PROPOSAL SIGNATURE FORM

THIS FORM MUST BE SIGNED BY THE PROPOSED PRINCIPAL INVESTIGATOR AND SUBMITTED ALONG WITH THE PROPOSAL. If the proposal has more than one investigator, this form must be signed by at least one of the investigators, and that investigator will ensure that Trustee Council requirements are followed. Proposals will not be reviewed until this signed form is received by the Trustee Council Office.

By submission of this proposal, I agree to abide by the Trustee Council's data policy (*Trustee Council Data Policy**, adopted July 9, 2002) and reporting requirements *(Procedures for the Preparation and Distribution of Reports***, adopted July 9, 2002).

PROJECT TITLE: Development of Technology to Support Restoration of Herring in Prince William Sound: Use of *in vitro* studies to validate and optimize restoration actions

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* **www.evostc.state.ak.us/Policies/data.htm**

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FY07 INVITATION
PROPOSAL SUMMARY PAGE

(to be filled in by proposer)

Project Title: Development of Culture Technology to Support Restoration of Herring in Prince William Sound: Use of *in vitro* studies to validate and optimize restoration actions

Project Period: December 1, 2006 – September 30, 2010 (FY07-FY10)

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Study Location: Prince William Sound, Resurrection Bay

Abstract: Intervention in the form of artificial propagation may be needed to restore Prince William Sound (PWS) herring to levels capable of supporting a healthy ecosystem as well as sustainable fisheries. We propose to test and refine propagation methods through laboratory and field studies over a four year period to evaluate the likely benefits and costs of stock restoration. The overall objective is to obtain biological and economic benchmarks of stock enhancement strategies by integrating established techniques for laboratory rearing of herring with state of the art methods used in the culture of multiple marine species. Our specific efforts will focus on the role of calcium sensing receptor proteins in herring osmoregulation, nutrition and immune function. The results will provide PWS stakeholders and other researchers with improved understanding of the optimal husbandry and nursery conditions for herring stock enhancement, and the potential effects of such restoration on PWS herring.

Funding: Funding has been approved for FY 07 only; FY 08-FY 10 will need to be re-submitted for consideration after this fiscal year.

EVOS Funding Requested: FY07 $ 92.7 FY08 $ 372.9 FY09 $ 417.0 FY10 $517.6
TOTAL: $ 1,400.2 (includes 9%GA)

Non-EVOS Funds to be used: FY07 $ 0

TOTAL: 0

Date: December 1, 2006

NEED FOR PROJECT

Statement of Problem

Pacific herring are an important resource in marine ecosystems and support commercial fisheries in the Gulf of Alaska and along the Pacific Ocean continental shelf in North America. The species exhibits wide variation in abundance, and its year class strength is often synchronous among widely separate populations (Williams and Quinn 2000). In Prince William Sound (PWS), Pacific herring abundance increased in synchrony along with other Alaskan herring stocks beginning in the late 1970's before peaking at more than 100,000 tons annually between 1989 and 1993 (Gray et al. 2002). Although biomass estimates differ, the herring population declined significantly thereafter and is presently at levels similar to those observed in the 1970's. Previous studies indicate that the *Exxon Valdez* oil spill impacted recruitment of the 1989 year class, but its effect on the longer term population decline is unclear (Carls et al. 1998, 2001). Rather, other stressors including a large population size, poor over-winter rearing conditions and disease within the herring population from viral hemorrhagic septicemia virus (VHSV) and Ichthiofauna (ICTH) also appear to have been important. These pathogens have reportedly caused massive and recurring mortality in herring (Marty et al. 2004), and combined with marked variation in local food availability (Foy and Norcross 1999; Norcross et al. 2001) and predation (Stokesbury et al. 2002), may be acting as major pressures contributing to persistent low population abundance.

Regardless of the actual cause(s) of the decline, intervention in the form of artificial propagation may be needed to restore PWS herring to levels that effectively support the marine food web and provide for a sustainable commercial fishery. Restoration projects have been proposed to protect herring through their most vulnerable life stages (egg and larval stages) when greatest mortality occurs (EVOS FY 2007 Invitation for Proposals, Pilot Projects for Testing Restoration and Re-Colonization Concepts and Techniques for PWS Herring). However, to be successful, restoration projects must support favorable conditions for nurturing the production of herring in large numbers, as well as demonstrate success in releasing healthy fish back into the environment. Thus, detailed knowledge of optimal husbandry conditions, favorable nursery conditions for rearing and release of juvenile fish, as well as the effects of restoration on life stage development are critical building blocks in the formation of successful herring restoration methods.

Stock restoration, or alternatively stock enhancement or ocean ranching, typically involves the mass releases of juvenile fish that feed and grow on natural prey in the marine environment and subsequently add biomass to the adult population (Salvanes 2001). The success of such projects depends not only on knowledge of the basic biology of the species being cultured, but also the environmental factors, wild conspecifics and other species that interact with the released fish. Extensive programs involving salmonid species have been among the most successful, notably those in western North America and Japan. Stock enhancement of marine fish is less developed, but occurs on a commercial scale in Japan for red sea bream and flounder (Coleman et al. 1998), and in the U.S. (Gulf of Mexico) for red drum (Serafy et al. 1999). These programs have been successful in stabilizing catches of these species and efforts to improve hatchery reared contributions to the harvests are continuing. In contrast, a comprehensive study on cod enhancement in Norway involving large-scale experimental releases of age-0 juvenile fish during the 1980's found no evidence of recruitment to commercial and recreational fisheries and the program was never scaled up to commercial level (Svasand et al. 2000). However, considerable

information obtained during the study revealed that complex physical and biological factors driving the ecosystem dynamics of cod stocks in near shore environment were critical for cod productivity. In particular, non-local wind driven advection of *Calanus* zooplankton to or from the fjord environment had a direct effect on the carrying capacity of fish such as cod at higher trophic levels. Japanese efforts for herring stock supplementation have also been reported (Suzuki 2002; Ishizaki et al. 2002), and although the program appears limited in scale (i.e. annual juvenile releases ~ 500,000), the contribution of enhanced fish to commercial landings has been substantial (1993-1998 mean ~ 17%).

Taken together, studies of herring and other marine fish suggest that stock restoration of PWS herring may be feasible through artificial propagation, although its success will ultimately depend on identifying the biological characteristics of the population to optimize the culture method(s), as well as the ecological features in PWS that maximize growth and survival of released fish. Fortunately, much of the latter has been described through the detailed studies of the SEA Program and these findings have and will be used to guide our studies for developing an effective restoration program. We propose to develop specific stock enhancement techniques for herring in PWS utilizing methods developed from significant experience with large-scale production of marine fish as well as salmonid stock enhancement in Alaska.

There are three issues central to our project. First, realistic estimates of the costs for hatchery-based production of herring must be obtained in order to determine the potential feasibility of large scale stock enhancement in PWS. The estimated spawning biomass for the PWS herring in 2006 was 17,550 tons, 4,450 tons below the 22,000 ton threshold needed before harvest. Suzuki (2002) provided approximate estimates of 40% survival from hatched larvae to released juveniles and 0.5 – 8.5% return to the fishery. Using the mid-point of the return (~4%), and average adult weight at spawning of 125 gm (Carls et al. 1998), it would be necessary to release nearly 2 billion juvenile herring to produce the 32 million adults that would comprise the 4,450 ton deficit. The best case harvest value for these fish from the PWS sac roe fishery, which has traditionally represented a major part of the PWS commercial herring harvest, can be estimated by applying the ex-vessel value of $1,260 per ton paid in 1979 (Gray et al. 2002), which yields $5.6 million, or $0.17 per adult fish (i.e. $1,260 / 7,300 fish per ton). At the 1997 ex-vessel price of $200 per ton, the value of this production falls to less than $0.9 million at about $0.03 per fish. Although these calculations are not actual production costs, they do provide an estimate of the break even costs of production for stock enhancement. Since large-scale enhancement efforts must be based on sound economic as well as biological considerations, data from this project will provide benchmarks for future planning of any herring stock enhancement activities in PWS.

Second, our initial efforts will also focus on identifying culture techniques that maximize the growth and energy reserve of juvenile herring prior to their release in PWS. Recruitment to the adult population appears to be limited by the whole body energy content (WBEC) of age-0 and age-1 juvenile herring in the fall since their feeding either stops or is greatly diminished during winter such that fish with reduced fall energy stores are unable to survive. This is particularly true for age-0 fish, which metamorphose after the spring plankton bloom in July and have only short growing season to prepare for winter (Paul and Paul 1998; Foy and Paul 1999; Norcross et al. 2001). Prey quantity and quality (energy density) play a critical role in determining WBEC in the fall, and these parameters can vary within and among nursery bays (Norcross et al. 2001).

Hence, our initial efforts will focus on identifying culture techniques that maximize the growth and energy reserve of juvenile fish prior to release in the wild.

Third, we will also attempt to identify culture techniques that reduce stress and boost innate immune function in juvenile herring. This component of the project is based on observations that when herring are held in confinement, such as in roe-on-kelp pounds, they experience increased prevalence and tissue titer concentrations of VHSV compared to free-ranging fish (Hershberger et al. 1999). VHSV prevalence is even higher in pounds containing young fish, and since it is evidently endemic among herring populations in the Pacific Northwest (Meyers et al. 1994), stock restoration programs involving confinement at of juvenile fish at high densities must also include the development of methods to minimize VHSV related disease. A subsequent component of our project will be directed at identifying culture techniques that reduce stress and boost innate immune function in juvenile herring.

During this four year project, we will investigate existing herring propagation methods, as well test and develop new methods through laboratory and field studies to aid in restoration of Pacific herring in PWS. Our basic approach will be to first utilize and adopt established techniques for the laboratory rearing of larval and juvenile herring, then refine and expand these techniques using our knowledge of stock enhancement programs (i.e. combination of tank and cage culture) that have been developed for herring in Japan and are currently being developed for multiple species of marine fish. We will make use of prior research by MariCal showing that highly conserved proteins called calcium sensing receptors (CaRs) are salinity sensors in fish (Nearing et al. 2002, Nearing et al. *in press*). CaRs were first identified in mammals as the principal regulators of extracellular Ca^{2+} homeostasis and later as proteins that also coordinate sensing of specific cations and L-amino acids in various osmoregulatory, sensory and nutrient absorbing tissues (Yamaguchi et al. 2000). MariCal's research has greatly expanded understanding of how CaRs modulate osmoregulation, nutrition and immunity in fish, and resulted in successful development the culture techniques that allow anadromous (U.S. Patents 6,463,883; 6,564,747; 6,655,318; 6,475,792; 6,481,379; 6,637,371; 6,748,900) and marine fish (U.S. Patents 6,463,882; 6,854,422) to be reared under salinity conditions that are often unique and outside the scope of tolerance for fish cultured using standard methods. The technology is based on modulation of CaR function through the diet and mineral supplementation of the rearing water using natural compounds. This method potentiates physiological and endocrine functions involved in osmoregulation, findings that have been supported in related research by Radman et al. (2002) and Loretz et al. (2004) in other teleosts.

We hypothesize that CaRs also function as important regulatory proteins to allow herring to adapt in varying degree to a wide range of salinity conditions. Moreover, because CaRs have been shown to be activated by small peptides produced by immune cells like macrophages, as well as the Ca^{2+} ion itself (Brown et al. 1991; Ferriere et al. 1997), CaRs may serve as immune "messengers" by sensing changes in environmental salinity and activating signaling pathways that affect immune system function. We will investigate these possible roles for CaRs in herring and also attempt to utilize CaRs as molecular targets to improve disease resistance in fish that may be exposed to pathogens when cultured and released into the natural environment.

Relevance to 1994 Restoration Plan Goals and Scientific Priorities

Previous work documenting the decline of Pacific herring in PWS and the role of forage limitation, predation and disease that are limiting recovery emphasize the importance of

developing effective methods to rebuild the population and restore the ecological and commercial activities that depend on it. The Trustee Council has classified herring as "non-recovered" and is committed to developing a long-term Herring Restoration Plan and implementing enhancement activities with the ultimate goal of assisting herring recovery. These activities include various forms of intervention ranging from protecting herring spawn to physically relocating eggs and larval fish to favorable nursery areas. This project specifically addresses Project Category 8 (Intervention) listed in Appendix A of the EVOS Trustee Council FY 2007 Invitation for Proposals. Data from the project will provide both technical and economic benchmarks for any future large-scale herring enhancement efforts.

We anticipate that our collaboration with Japanese researchers will provide valuable information regarding the current state of technology on Pacific herring stock restoration methods and allow the transfer and rapid adoption of this technology for herring restoration efforts in PWS. Additionally, we predict that detailed studies of the ionic and nutritional requirements that influence early life history growth and development of herring will provide PWS stakeholders with improved understanding of the likely near-term performance of proposed stock restoration strategies. This expectation is derived from previous work that has resulted in the development of ionic and nutrient-based culture methods to enhance early seawater growth and survival of Chinook, coho and sockeye salmon in Alaska.

For herring, we expect our studies will show how water chemistry conditions and nutrition during the embryonic and larval stages interact to shape subsequent salinity tolerance and G.I. tract development in juvenile (metamorphosed) fish. Once these conditions are established, we intend to optimize culture methods to increase assimilation efficiency, WBEC, and over-winter survival of age-0 herring released into the wild. Based on previous work with other marine fish, we anticipate that embryonic and larval herring cultured under conditions that increase CaR sensing and expression will show improved salinity tolerance and G.I. tract differentiation compared to fish reared under hatchery conditions that attenuate CaR function.

Previous work has shown that confinement of herring under conditions that increase stress results in increased prevalence of VHSV and ICTH (Hershberger et al. 1999), suggesting that introduction of young herring into nursery areas to exploit natural prey or optimal environmental conditions might be problematic if the action leads to stress related immunosuppression that causes increased susceptibility to pathogens such as VHSV and ICTH (Hershberger et al. 1999, Tierney et al. 2004). However, recent developments in the use of probiotics in the diets of larval and juvenile marine fish have shown significant promise to control pathogens in cultured fish (Vine et al. 2006). As part of our effort, we will assess the utility of using commercially available dietary probiotics to potentially enhance the overall immune function of cultured herring and thereby increase the likelihood for releasing healthy fish into the wild.

This project will help determine the interplay between natural forces and intervention efforts in the proposed experimental nursery bay. Comparative assessments of the relative fitness of hatchery reared and wild fish in marine stock enhancement programs have shown an increased vulnerability of hatchery fish after release, highlighting the need to acclimate or condition these fish to specific environmental or predatory cues before release (Serafy et al. 1999). Specifically, in conjunction with mass marking of enhanced fish, the effects of factors such as the time and size of juvenile releases on predator and prey interactions can be measured to improve the

likelihood that intervention strategies will be successful across broad areas of PWS. Integration of detailed knowledge of local environmental conditions with the performance of enhanced fish under such conditions, and the effects of this enhancement on ecosystem dynamics may help guide recovery efforts for other species.

Finally, this project addresses the central need to restore commercial harvest opportunities for herring. During 1978-1993, the *mean* ex-vessel value of all herring fisheries in PWS exceeded $6 million. In contrast, the *cumulative* harvest value for the last 12 years has been $3 million. An investment in infrastructure needed to restore herring fisheries is clearly needed and likely will be significant. As an example, the total capital investment in the PWS pink salmon enhancement program, developed to restore and provide for a sustainable fishery, was approximately $30 million (Farrington 2004). This program has returned hundreds of millions of dollars in ex-vessel harvest value since its inception in the early 1970's, and continues to generate millions of dollars in harvest value annually. Identifying methods that will be as technically successful and cost effective as the PWS salmon enhancement program will be an essential part of this project.

PROJECT DESIGN

Objectives

This project is composed of four inter-related objectives that combine detailed knowledge of aquatic CaRs with established techniques for intensive culture of marine fish and large-scale fish stock enhancement. MariCal's research and development experience with aquatic CaRs will be augmented by the expertise of the Japanese researchers investigating stock restoration methods for herring, as well the VA Tech Aquaculture Consulting Group in the areas of larval marine fish culture and marine fish stock enhancement. The project will use the resources of the Alaska SeaLife Center and leverage salmon enhancement research activities that have been underway there during the last three years.

Using these resources in a stepwise approach over four years, we propose to identify key economic benchmarks and biological requirements for the embryonic, larval and juvenile culture herring that will allow for the mass production and release as age-0 fish into the marine environment and their recruitment to the adult herring population. Our overall hypothesis is that ionic and nutrient culture conditions that temporally advance the development of early life history stages, independent of temperature, will have a highest likelihood of increasing adult recruitment because of the short growing season that age-0 juvenile herring experience after metamorphosis. More specifically, we hypothesize many of the developmental pathways that are activated during the early life history stages in herring are regulated, in part, by CaR sensing and signaling, and that CaR targeted modulation of these pathways during the larval and juvenile culture of herring will result in improved growth and survival of fish released in PWS. These hypotheses are supported by our previous research (Nearing et al. 2006 *in press*) showing that CaRs generate critical signals responsible for salmonid growth and survival as they transit between freshwater and seawater. Utilizing our fundamental knowledge of CaR biology, we have developed methods that advance parr-smolt transformation in sub-yearling Chinook salmon at the ASLC by more than one month as compared to control fish. This advancement provides a significant advantage with respect to the time these fish can forage during their first summer in the ocean. Based on our results with salmon, we anticipate that we will be able to develop CaR-based culture methods that will provide similar advantages for herring stock enhancement.

Objective 1: *Provide biological and economic benchmarks to develop large-scale stock enhancement of herring in PWS.*

Any consideration of the use of artificial propagation for herring stock enhancement in PWS will require a set of specific parameters that will dictate the fundamental economic aspects of the overall effort. Although production criteria for laboratory (Kocan 1996) and pilot-scale hatchery rearing of herring (Suzuki 2002) are available, these and similar rearing projects do not provide the economies of scale found in large enhancement programs or improvements in larval rearing of marine fish that have been widely instituted within the last 2-3 years. A key deliverable for Objective 1 is to identify technical and economic bottlenecks using state of the art culture methods to test specific aspects of larval marine fish production.

As suggested in the **Statement of Problem,** a minimum of 4,450 tons of additional spawning biomass was needed to meet the 22,000 ton threshold for a commercial fishery in PWS in 2006. Based on production data from Suzuki (2002), this suggests that approximately 2 billion juvenile herring would have to be reared and released to obtain this additional biomass. However, to enable stakeholders to make informed decisions about the costs and benefits of truly large scale production, critical culture requirements must be identified and quantified. Based on previous experience with other marine fish species (Schwartz 2004; Craig et al. 2005), there are at least three critical production stages that must be quantified:

- The variation in the quality of eggs used for hatchery production: Eggs obtained from wild spawning marine fish vary widely in their quality. The use of eggs with marginal yolk reserves not only results in biological complications in production, but greatly adds to operating expense since low quality eggs usually produce low yields of larvae and juveniles.
- The quantitative "cascade" of live algae, rotifers, un-enriched and enriched *Artemia* and *Artemia napulii*, as well as expensive weaning diets needed for larval production: For selected species of fish, specific parts of the nutritional cascade can be modified or eliminated to provide significant savings. The same concept applies to the feed composition for juvenile fish.
- The extent to which larval and juvenile fish must be graded: Larval rearing studies in marine fish reveal a high degree of size variation during live feeding and again after weaning to dry diets. Not only does large variation in larval size complicate production management, it also results in cannibalism that dramatically reduces juvenile fish yield. On a small scale, hand grading is manageable; this problem becomes more acute as larval production is increased.

In the first year of the project we will gather information and conduct an assessment of herring culture practices in Japan to provide baseline data for possible implementation of large-scale stock restoration in PWS. This effort will result in development of a 'best practices" report that will be subsequently supplemented with information derived from additional detailed laboratory studies. The studies described above will be conducted during Years 1 and 2 of the project and will test and refine specific aspects of the standard larval rearing for marine fish to optimize these for herring. These protocols will include measures and techniques to evaluate egg quality, methods as well as time and labor to provide the quantitative cascade of live feed for a given level of larval production, and an assessment of the utility and means for grading larval herring.

At the completion of Year 2 studies, we expect to have an optimized herring culture protocol with economic parameters for estimating costs over a range of larval production scales. These

parameters will be integrated with data obtained from Objectives 2-4 and adapted to existing methods from commercial scale marine fish hatcheries to enable efficient mass production of larval and juvenile herring.

Objective 2: *Determine the effects of salinity on the survival, growth and time to metamorphosis in larval fish.*

Salinity exerts a profound effect on several life history events in herring, notably spawning, fertilization, hatching and early development (Alderdice and Hourston 1985). In general, fertilization and hatching success are highest at intermediate (15-20‰) salinities (Dushkina 1973; Tytler and Blaxter 1988; Alderdice and Hourston 1985; Griffin et al. 1998). Salinity effects on early development may relate, in part, to the fact that like most marine fish, herring possess a relatively undifferentiated tube-like gut (Tytler and Blaxter 1988). Prior to the appearance of gills, osmoregulation in larval herring occurs primarily across the skin through chloride cells (Jones et al. 1966) and by drinking, which varies directly with external salinity (Tytler and Blaxter 1988). Although intestinal calcium uptake in relation to salinity has not been measured in herring, it has been measured in larval sea bream (Guerreiro et al. 2003). The authors reported that both whole body and intestinal Ca^{2+} uptake decreases 50-75% when salinity falls in the range of 10-17‰, and that Ca^{2+} additions of ~5-7 mMol at these salinities restored normal intestinal calcium uptake (Guerreiro et al. 2003). These findings suggest that activation of CaR proteins in the G.I. tract of larval euryhaline fish may be reduced at lower salinities, which could influence somatic growth and differentiation of the gut during early development.

This hypothesis is supported by our studies (Nearing et al. 2006 *in press*) showing that CaRs are highly expressed in the G.I. tract of larval fish, and in specific regions of the brain controlling gustatory and visceral activity. In addition to sensing and osmotic handling of mono- and divalent cations, CaRs also sense L-amino acids and biogenic amines, such as polyamines that potentiate CaR cationic sensing (Conigrave et al. 2000; Quinn et al. 1997). Taken together, these data suggest that CaRs integrate cellular processes such as digestion, utilization of nutrients and growth early in life. Polyamines (e.g. putrescine, spermidine and spermine) are naturally occurring derivatives of arginine metabolism and have an essential role in somatic growth and differentiation in the gut (Löser et al. 1999). They are present in the eggs of teleost fish (Srivastava 1992) where they are believed to have a role in directing cell division and differentiation during embryonic development. Moreover, recent studies have shown that possible key nutrients supplied by microalgae supplementation during fish larval culture may actually be polyamines. Hence, augmenting CaR expression in the gut of larval herring in response to higher concentrations of divalent ions (Ca^{2+}, Mg^{2+}) in the early rearing water may contribute to their differentiation and improve assimilation efficiency. To potentially augment growth and survival during larval and juvenile growth, we propose to test the effects of mineral (Ca^{2+}, Mg^{2+}) enrichment in the water supply after weaning onto dry feed.

