio-telemetry attachment on seabirds.

1998-2000. Co-investigator- Condition and health indices development for rehabilitated harbor seal pups at the ASLC.

1996. Technician-Nutritional analysis of samples for bioenergetic studies on herring, pollock and pink salmon in Prince William Sound.

1994. Co-investigator-Lower Fraser River white sturgeon population assessment through telemetry tracking and statistical analysis of angler fishery card data.

1994-5. Principal Investigator for 2 year biological and limnological study of Wahleach Reservoir.

1992-93. Principal Investigator for 2 year study of the distribution, behavior, and habitat requirements of two endangered fish species in BC.

1993. Principal Investigator to determine the impact of proposed summer cold water release from Kenney Dam on growth and behavior of fish populations in the upper Nechako River.

SELECTED PUBLICATIONS/REPORTS:

Castellini, J.M., *S.D Inglis*, S. J. Trumble, and M.A.Castellini. 1999. Condition and Health Indices in Rehabilitated Harbor Seal Pups at the Alaska SeaLife Center. *Exxon Valdez* Oil Spill Restoration Project Report, University of Alaska, Fairbanks, Alaska.

Newman, S. H, *S.D. Inglis*, and S. Hatch. 1999. Biological & behavioral impacts of radio-telemetry attachment on seabirds. OWCN Project Report, University of California, Davis.

Castellini, J.M., *S.D Inglis*, S. J. Trumble, and M.A.Castellini. 1999. Condition and Health Indices in Rehabilitated Harbor Seal Pups at the Alaska SeaLife Center. Abstract

submitted-13th Bi-annual Conference on the Biology of Marine Mammals, Maui, Nov. 1999.

Inglis, S.D. 1995. Wahleach Reservoir Fertilization Project - Final Pre-fertilization Assessment. BC Environment, Lands and Parks, Fish and Wildlife Management, Surrey, BC Regional Fisheries Report No.LM250

Rosenau, M., and **S.D.Inglis**, 1994. Analysis of Lower Fraser River Sturgeon Population. BC Environment, Lands and Parks, Fish and Wildlife Management, Surrey, BC Regional Fisheries Report No.LM242.

Inglis, S.D. and S.M. Pollard. 1994. Distribution and habitat of the Nooksack dace (*Rhinichthys* sp.) in Canada. BC Environment, Lands and Parks, Fish and Wildlife Management, Surrey, BC Regional Fisheries Report No. LM237.

Inglis, S.D., Lorenz, A., and M.L. Rosenau. 1993. Distribution and habitat of the endangered Salish sucker (*Catostomus* sp.). BC Environment, Lands and Parks, Fish and Wildlife Management, Surrey, BC Regional Fisheries Report No. LM230.

Inglis, S.D. and R.W. Blake. 1993. Impact of proposed summer cold water release from Kenney Dam on growth and behavior of fish populations in the upper Nechako River. For BC Ministry of Environment, Victoria, BC.

Inglis, S.D. 1993. Growth, carcass composition and plasma growth hormone levels in cyclically fed rainbow trout, (*Oncorhynchus mykiss*). *Master of Science* thesis, University of British Columbia, Vancouver, BC, Canada.

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001						
Personnel		\$0.0						
Travel		\$0.0						
Contractual		\$112.2						
Commodities		\$0.0						
Equipment		\$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal		\$112.2			Estimated	Estimated		
General Administration		\$7.9						
Project Total		\$120.1						
Full-time Equivalent (FTE)		1.3						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
Comments:								

FY01

Project Number: 01558 Revised
 Project Title: Effect of Oil Products on Endocrine and Immune Systems in Harbor Seals
 Agency: Alaska Department of Fish and Game

Prepared:

Budget Category:	Authorized FY 2000	Proposed FY 2001					

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel							
		\$41.9					
Travel		\$1.6					
Contractual		\$45.2					
Commodities		\$1.0					
Equipment		\$0.0					
Subtotal		\$89.7					
Indirect		\$22.5			Estimated 2001	Estimated 2002	
Project Total		\$112.2				\$120.0	
Full-time Equivalents (FTE)		1.3					
Other Resources							

LONG RANGE FUNDING REQUIREMENTS

Dollar amounts are shown in thousands of dollars.

Comments:

The indirect rate is 25% TDC, as negotiated by the *Exxon Valdez* Oil Spill Trustee Council with the University of Alaska.

Student personnel costs include resident tuition of \$3006 per year.

FY01

Project Number: 01558 Revised
 Project Title: Effect of Oil Products on Endocrine and Immune Systems in Harbor Seals
 Name: Shannon Atkinson

Prepared:

Personnel Costs:			Months Budgeted	Monthly Costs	Overtime
Name	Position Description				
Shannon Atkinson	PI/Professor			10.5	
TBA	Research Associate		3.5	5.5	

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

TBA	M.S. Student		12.0	1.7	
Subtotal			15.5	17.7	0.0
Personnel Total					
Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem
Description					
R/T Mississippi to Seward, AK		1.0	1	7	
Travel Total					

FY01

Project Number: 01558 Revised
 Project Title: Effect of Oil Products on Endocrine and Immune Systems in Harbor Seals
 Name: Shannon Atkinson

Prepared:

Contractual Costs:	
Description	
Hormone analysis (195 Samples x4 Hormones @\$13/sample)	
Blood chemistry and proximate analyses (ALSC)	
Contaminant Analysis	
Dr. Middlebrooks Subcontract	3 of 5

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

		Contractual Total
Commodities Costs:		
Description		
Blood collecting supplies and reagents		
		Commodities Total

FY01

Prepared:

Project Number: 01558 Revised
 Project Title: Effect of Oil Products on Endocrine and Immune Systems in Harbor Seals
 Name: Shannon Atkinson

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

New Equipment Purchases:		Number of Units	Unit Price	
Description				
Those purchases associated with replacement equipment should be indicated by placement of an R.			New Equipment Total	
Existing Equipment Usage:		Number of Units		
Description				

FY01

Project Number: 01558 Revised
 Project Title: Effect of Oil Products on Endocrine and Immune Systems in Harbor Seals
 Name: Shannon Atkinson

Prepared: