Harbor Seal Recovery: Application of New Technologies for Monitoring Health

Project Number: 02558

Restoration Category: Research

Proposer: Shannon Atkinson, Ph.D., University of Alaska Fairbanks, School of Fisheries and Ocean Science, Institute of Marine Science

Lead Trustee Agency: ADFG

Alaska SeaLife Center: YES

Duration: 2nd of a 3-year project

Cost FY 02: $124,751

Cost FY 03: closeout approx. $80,000

Geographic Area: Alaska SeaLife Center, Gulf of Alaska

Injured Resource/Service: Harbor seals

ABSTRACT

This study continues investigating the potential for new technologies to assess and monitor endocrine and immune systems as diagnostic measures of harbor seal health. 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 assesses both permanently captive seals as well as seals that are brought into the Alaska SeaLife Center (ASLC) for rehabilitation. 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 Vodicnik, 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 of 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 it’s 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 (2001) 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.

During the first year of the funding, we have been developing the techniques to quantify harbor seal immunoglobulins. The cell lines for this technique are being developed through the production of monoclonal antibodies at the University of Southern Mississippi. Hormonal assays for the thyroid hormones and cortisol have been validated in my laboratory at ASLC. Blood samples for the circadian pattern of thyroid hormones and cortisol have been collected and the assays completed. The data are currently being statistically analyzed. The permit modification to collect blubber samples from the permanently captive seals and ASLC has been submitted and reviewed. We anticipate collecting the first blubber samples this spring.

**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-incompetence 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 to closeout the project, including the publication of results that

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have been obtained in years 1 and 2, as well as the analysis of a few 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 the suite of measurements as overall indicators of health in free-ranging seals (Yr 3).

B. Methods
**Objective 1.** Seven harbor seals (3 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 seven 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. Blood and blubber samples will be collected quarterly over the two years for organochlorine analysis.

The analyses for these hormones have previously been validated for other pinniped species (Atkinson and Oki, 2001) and recently for harbor seals. Concentrations of cortisol will be measured in unextracted plasma using a single-antibody radioimmunoassay (Atkinson and Oki, 2001; 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, 2001). 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 statistically 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 monoclonally 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. Mice 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 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® affinity chromatography to obtain the IgG fraction of this monoclonal 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 quantification 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 are being collected on a monthly basis from the permanently captive seals at 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 formula that are fed to very young seals as well as upon weaning when the diet and digestive efficiency of the animals is maturing.

Seals admitted for rehabilitation at the SeaLife Center are held in quarantine and placed in individual holding tanks. Currently, health data such as blood chemistry and morphometrics are collected every 10 days 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 sub-samples 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 trial 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. While the primary goal of Year 3 will be to publish the data collected it Years 1 and 2, it is hoped that Year 3 can include a small number of samples collected 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 in Dr. Bobby Middlebrooks’ laboratory at the 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. The analysis of these samples has been discussed 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 late summer or fall. Most of the analyses for samples collected in early 2001 will be accomplished by Sept 2001. Samples collected in 2002 will be scheduled to complete during FY 02.

**B. Project Milestones and Endpoints for Year 2**

1. Establishment of baseline levels of total and free T₃, T₄ and cortisol levels in the serum: Analysis of the circadian hormone concentrations from captive animals will be completed during Year 2, with a comparison of winter and summer seasons. Monthly blood samples from two years will enable us to assess the variation in values from the samples collected from healthy animals in a stable environment. The rehabilitation seals from two years will also have samples collected enabling an analysis of seals that are failing to thrive in the natural environment.

2. Development of species-specific antisera against immunoglobulins of the harbor seal: An important outcome of Year 1 is the production of antisera against immunoglobulin isotypes of the harbor seal. These antisera will be available for quantifying
immunoglobulins in Year 2. The immunoglobulins will be analyzed for seasonal variation, allowing the question of the variability in immune status throughout the year to be addressed for permanently captive seals in a stable environment.

3. The quantification of organochlorines in captive and rehabilitated harbor seals will provide a baseline as to what kinds of body burdens we can expect. A comparison between blood and blubber concentrations will allow the assessment of the best type of sample to be collected from field studies. This will be done in Year 2.

C. Completion Date

The anticipated completion date of the captive portion of this project is October 2003. At this point we will hope to be able to recommend that some form of these techniques be applied to a field monitoring program. If this is accomplished the feasibility of field sampling could be determined by October 2004.

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

The PI will request travel for the graduate student, Ms. Danielle Goodrode to attend the Biennial Conference of the Biology of Marine Mammals in Vancouver, Canada in FY02. This conference attracts international researchers, some of whom specialize in harbor seals. It is anticipated that Ms. Goodrode will have enough data to submit and abstract of our work in time for the call for abstracts.

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 the data obtained from FY02 will compliment the data obtained from previous EVOS funded projects. It is also anticipated that if any field samples are scheduled for Year 3, the 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)**

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The PI of this project has been a professor at UAF for 16 months, 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 vita 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 years 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 vita 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 vita 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 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.

**LITERATURE CITED**


Curriculum Vitae (abbreviated)

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

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           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 1986-1988

Recent Research Projects:
- Reproduction and development of rough-toothed and bottlenose dolphins 1998-2001, NOAA/Sea Grant
- Metabolism and reproduction in Hawaiian monk seals, 1998-2001, Pacific Marine Life Foundation
- Distribution and abundance of odontocetes around Oahu, Hawaii 1999-2001, NOAA/private foundation

Selected Relevant Publications:


Curriculum Vitae (abbreviated)

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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):

Prepared 6/13/2005

18

Project 02558


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*


Curriculum Vitae

NAME AND TITLE: Susan D. Inglis, Director of Research and Operations/Rehabilitation
ADDRESS:       Alaska SeaLife Center
            301 Railway Ave., P.O. Box 1329,
            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 POSTITIONS:
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:
1998-2000. Co-investigator- Condition and health indices development for rehabilitated harbor seal pups at the ASLC.
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:

Prepared 6/13/2005  Project 02558


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<td>Equipment</td>
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Dollar amounts are shown in thousands of dollars.

Comments:

Project Number: 02558

Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health

Agency: NOAA

Prepared:
## 2002 Exxon Valdez Trustee Council Project Budget

### October 1, 2001 - September 30, 2002

<table>
<thead>
<tr>
<th>Budget Category</th>
<th>Authorized FY 2001</th>
<th>Proposed FY 2002</th>
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<td>Indirect</td>
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<td>Project Total</td>
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<td>Full-time Equivalents (FTE)</td>
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### Long Range Funding Requirements

- **Subtotal**: $96.0
- **Indirect**: $24.0
- **Project Total**: $120.0

**Estimated FY 2003**: $80.0

**Full-time Equivalents (FTE)**: 1.4

Other Resources

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 tuition is included in the Wages - $3,205.

---

**FY02**

Project Number: 02558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Name: Shannon Atkinson
### Personnel Costs:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position Description</th>
<th>Months Budgeted</th>
<th>Monthly Costs</th>
<th>Overtime</th>
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<td>Atkinson, S.</td>
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<td>TBA</td>
<td>Research Associate</td>
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<td>TBA</td>
<td>M.S. Student</td>
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### Travel Costs:

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<thead>
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<th>Description</th>
<th>Ticket Price</th>
<th>Round Trips</th>
<th>Total Days</th>
<th>Daily Per Diem</th>
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<tbody>
<tr>
<td>Seward to Vancouver</td>
<td>1000.0</td>
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<td>6</td>
<td>633.0</td>
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<tr>
<td>Seward to Anchorage (car mileage)</td>
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</table>

### Total

Personnel Total: 19.2
Travel Total: 754.0

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**FY02**

- **Project Number:** 02558
- **Project Title:** Harbor Seal Recovery: Application of New Technologies for Monitoring Health
- **Name:** Shannon Atkinson

Prepared:
<table>
<thead>
<tr>
<th>Description</th>
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<td>Hormone analyses (195 samples x 4 hormones @ $13/sample)</td>
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<td>Blood Chemistry and Proximate Analyses (ASLC)</td>
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<td>Dr. Middlebrooks Subcontract</td>
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<td>Contaminant Analysis</td>
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<tr>
<td>Commodity Costs:</td>
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<tr>
<td>Description</td>
<td>Commodities Total</td>
</tr>
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<td>Blood Collecting supplies and Regants</td>
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</table>

**FY02**

<table>
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<tbody>
<tr>
<td>Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health</td>
</tr>
<tr>
<td>Name: Shannon Atkinson</td>
</tr>
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</table>
# New Equipment Purchases:

<table>
<thead>
<tr>
<th>Description</th>
<th>Number of Units</th>
<th>Unit Price</th>
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</thead>
</table>

Those purchases associated with replacement equipment should be indicated by placement of an R.

**New Equipment Total**

---

# Existing Equipment Usage:

<table>
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<tr>
<th>Description</th>
<th>Number of Units</th>
</tr>
</thead>
</table>

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**Project Number:** 02558  
**Project Title:** Harbor Seal Recovery: Application of New Technologies for Monitoring Health  
**Name:** Shannon Atkinson  

**Prepared:**