Objective 3: *Determine the interaction between salinity conditions and weaning diet during larval rearing on the subsequent growth and over-winter survival of juvenile fish.*

Salinity, temperature and forage conditions differ within and among nursery bays in space and time in PWS, and these differences contribute to the variation in survival of juvenile herring (Stokesbury et al. 2000, Norcross et al. 2001). Water temperature exerts a positive effect on survival by promoting growth when it is warmer during summer, whereas warmer winter

temperature may reduce survival because of increased metabolic demand (Norcross et al. 2001 citing Blaxter and Holiday 1963). Forage quality and quantity also influence age-0 juvenile survival through WBEC in autumn (Foy and Norcross 1999; Norcross et al. 2001). Large calanoid copepods and euphausids provide seasonally limited energy rich forage for juvenile fish, but the overall biomass of zooplankton that is consumed appears more important than composition when high energy species are absent. Regardless of mechanism, conditions that reduce summer growth and increase winter metabolic demand reduce over-winter survival of age-0 fish. Foy and Paul (1999) suggested that size-dependent mortality of age-0 herring may result from osmoregulatory dysfunction during winter as water replaces lipid stores used during months of reduced feeding (Foy and Paul 1999). If correct, fasting age-0 herring that have not reached a sufficient size and WBEC by late fall should show increasingly impaired hypo-osmotic function through the winter.

We hypothesize that rapid transition through the larval stage and early weaning to commercial diets in enhanced fish will result in increased growth, WBEC and subsequent over-winter survival through improved osmoregulatory performance. Live diets are critical for successful first feeding in marine species, including herring (Kocan et al. 1997), but are costly to produce and not practical beyond first feeding stages for mass production and release of juvenile fish. Alternatively, we propose that specific interactions between weaning diets and salinity conditions during larval rearing will result in greater growth, higher WBEC in fall and improved hypo-osmotic capability of fasting fish during winter.

In addition to the potential to enhance immune function in fish, probiotics have also been shown to have positive effects on larval growth (Gatesoupe 1999). MariCal studies have demonstrated positive synergy between its CaR-based regime to improve early seawater performance in salmonids and probiotics added to the feed. We hypothesize that herring may benefit from similar culture interactions.

Objective 4: *Determine the comparative performance of hatchery based rearing methods on growth, immune system function and survival during short-term cage culture in the natural marine environment.*

Although experimental releases of Pacific herring in Japan (Suzuki 2002; Ishizaki 2002) show potential for larger-scale stock enhancement efforts in PWS, current applications for herring culture in North America are largely confined to laboratory studies for disease (Kocan et al. 1997, 1999; Tierney et al. 2004) and ecology (Paul and Paul 1998) related research. To be economically viable, mass production and release of juvenile herring will require the development large-scale cage culture practices such as those used in Alaska and Japan for ocean ranching of salmon. Moreover, due to the prevalence of pathogens such as VHSV in marine waters and in reservoir species such as herring (Meyers et al. 1994), intensive culture of herring even for laboratory study is dependent on pathogen free water and feed to prevent disease expression (Kocan et al. 1997). This suggests that mass production of juvenile herring using extensive methods such as cage culture in marine waters may be constrained in the absence of an effective vaccine. Viral fish vaccines, however, are costly to develop and have a history of variable efficacy (Garver et al. 2005). As described above, probiotics and related immuno-stimulants may offer an alternative for short-term (< 2 months) cage culture. Diets containing microbial cell derivatives including oligonucleotides and beta-glucans are commercially available and have been shown to convey various non-specific immune system responses (Vine

et al. 2006; Li et al. 2004). We propose to test the utility of cage culture and commercially available diets to determine if healthy age-0 juvenile herring can be produced and released in large number into marine waters. The results from this pilot-scale study, scheduled for Year 4 of our project, will be used to assess the feasibility of stock enhancement as a restoration tool for Pacific herring in PWS.

Procedural and Scientific Methods

We propose to establish our collaboration with Japanese herring researchers through our academic and business relationships that we (MariCal) have developed for the use of our calcium sensing receptor technology for aquaculture in Japan. Specifically, we will work closely with Prof. Naoyoshi Suzuki, President of Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, and Dr. Shinji Harakawa, Chief Researcher at the Hakuju Institute for Health Science in Tokyo to develop contacts, arrange for research and production site tours, and obtain "hands on" herring culture experience at fisheries research facilities such as the National Center for Stock Enhancement and the Hokkaido Fisheries Experimental Station. Our travel and visits will be timed to coincide with spawning, larval production, and the transfer of juvenile fish to sea cages for intermediate culture. Culture methods will be documented and compiled into a "best practices" paper for use in Alaska. These practices will be used to help guide development of the pilot-scale facilities and studies we have proposed to conduct at the Seward Shellfish Lab. We anticipate at least one visit for the PI for 4 weeks duration.

The laboratory studies in this project will utilize the existing infrastructure located at the Alaska SeaLife Center (ASLC) and the Seward Shellfish Hatchery (SSH). Both facilities are fully equipped with temperature controlled flowing seawater and freshwater to obtain and maintain the environmental conditions described below. Filtered influent seawater used for incubation and rearing at the ASLC and SSH will be disinfected with UV light. The existing infrastructure at SSH will also be utilized to grow live feeds including algae, rotifers and *artemia* used for larval diets. Following initial rearing and weaning onto dry food, juvenile herring will be transferred to ASLC for further grow out and winter fasting studies. Objective 1 will be the focus in Year 1 of the project. Objectives 2 and 3 will be addressed in Years 2, 3 and 4.

We will follow general methods for collection and spawning of adult herring, egg fertilization and incubation (Kocan et al. 1996, 1997; Griffin et al. 1998; Suzuki 2002) with appropriate modifications to test the hypotheses described in the objectives. Work proposed in this project will build upon the foundation of knowledge established previously for larval herring rearing in the laboratory. Moreover, we will incorporate recent technical improvements in the large scale rearing of marine larval fish as detailed below. This work will be carried out with the assistance of project consultants Drs. McLean, Craig, Schwartz and Delbose, who are recognized experts in the field of marine fish larval culture and faculty members at the Virginia Tech Aquaculture Center (http://www.cfast.vt.edu/facilities/recaq.shtml). Their current efforts are focused on the larval rearing of the marine fish cobia as new aquaculture species. The members of this group will provide the P.I. with direct (on-site) and indirect technical assistance to establish and refine the specific techniques for herring incubation and rearing, particularly in the formulation of live diets and weaning onto dry food.

Objective 1: Sexually mature herring (~10-15 of each sex per sample period) will be captured by gill net from PWS spawning stock at the early, middle and end of the spawning season and transported in coolers to SSH. Measures of egg quality will be obtained from females collected throughout the spawning season, whereas studies designed to optimize salinity and dietary regimes for larval production will be based only on gametes obtained in the middle of the spawning season.

The eggs from each female will be stripped and placed separately on to glass slides in 5 L jars (~1000 eggs per jar) or pooled with eggs from 3-4 other females and placed evenly on egg trays made of nylon netting (20 x 30 cm) at a density of ~15,000-20,000 eggs per tray. The number of eggs in each jar and tray will be determined gravimetrically. A sub-sample of ~200 eggs from each female will be frozen on dry ice for analysis of amino acid and polyamine content using HPLC. Once the eggs are attached to the slides or trays, they will be fertilized by immersing them into 16‰ seawater that contains the pooled milt from 5-6 males. After fertilization, the pooled egg trays will be disinfected with 200 ppm iodophore for 60 minutes and placed in six 300 L tanks supplied with flowing water at a salinity of 16‰. Flow will be maintained at 2 L/min, and temperature will be held constant at 5ºC. Reduced salinity seawater will be obtained by mixing sand-filtered, UV-treated seawater with groundwater that is the freshwater supply for SSH. The final proportions of seawater and freshwater will be adjusted to account for the specific water chemistry conditions of the freshwater supply at the SSH. The eggs of the individual females held in jars will also be provided with sand-filtered, UV-treated seawater at a salinity of 16‰ and flow of 15 ml/min.

Eggs of both individual and pooled females will be sub-sampled at 60 minutes post-fertilization to determine the percent fertilization success. Larval fish will be sampled for length, weight and CaR receptor expression at hatch and near yolk absorption prior to first feeding. Herring eggs in PWS hatch in approximate three weeks at water temperatures of 4-5ºC and require 7-10 days to reach yolk absorption (Biggs and Baker 1993). We anticipate that herring eggs incubated at SSH will have a similar developmental rate. Larval mortality will be counted daily in each tank.

