Harbor Seal Recovery: Application of New Technologies for Monitoring Health

Project Number:	030558
Restoration Category:	Research
Proposer:	Shannon Atkinson, Ph.D., University of Alaska Fairbanks, School of Fisheries and Ocean Sciences, Institute of Marine Science
Lead Trustee Agency:	ADFG
Alaska SeaLife Center:	YES
Duration:	3rd of a 3-year project
Cost FY 01:	$120,128
Cost FY 02:	$128,400
Cost FY 03:	$119,100 + ASLC bench fees of $167,600 (project total $286,700)
Geographic Area:	Alaska SeaLife Center, Gulf of Alaska
Injured Resource/Service:	Harbor seals

ABSTRACT

This study is a continuation of the study to assess the potential for new technologies to monitor the endocrine and immune systems for the health of harbor seals. During year one, baseline samples were collected from both permanently captive and rehabilitation seals at the Alaska SeaLife Center (ASLC). Analysis of thyroxine (T_4), triiodothyronine (T_3), and cortisol (metabolic and gluconeogenic hormones), and measurement of immunoglobulins (IgG, IgM, and IgA) and organochlorine contaminants are currently being assessed. Cell lines to quantify immunoglobulins have been initiated, and baseline hormones have been established. The final year will compare the profiles of free-ranging seals and those failing to thrive in their environment 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 would 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). To assess the baseline patterns of hormone release in harbor seals, Oki and Atkinson (Oki, 2001) measured the circadian patterns of the thyroid hormones and cortisol during winter and summer. Interestingly, the thyroid hormones were elevated in winter; however, the circadian pattern of cortisol was also abandoned in winter. Using these results for baseline pattern in captive harbor seals, we are now assessing a suite of measurements, including these hormones, with the goal of providing 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 enzyme-linked immunosorbent assay ELISAs 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 ELISAs 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. We are in the process of developing antisera that is specific to harbor seals. All of the samples that are being collected for the captive and rehabilitated seals will also be analyzed for immunoglobulins once the assay is developed.

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. During the proposed third year of this study we will assess the hormone and immunoglobulin concentrations in free-ranging harbor seals. This portion of the project will be conducted in collaboration with Dr. Robert Small, Alaska Department of Fish and Game. We will continue to develop the 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. 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 Prince William Sound and 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 complement these investigations as it will utilize new techniques to enhance our understanding of the health and physiology of the species and incorporate the possible effects 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 and free-ranging 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 (i.e., poor milk quality or shortened 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 one and two of this project have been, and are still being, undertaken at the ASLC using harbor seals that are currently resident and permitted for research under the Marine Mammal Protection Act for research. It has also utilized 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 three of this work is proposed to closeout the project, including the publication of results that have been obtained in years one and two, as well as the analysis of free-ranging seals in Prince William Sound and areas near South Central Alaska.

COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE

This project has been closely associated in a growing collaboration with the Alaska Native Harbor Seal Commission (ANHSC). To a large extent the collaboration with ANHSC has increased our awareness of traditional and local knowledge of harbor seals as well as incorporated local expertise into the project. In addition to the native communities, we propose working with Dr. Robert Small, Alaska Department of Fish and Game (ADFG) to obtain samples from free-ranging harbor seals. ADFG has had a successful program working with free-ranging seals, and has offered to collaborate to provide samples from Alaskan waters, including Prince William Sound.

This project will also coordinate with the existing volunteer and intern programs at ASLC to make opportunities available for individuals who would like to spend time volunteering at ASLC. 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 (T_3), thyroxine (T_4), 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 2 and 3).
4. Determine endocrine and immunoglobulin profiles and measure organochlorine concentrations for rehabilitation seals periodically throughout the rehabilitation process (Yrs 2 and 3).
5. Assess the suite of measurements as overall indicators of health in free-ranging seals (Yr 3).

The third year of this project is essential to the success of this project.

B. Methods

Objective 1. This objective has been successfully completed and resulted in a Master's thesis for Ms. Carolyn Oki at the University of Hawaii. Ms. Oki is in the process of drafting a manuscript for publication.

Objective 2. This objective is currently underway and should be completed during year two. The project has a Master's candidate at the University of Southern Mississippi working on it.

Objective 3. This objective is also underway and will be continued into year three. 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. This objective is also underway and will continue throughout year three. A Master's candidate, Ms. Danielle O'Neil is being employed and her project will run through the third year of funding. Using the previously described techniques, we have measured total and free T_3, T_4, and cortisol, in harbor seals that are brought in for rehabilitation at ASLC and The Marine Mammal Center in California. An assessment of the level of contamination by organochlorines is underway in collaboration with the University of Hawaii Department of Environmental Biochemistry on all animals that have not been successful in the rehabilitation program, and is being developed for use in blood samples.

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 ten 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 rates are measured. We are currently analyzing past samples from the Castellini study to synthesize the data from both studies.

Objective 5. Year three has two primary goals. First will be to publish the data collected in years one and two, and second is to include samples collected from free-ranging seals. The sites of collection, numbers of animals, and the permits to cover the sampling of wild seals are being planned in collaboration with Dr. Robert Small, ADFG. Samples will also be collected from native harvests in collaboration with the Alaska Native Harbor Seal Commission.

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 and The Alaska Region of National Marine Fisheries Service. The letter of authorization for these seals is also held by ASLC.

SCHEDULE

A. Measurable Project Tasks for FY 03 (October 1, 2002 – September 30, 2003)

October 2002:	Blood sampling continues on a monthly basis for captive animals. Perform endocrine assays on FY02 samples.
November 2002:	Blood samples from ADFG archive supplied. All samples will be sent for contaminant analysis.
January 2003:	Endocrine assays will be undertaken with batches of samples to assist with quality control.
March–June 2003:	Spring collections from ADFG received.
May–June 2003:	ASLC rehab season samples collected.
June–September:	Endocrine and immunology samples analyzed.
September–October:	Rehabilitation seals released.

Samples collected in 2002 will be scheduled for completion during FY 02.

B. Project Milestones and Endpoints for Year Three

1. Publish baseline levels of T_3, T_4, and cortisol levels in the serum. Publish circadian hormone concentrations from captive animals, comparing winter and summer seasons. Monthly blood samples from all 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 all 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 will be complete and the production of antisera against immunoglobulin isotypes will be available. These antisera will be available for quantifying immunoglobulins in samples collected in year two. 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 and for free-ranging seals.

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. Collaboration with the Alaska Native Harbor Seal Commission's biosampling program will allow samples from free-ranging seals to be collected.

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. The Master's project by Ms. Carolyn Oki is completed and will be prepared for publication in either General and Comparative Endocrinology or Comparative Biochemistry and Physiology. In sum, we anticipate three Master's projects to be produced from this project. Any student projects will be presented in thesis or dissertation format as well as submitted for journal publication.

PROFESSIONAL CONFERENCES

Two presentations of work from this project have already been presented at the Society for Marine Mammalogy's 14th Biennial conference on the Biology of Marine Mammals in Vancouver, BC in November 2001. An additional poster is being presented at the International Association of Aquatic Animal Medicine in May 2002. The PI will request travel to Quebec, Canada to discuss results with harbor seal researchers. The discussion will center on comparisons of Alaskan and Atlantic harbor seal physiology and population dynamics.

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 making an effort to collaborate with harbor seal researchers in Alaska. 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 FY03 will complement the data obtained from previous EVOS funded projects. It is also anticipated that the samples collected in year three will come from a shared field site, integrating existing field projects with our sample collections.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR (qualifications)

The PI of this project has been a professor at the University of Alaska Fairbanks for 2.3 years, with half-time duties as the Science Director at ASLC. She has eighteen 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

Dr. Bobby Middlebrooks is a Professor at the University of Southern Mississippi. He has been an active part of this project for both years one and two; year three will provide the necessary closure for his part of the project. 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 is responsible for performing and analyzing the results from the immunological 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 all seals. The research associate will also work to analyze data and assist in the submission of manuscripts for publication.