The newly hatched larvae resulting from each individual female will be collected daily for five days by siphoning and placed in 2 L glass jars at a density of ~150-200 larvae per jar. These larvae will be starved to provide an *in vivo* measure of egg quality. Mortality will be monitored daily to determine the cumulative loss over time and the time to 100% mortality, which we expect will be ~30 days at 5ºC (McGurk 1984).

Objective 2: Newly hatched larvae from the pooled females will be offered a first feeding live diet developed for marine fish. Briefly, live algae (*Nannochloropsis sp*), will be produced onsite from inoculation disks (Florida Aqua Farms, Tallahassee, FL) via standard production protocols as described in Hoff and Snell (1989). L-type (*Brachionus plicatilis*) rotifers, that are fed algal paste (Nanno 3600, Reed Mariculture; Campbell, CA), will be harvested daily after enrichment with Protein Selco Plus (INVE, Salt Lake, UT). During their enrichment, pure oxygen injection of the growth media will be used to maintain oxygen levels at above 10 ppm. After enrichment, rotifers will be harvested, rinsed, and then cold-banked in a cooler at < 9ºC for use over the next 24 hours. The *artemia* utilized will be pre-decapsulated (Embryon; INVE, Salt Lake, UT) then are hatched at 30ºC for 24 hours with 2000 lux, oxygen injection, hatch controller, antifoam, and sodium bicarbonate additions to maintain buffering. These *artemia* will then be harvested and enriched at 24ºC using DC DHA Selco (INVE, Salt Lake, UT) at two additions at a

concentration of 300 ppm over a total of 24 hours. After enrichment, the *artemia* will be harvested, rinsed, and then cold-banked as described above for use over the next 24 hours.

The live diet will be fed 4-5 times per day for 5-7 days at which time artificial food will be introduced into the feeding regime. One half of the fish (in 3 of the 6 start tanks) will receive a proprietary first feeding diet from a commercial manufacturer that is formulated to include immuno-stimulants. The remaining fish will receive a similar diet made by a competing supplier. Once the fish in all six tanks are completely weaned to their respective commercial diets, they will be divided into two groups. One group will continue to be reared at a water salinity of 16‰. The salinity of the other group will be reduced to 8‰ and supplemented with a mineral that results in the addition of 2.5 mMol each of Ca^{2+} and Mg^{2+} + 1.8 mMol $NaHCO^{-}_{3}$ to the flowing water supply. This mixture is based on prior studies (Nearing et al. 2002) showing that CaR activation is half maximal at ~5 mMol Ca^{2+} and 15 mMol Mg^{2+} at a Na^{+} concentration of ~107 mMol (≈8‰ seawater). In contrast, the respective half maximal CaR activation concentrations for Ca^{2+} and Mg^{2+} in a Na^{+} concentration of ~214 mMol (≈16‰ seawater) are ~12 mMol and 36 mMol, well below their actual concentrations in 16‰ seawater (5 mMol and 25 mMol, respectively). Sodium bicarbonate is included in the mixture to raise the pH to 8.0–8.4, which is approximately that found in full strength seawater. The larval herring will be reared under these four conditions until they reach approximately 30 mm in length and metamorphose to adult form (July). Mortality will be monitored daily to determine survival based on a combination of gravimetric estimates and direct counts (larvae, juveniles) to determine survival to hatch, at weaning and metamorphose. Growth in length (mm) and wet weight (mg), and changes in CaR expression will be determined at weekly intervals.

Objective 3: Comparative assessment of four dietary and water treatment regimes will be used to determine their utility for subsequent field trials involving cage culture of juvenile herring. Prior to conducting field trials, however, we will determine the effects of these treatments on growth, survival and osmoregulation under full strength seawater rearing conditions. Hence, once metamorphosis is complete the salinity in the tanks all four treatment groups will be increased to provide full strength seawater for juvenile rearing. These fish will be reared for an additional month at SSH before transfer to the ASLC in August for rearing through the fall and winter. After transfer to the ASLC, the juvenile fish will continue to be reared in full strength seawater under a natural photoperiod and at ambient temperature until early December, at which time they will be taken off food and starved for four months. Survival, growth in length and weight, WBEC, CaR expression, and hypo-osmotic capability will be measured bi-weekly until December and the onset of fasting. From December until early April, these parameters will be measured on a monthly basis, along with blood ion (Na^{+}, Cl^{-} and Ca^{2+}) concentration. Remaining fish will be sacrificed.

Objective 4: In the fourth year of this project we will implement a pilot-scale field trial to assess the performance of juvenile fish produced under the four hatchery production regimes during cage culture. After weaning to the commercial diets and rearing through metamorphose, otolith marked juvenile herring (n=50,000) will be reared in full strength seawater for an additional two weeks and then moved by vessel to a marine site either in Resurrection Bay or PWS. Once at the site, they will be transferred into net pens and fed their respective commercial diets for a period of four weeks. Growth in length and weight, survival and the visible incidence and frequency of VHSV and ICH in each treatment will determined at the end of the four weeks at which time the

fish will be released. Randomly collected sub-samples will be made from each group and sent to the ADF&G pathology lab for detailed histopathology to confirm the frequency and severity of either VHSV or ICH. The procedures for this diagnosis of VHSV and ICH and scoring for the prevalence of disease from these pathogens are outlined in Marty et al. (2004).

Laboratory Procedures for Objectives 1-4: Tissue localization of CaR proteins using immunocytochemistry will be performed using methods described previously by our group (Nearing 2002; Hentschel et al. 2003). Briefly, paraffin-sections containing specific herring tissues will be deparaffinized, exposed to antibody blocking solution and then incubated with one of several available anti-CaR antisera. Bound anti-CaR antibody will be localized using affinity purified peroxidase conjugated secondary antiserum. Appropriate controls to evaluate nonspecific binding of antibody will be included.

Protein immunoblotting will be performed as described previously (Ward et al. 1998) to determine the presence and relative amount of CaR proteins within tissue samples. Briefly, homogenized tissue fractions will be subjected to SDS-PAGE followed by the transfer of proteins to membranes where they will be subjected to immunoblotting techniques that include appropriate blocking, anti-CaR incubation, washing and development using peroxidase-conjugated second antiserum. Expression is quantified by imaging the relative intensity of resulting bands where they are expressed in arbitrary units.

Quantitation of tissue Na+K+ATPase activity (µMol ADP•hr^{-1}•mg protein) will be based on the method of McCormick (1993). Briefly, homogenized tissue fractions are exposed to a coupled enzymatic reaction system where ATPase activity is quantified spectrophotometrically via changes in NADH-NAD oxidation. Reactions with and without the addition of oubain are compared to determine the Na+K+ATPase component of overall ATPase. Blood ion concentration will be measured using and ABL Model 77 Series radiometer. Ionized concentrations for Na^+, Cl^- and Ca^{2+} are expressed in mMol. WBEC will be expressed in kJ•g^{-1} determined according to the methods described by (Paul et al. 1998). Briefly, each fish will be weighed (wet), freeze dried to remove moisture, then dried at 60ºC to a constant weight. Dry tissue will be ground and the caloric content measured by bomb calorimetry.

Free amino acid and polyamine content of eggs will be measured by HPLC after their derivatization with fluorescent ligands. Briefly, eggs from each female will be homogenized in 0.1 M HCl and protein precipitated with a solution of 0.05 M sodium acetate and 1% tetra-hydro-furan and centrifuged. Pre-column derivatization, mobile and stationary phases and program gradients are described in Vasantis et al. (2000) and Merali and Clarkson (1996).

Data Analysis and Statistical Methods

Variation in weight and length, WBEC, Na^+K^+ATPase activity, CaR expression and blood ion concentration will be analyzed by ANOVA. Differences in size or CaR expression at the time of weaning will be analyzed with a one-way model $Y_{ik} = \mu + F_i + e_{ik}$ where Y_{ik} is the weight, length, or CaR expression, μ = mean, F_i = effect of the diet and e_{ik} = error. All effects are fixed. After metamorphosis the model expands to $Y_{ik} = \mu + F_i + S_j + FS_{ii} + e_{ijk}$ where Y_{ik} also includes Na^+K^+ATPase activity, S_j is the effect of salinity and FS_{ii} is the interaction between the weaning diet and salinity. From the onset until the end of winter fasting Y_{ik} also includes WBEC and blood ion concentration. Heterogeneity in survival at each life history stage will be tested by

likelihood ratio (G-test). Survival rates for each treatment group will be transformed by arcsine square root and analyzed using the models for the various stages described above.

A minimum power of 0.8 was used to establish adequate sample sizes for each of the parameters listed above at two or more life stages of the study. At a sample size of n=10 per treatment group per date, the minimum detectable differences based on the previous estimates of the mean values (± SD) for these parameters would be:

- CaR Expression: 1.4 arbitrary units (3.5±0.8)
- Na^+K^+ATPase activity: 4.5 µMol ADP•hr^{-1}•mg protein (12±3)
- WBEC: 1.4 kJ·g^{-1} (5.2±0.9)
- Blood Ion Concentration: Sodium: 20 mmol/L (157± 10mmol/L)
  Calcium: 0.75 mmol/L (1.08 ±0.38mmol/L)
  Chloride: 39 mmol/L (150 ±20mmol/L)
- Length: Weaning: 2 mm (12±1mm)
  Onset of fasting: 12mm (76±7.5mm)

Mean weight and variance data for weight were not unavailable for power analysis. However, because weight and length are highly correlated, we assume that a sample size of n=10 will provide a similar detectable significance among treatments for weight.

Description of Study Area

Adult herring will be obtained from spawning stock in PWS, specifically from spawning stock on the eastern end of Montague Island. All laboratory studies will be conducted at either at the Seward Shellfish Hatchery or the Alaska SeaLife Center. The release site for the experimental net pen rearing will be determined through the Alaska Coastal Zone Permit application process.

Coordination and Collaboration with Other Efforts

We anticipate that this project will compliment several other proposed studies related to herring in PWS. First, the use of *in vitro* studies to validate and optimize proposed restoration actions will aid in evaluating the interaction between natural forces and stock enhancement in the proposed experimental nursery bay. Comparative performance of hatchery reared and wild fish in marine stock enhancement programs have highlighted the vulnerability of hatchery fish after release and need to condition these fish to specific cues before release. In conjunction with otolith mass marking of enhanced fish, factors such as release location can be measured to assess the likelihood stock enhancement will contribute to recruitment from diverse areas in PWS. The success of stock enhancement programs depends importantly on identifying release areas and times that will maximize survival. Dr. E. Brown will play an important role in this regard by integrating and parameterizing enhancement data into a proposed life-stage specific, ecosystem based model for PWS herring. Because herring are such an essential part of the PWS food web, having detailed knowledge of local and large-scale environmental conditions, the performance of enhanced fish under such conditions, and the effects of this enhancement on ecosystem dynamics will help guide recovery efforts herring as well as other species.

Our project will also provide collaboration opportunities with studies on the role of disease on herring recruitment. Our laboratory studies and pilot scale production will involve the use of specific pathogen free fish. In Year 4 of our project we propose to transfer juvenile herring to a PWS marine site for short-term cage rearing and release. These fish will be naïve to pathogens in PWS. Whether or not these fish experienced increased prevalence of either VHSV or ICH prior

to or after release may have direct implications for the utility of large-scale herring enhancement in PWS and determining the fate of these fish in relation to their health status.

SCHEDULE

Project Milestones

Objective 1. Provide biological and economic benchmarks to develop large-scale stock enhancement of herring in PWS. To be met in part by September 2007 (assessment of Japanese herring stock enhancement methods) and in full by 2010.

Objective 2. Determine the effects of salinity on the survival, growth and time to metamorphosis in larval fish. To be met by July in 2007, 2008, 2009 and 2010.

Objective 3: Determine the interaction between salinity conditions and weaning diet during larval rearing on the subsequent growth and over-winter survival of juvenile fish. To be met by March in 2009 and 2010.

Objective 4: Determine the comparative performance of hatchery based rearing methods on growth, immune system function and survival during short-term cage culture in the natural marine environment. To be met by July 2010.

Measurable Project Tasks

FY07, 1st quarter (October 1, 2006 – December 31, 2006)
November: Project funding approved by Trustee Council

FY07, 2nd quarter (January 1, 2007 – March 30, 2007)
January: Annual Marine Science Symposium

FY07, 3rd quarter (April 1, 2007 – June 30, 2007)
April: Travel to Japan
April 30: Collect eggs, start larval culture #1

FY07, 4th quarter (July 1, 2007 – September 30, 2007)
September 1: Complete assessment of Japanese culture techniques. Submit annual report

FY08, 1st quarter (October 1, 2007 – December 31, 2007)
November 30: Lab analysis of larval study #1

FY08, 2nd quarter (January 1, 2008 – March 30, 2008)
January: Annual Marine Science Symposium

FY08, 3rd quarter (April 1, 2008 – June 30, 2008)
April 30: Collect eggs, start larval culture #2

FY08, 4th quarter (July 1, 2008 – September 30, 2008)
July 30: Complete rearing through metamorphose #1,
September 1: Submit annual report

FY09, 1st quarter (October 1, 2008 – December 31, 2008)
November 30: Initiate fasting study #1, finish lab analysis of larval study #2

FY09, 2nd quarter (January 1, 2009 – March 30, 2009)
January: Annual Marine Science Symposium

FY09, 3rd quarter (April 1, 2009 – June 30, 2009)
April 30: Complete fasting study #1, collect eggs, start larval culture #3

FY09, 4th quarter (July 1, 2009 – September 30, 2009)
July 30: Complete rearing through metamorphose #2, finish analysis of fasting study #1
September 1: Submit annual report

FY10, 1st quarter (October 1, 2009 – December 31, 2009)
November 30: Initiate fasting study #2, finish lab analysis of larval study #3

FY10, 2nd quarter (January 1, 2010 – March 30, 2010)
January: Annual Marine Science Symposium

FY09, 3rd quarter (April 1, 2009 – June 30, 2009)
April 30: Complete fasting study #2, collect eggs, start larval culture #4

FY09, 4th quarter (July 1, 2009 – September 30, 2009)
July 30: Complete rearing through metamorphose #3, transfer juveniles to marine site, rear 1 month and release, finish analysis of fasting study #2
September 30: Submit annual report

RESPONSIVENESS TO KEY TRUSTEE COUNCIL STRATEGIES

Community Involvement and Traditional Knowledge (TEK):

This project represents a foundational development intended to support broader scale herring restoration in PWS. Due to complex biological and socio-economic factors underlying the herring debate, we intend to involve PWS community stakeholders at multiple levels of outreach and education. First we will inform stakeholders of the project objectives and methods with annual updates on project progress. We will also communicate with stakeholders and management agencies to integrate project developments with future restoration efforts other parties may implement in Prince William Sound. We will gather traditional knowledge that will guide the third year field trial. We will also provide education and outreach through the Alaska SeaLife Center's exhibits and distance education programs. We plan to disseminate the results using outreach strategies to target public interests on local, regional, and national levels.

Stakeholder meetings in Cordova: We plan to hold public informational meetings in Cordova in spring 2007, followed by annual meetings in 2008, 2009 and 2010 to keep stakeholders current on project progress and to solicit stakeholder input on herring restoration. We will coordinate meetings in Cordova through the Native Village of Eyak (Bruce Cain, Executive Director) and Prince William Sound Fisheries Research Applications and Planning group (Ross Mullins). These organizations have cultural, economic, social and biological interest in the direction and success of this project and have already been contacted. Fact sheets describing the project will be produced and distributed at the Cordova meetings. Input will be shared and

gathered to guide the future direction of the project for conducting Year 4 field trials. This will help build a framework upon the foundation research for larger scale restoration in PWS.