The graduate student is responsible for organizing the sample collections and performs 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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Curriculum Vitae (abbreviated)

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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:

- Harbor seal recovery: Application of new technologies for monitoring health 2000–present, EVOS/Pacific Marine Life Foundation
- Assessment of endocrine and immune status in relation to organochlorine burdens in Steller sea lions, 2000–present, NMFS/PCCRC
- Reproduction and development of rough-toothed and bottlenose dolphins 1998–2002, NOAA/Sea Grant

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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, J.R. and ***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, W.G., and Lasley, B.L. 1993. Testosterone reduction after injection of gonadotropin releasing hormone in male Hawaiian monk seals. J. Reprod. Fert. 97:35–38.

Atkinson, S. and Gilmartin, W.G. 1992. Seasonal testosterone pattern in Hawaiian monk seals. J. Reprod. Fert. 96:35–39.

Atkinson, S. and Atkinson, M.J. 1992. Detection of estradiol-17B during a mass coral spawning. Coral Reefs. 11:33-35.

Adams, N.R.; ***Atkinson, S.;*** Hoskinson, R.M.; Abordi, J.A.; Briegel, J.R.; Jones, M.; and Sanders, M.R. 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

BUSINESS ADDRESS: Department of Biological Sciences
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Hattiesburg, Mississippi 39406

TELEPHONE: Office --- (601) 266-4748
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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)
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*. p.103 *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. p.103 *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. p. 103 *In* Proceedings of the 30th Annual International Association of Aquatic Animal Medicine Conference.

Middlebrooks, B. L., 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.

Najafabadi, A.K., 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.

Ellender, R.D., 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.

FY 03 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2002 - September 30, 2003

Budget Category:	Authorized FY 02	Proposed FY 03	LONG RANGE FUNDING REQUIREMENTS					
Personnel		$23.9						
Travel		$2.8						
Contractual		$60.3						
Commodities		$0.5						
Equipment		$0.0						
Subtotal	$0.0	$87.5	Estimated					
Indirect		$21.8	FY 04					
Project Total	$128.4	$109.3						
Full-time Equivalents (FTE)		1.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

PROJECT TOTAL: $109.3 (UAF direct and indirect costs)
9.8 (ADF&G 9% GA on $109.3)
153.8 (ASLC bench fees)
13.8 (ADF&G 9% GA on $153.8)

SUM OF ABOVE $286.7

FY03

PROJECT 030558: HARBOR SEAL RECOVERY--APPLICATION OF NEW TECHNOLOGIES FOR MONITORING HEALTH
PROPOSER: S. ATKINSON, UAF

Prepared: 4/10/02

FY 03 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2002 - September 30, 2003

Personnel Costs:			Months	Monthly		
	Name	Position Description	Budgeted	Costs	Overtime	
	Atkinson	PI	0.5	10.0		
	TBA	Research Associate	2.0	4.5		
	Danielle O'Neil	Master's degree student	9.0	1.1		
		Subtotal	11.5	15.6	0.0	
					Personnel Total	

Travel Costs:		Ticket	Round	Total	Daily	
	Description	Price	Trips	Days	Per Diem	
	Seward-Anchorage (Car mileage)	0.1	1	2	0.1	
	Seward-Quebec	0.7	1	6	0.3	
					Travel Total	

FY03

Prepared: 4/10/02

Project Number: 03558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Name: University of Alaska Fairbanks

FY 03 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2002 - September 30, 2003

Contractual Costs:	
Description	
Hormone analyses (175 samples x 4 hormones @ $13/sample) Dr. Middlebrook (subcontract) University of Hawaii (subcontract for contaminant analysis) ADF&G free-ranging samples Tuition (Danielle O'Neil)	
Contractual Total	
Commodities Costs:	
Description	
Blood collecting supplies and reagents	
Commodities Total	

FY03

Prepared: 4/10/02

Project Number: 03558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Name: University of Alaska Fairbanks

FY 03 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2002 - September 30, 2003

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		

FY03

Prepared: 4/10/02

Project Number: 03558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Name: University of Alaska Fairbanks