Integration with management agencies. We will implement a communication plan to inform agencies of progress. Field trials planned for Year 4 implementation will require coordination and permitting with management agencies.

Ecosystem-based education. This project provides a perfect context for teaching a broader view of the ecosystem. ASLC will use pacific herring in PWS as a platform for understanding the inter-relatedness of system components and the importance of restoring herring to the system. This will allow ASLC to develop and deliver educational content to regional and national school districts about the importance of herring and herring restoration because of its key role in the ecosystem. ASLC will use its distance learning capabilities to reach a wide audience both within Alaska and throughout the nation. Individual classrooms can also participate in programs delivered onsite at the ASLC and in their communities with an outreach educator. ASLC will partner with Prince William Sound Science Center to serve local communities and maximize accessibility for schools. Support materials will be developed such as teacher guides, research fact sheets, and hands-on lesson plans. This component will be managed by Dana Sitzler, Education Director and coordinated by Rachel Simon, Distance Learning Specialist at the ASLC.

Exhibitry and public displays. Signage and exhibits will be developed and displayed at the ASLC. Exhibits may include live research on display. Signage will address herring as a resource, its cultural and economic value, and the herring life cycle.

Web access. ASLC and MariCal maintain public access web sites that are continually updated and expanded to provide the public with recent findings and developments related to their various research activities. ASLC and MariCal plan to crosslink web pages dedicated to herring research as a means to highlight their respective involvement in this project and broader efforts related to herring restoration.

Finally, our findings will also be made available to other scientists and resource management agencies at EVOS meetings and appropriate scientific conferences and through publication in peer reviewed scientific journals and news letters.

PUBLICATIONS AND REPORTS

Proposed Peer Reviewed Publications

Aquaculture – Effects of calcium water concentration on calcium sensing receptor expression (CaR) during larval rearing of Pacific herring. December 2007.

Aquaculture Research – Advances in the culture techniques for stock enhancement of Pacific herring. December 2008

Journal of Aquatic Animal Health – Incidence of VHSV and ICH in juvenile Pacific herring during cage culture in Prince William Sound

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Radman, D.P., C. McCudden, K. James, E.M. Nemeth, and G.F. Wagner. 2002. Evidence for calcium-sensing receptor mediated stanniocalcin secretion in fish. Mol. Cell. Endocrin. 186: 111-119

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Stokesbury, K.D., J. Kirsch, E. Brown, G. Thomas, and B. Norcross. 2000. Spatial distributions of Pacific herring, *Clupea pallasi*, and walleye Pollock, *Theragra chalcogramma*, in Prince William Sound, Alaska. Fishery Bulletin. 98(2): 400-409.

Stokesbury, K.D., J. Kirsch, E. Patrick, and B. Norcross. 2002. Natural mortality estimates of juvenile Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska. Can. J. Fisheries and Aquatic Sciences. 59(3): 416-423.

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Svasand, T., T. Kristiansen, T. Perersen et al. 2000. The biological and economical basis of cod stock enhancement. Fish and Fisheries. 1: 173-205.

Tierney, K.B., A. Ferrel, and C. Kennedy. 2004. The differenctial leucocyte landscape of four teleosts: juvenile *Onchorhynchus kisutch, Clupea pallasi, Culdea inconstans* and *Pimephales promelas*. J. of Fish Biology. 64(4): 906-919.

Tytler, P. and J. Blaxter. 1988. The effects of external salinity on the drinking rates of the larvae of herring, plaice and cod. J. Exp. Biol. 138: 1-15.

Vasanits, A., D. Kutlan, P. Sass, and I. Molnar-Perl. 2000. Retention/quantitation properties of the o-phthaldialdehyde-3-mercaptopropionic acid and the o-phthaldialdehyde-N-acetyl-L-cysteine amino acid derivatives in reversed-phase high-performance liquid chromatography. J. Chromatography. 870: 271-287.

Vine, N.G., W. Leukes, and H. Kaiser. 2006. Probiotics in marine larviculture. FEMS Microbiol Rev. 30: 404-427.

Ward, D.T., E.M. Brown, and H.W. Harris. 1998. Disulfide-bonds in the extracellular calcium-polyvalent cation sensing receptor mediate dimmer formation and its response to divalent cations in vitro. J. Biol. Bhem. 273: 14476-14483.

Williams, E.H. and T.J. Quinn. 2000. Pacific herring, *Clupea pallasi*, recruitment in the Bering Sea and north-east Pacific Ocean, I: Relationships among different populations. Fisheries Oceanography. 9(4): 285-299.

Yamaguchi, T., N. Chattopadhyay, and E.M. Brown. 2000. Hormones and Signaling. Acad. Press. 209-253.

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Employment

2000-present	Senior Research Scientist, MariCal Inc.
1999-2000	Aquaculture Program Instructor, Washington County Technical College, Eastport ME
1997-1999	Fisheries Consultant
1995-1996	Chief Scientist, Operations Manager, Prince William Sound Aquaculture Corp., Cordova AK
1988-1994	Hatchery Manager, Northern Southeast Regional Aquaculture Assoc., Sitka, AK

Education

Ph.D. Fisheries, University of Washington, 1993
M.S. Fisheries, University of Washington, 1988
B.S. Wildlife Ecology, University of Wisconsin, 1979

Publications Related to Project

Linley, T., D. Russell, M. Betka, J. Nearing, and W.H. Harris. Calcium-sensing Receptor (CaR) Activation Enhances Parr-smolt Transformation in Chinook Salmon. 2004. Proc. NW Fish Cult. Conf., Victoria, B.C. 11p.

Linley, T., M. Betka, D. Russell, and H. W. Harris. 2002. Improved diets for larval fish culture. MTI Seed Grant SG 1214.

Linley, T. 2001. A comparison of first feeding characteristics in two populations of chinook salmon. Trans. Am. Fish. Soc. 130:519-525

Other Publications

Linley, T, 2001. Influence of short-term estuarine rearing on the ocean survival and size at return of coho salmon in southeastern Alaska. N. Am. J. Aqua. 63: 306-311.

Smoker, W.W. and T. Linley. 1997. Are Prince William Sound hatcheries a fools bargain? Alaska Fishery Research Bulletin. Vol. 4 No.

Smoker, W.W., B.A. Bachen, G. Freitag, H.J. Geiger and T.J. Linley. 2000. Alaska ocean ranching contributions to sustainable salmon fisheries. Pages 407-420 in E.E. Knudson, C.R. Steward, D.D. MacDonald, J.E. Williams, and D.W. Reiser, editors. Sustainable Fisheries Management. CRC Press. Boca Raton.

Bachen, B. A. and T. Linley. 1995. Hidden Falls Hatchery Program. p. 564-565. *In*: H.L Schramm and R.G Piper [eds.] Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society.

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EDUCATION:

Ph.D.	1989, University of Hannover, Germany (Zoology, Botany)
Diploma (M.S.)	1981, University of Hannover, Germany (Zoology, Botany, Biochemistry, Physiology)

APPOINTMENTS:

2005-present	Senior Vice President, Research at MariCal Inc.
1999-2004	Research/Senior Research Scientist at MariCal Inc.
1990-1999	Research/Senior Research Associate, Department of Biology, Boston University, Boston
1985-1989	Research Assistant, Department of Phytopathology, University Kiel, Germany

HONORS AND AWARDS:

1995/1996	Young Investigator Fellowship, Mount Desert Island Biological Laboratory
Jan 1990	Fellowship from the Scottish Crop Research Institute, Invergowrie, Scotland
1978-1984	Scholarships from the Study Foundation of the German Nation ("Studienstiftung des Deutschen Volkes")

SELECTED PUBLICATIONS and ABSTRACTS:

J Nearing, **M Betka**, S Jury and HW Harris. Tissues of Atlantic salmon (*Salmo salar*) express calcium-sensing receptors (CaRs) cDNAs in various tissues. J Exp Biol (submitted)

Jury, S.H., **Betka, M.** Linley, T., Harris, H.W. 2005. The interaction of low pH, aluminum, and calcium on olfaction of Atlantic salmon (Salmo salar). Integrative and Comparative Biology 45(6):1151

Jury, S.H., HW Harris, T. Linley, D. Russell, **M. Betka**, S. Anderson, S. Harakawa and A. Hara. Aquaculture and Stock Enhancement Technologies Based on Recently Discovered Calcium-Sensing Receptors in Finfish. US-Japan Natural Resources (UJNR) Aquaculture meeting. San Diego, CA 2005

H Hentschel, J Nearing, HW Harris, **M Betka**, M Baum, SC Hebert, M Elger. Localization of Mg2+-sensing shark kidney calcium receptor SKCaR in kidney of dogfish, *Squalus acanthias*. Am J Physiol Renal Physiol 285(3):F430-439. Epub May 20, 2003

J Nearing, **M Betka**, S Quinn, H Hentschel, M Elger, M Baum, M Bai, N Chattopadyhay, EM Brown, SC Hebert, HW Harris. Polyvalent cation receptor proteins (CaRs) are salinity sensors in fish. PNAS 99 (14), 9231-9236, 2002

M Betka and GV Callard. Stage-dependent accumulation of cadmium and induction of metallothionein-like binding activity in the testis of the dogfish shark, *Squalus acanthias*. Biology of Reproduction 60, 14-22, 1999

Callard GV, **Betka M** and D. Miller. Unconventional models for toxicology research. In: Comprehensive Toxicology, Vol. 10. Reproductive and Endocrine Toxicology. Section I. Male Reproductive Toxicology, K Boekelheide, R Chapin, P Hoyer (eds.), Elsevier, New York, 235-247, 1997

Callard GV, Kruger A and **M Betka**. The goldfish as a model for studying neuroestrogen synthesis, localization and action in the brain and visual system. Envir. Hlth Persp. 103 (Suppl. 7), 51-57, 1995

M Betka and GV Callard. Negative feedback control of the spermatogenic progression by testicular oestrogen synthesis: Insights from the shark testis model. APMIS 106, 252-258, 1998

Callard GV, **Betka M** and JC Jorgensen. Stage-related functions of Sertoli cells: Lessons from lower vertebrates. In: Bartke A, ed. Function of somatic cells in the testis. New York, Springer, 27-54, 1993

Recent collaborations involving work on herring: none

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Assistant Director for Research Operations

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EDUCATION

- M.S., Biological Oceanography, Institute of Marine Science, University of Alaska, Fairbanks, 1980
- B.A., Biology, Rutgers University, Camden, NJ, 1972
- American Management Assn. CEC Programs
- MAT student University of Alaska Fairbanks, 1982-83

EMPLOYMENT

2002 - present	Assistant Director for Research Operations, Alaska SeaLife Center, Seward, AK
2006 - present	Salmon Restoration Program Manager, Alaska SeaLife Center
2000 - 2002	President and Chief Operating Officer, XRD*i*, Beaufort, SC
1999 - 2000	Chief Operating Officer, Solo Water Sports, Bellevue, WA
1998	Project Manager, Agri-Tech Inc, Corvallis, OR
1989 – 1997	Prince William Sound Aquaculture Corporation, Cordova, AK
	Special Projects Manager and Regional Planner
	Vice President
	Interim CEO
	Special Assistant to the CEO

PUBLICATIONS

None

COLLABORATIONS

Dr. Tim Linley, Senior Research Scientist, MariCal. Portland, ME
Gary Fandrei, President Cook Inlet Regional Aquaculture Corporation, Kenai, AK

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MariCal. Inc.
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EDUCATION

Master of Aquaculture, Simon Fraser University, Burnaby, BC 1995.

Fisheries and Aquaculture Technical Diploma, Malaspina College, Nanaimo, BC 1992.

Bachelor of Science, Simon Fraser University, Burnaby, BC 1989.

WORK EXPERIENCE

Research Scientist, MariCal, Portland, Maine. July 2004 to present.

Freshwater Production Manager, Cermaq, Mainstream Canada, Port Alberni, BC July 2003 to May 2004.

Freshwater Production Manager, Cermaq, Pacific National Aquaculture, Port Alberni, BC October 2000 to July 2003.

Freshwater Production Manager, Pacific National Group, Port Alberni, BC June 1998 to October 2000.

Senior Technician, Pacific National Group, Port Alberni, BC May 1997 to June 1998.

Broodstock and Environmental Monitoring Technician, Pacific National Group, BioTechnical Services, Port Alberni, B.C. April 1995 to May 1997.

Fish Technician, Department of Fisheries and Oceans, Snootli Creek Hatchery, Bella Coola, BC 1998 – 1993 (summers only).

AREAS OF EFFECTIVENESS

A. Hatchery Management

- Currently managing the Salmon Project at the Alaska SeaLife Center, raising 100,000 Chinook salmon for restoration/enhancement purposes.
- Managed the production side of a facility with a capacity to produce 3.5 million, 50 gm smolts per year using both flow thru and recirculation technology.

B. Fish Culture

- Extensive experience raising Pacific and Atlantic salmon for enhancement and retail.

- Includes spawning, incubation, feeding, cleaning, weight sampling, vaccination (dip, bath and injection),grading (bar, belt), monitoring water quality (DO, temperature, hardness, pH), transporting of smolts via road and air, finclipping, coded wire tagging and PIT tagging of selected fish groups for future identification.

C. Research and Development

- Implemented field trials using the SeaReady™ technology to improved smoltification in Chinook, coho and sockeye salmon.
- Monitor smoltification in salmon using blood ions, Na+-K+ ATPase analysis, Western Blot and Immunocytochemistry.
- Collection and interpretation of otoliths from coho, sockeye and Chinook salmon.

D. Reproductive Technologies

- Extensive, hands-on experience in the implementation of broodstock development programs.
- This program includes design work, brood selection, spawning, eggsetting, production of all-female stocks via masculinization and gynogenesis and later analysis of family groups for future brood selection.

E. Data Analysis and Computer Literacy

- Have designed databases to track trial results from SeaReady™ applications and otolith collections.
- Have designed databases to track tagged fish throughout all rearing stages and to integrate reports from fish health program to aid in the analyses and predictions
- Extensive software expertise including statistical analyses, spreadsheets, databases and word processing.

F. Fish Health Management

- Experience managing a comprehensive Fish Health Monitoring Program.
- Includes the analysis of mortality records and fish health reports to recommend treatments if necessary. In co-ordination with consulting veterinarian, determine the most cost-effective treatment both at the hatchery and farm setting. e Hatchery.

G. Communication

- Have authored several technical reports on research projects for Revenue Canada's Scientific Research and Expenditures Development Program (SRED).
- Routinely prepared inter-departmental reports, analysis and briefing memos on a wide variety of projects.
- Active participant in monthly managerial meeting where oral presentations of reports are necessary.

Recent collaborations involving herring – None

Evelyn D. Brown, Consultant
Flying Fish, Ltd.
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Education:

B.S. Zoology and Chemistry, University of Utah, Salt Lake City, 1977
M.S. Fisheries Biology and Aquacultural Engineering, Oregon State University, Corvallis, Oregon, 1980
Ph.D. Fisheries at University of Alaska, Fairbanks, 2003

Recent Experience:

Research Associate, University of Alaska, Fairbanks, 1995 to the present
Herring Research Biologist, Alaska Department of Fish and Game, Cordova, Alaska from 1988 to 1995
Principal Investigator, Injury to PWS Herring from the *Exxon Valdez* Oil Spill, NRDA FS 11, 1989-1992

Expertise: Fisheries, Fisheries Oceanography, Marine Ecology, Aerial Survey and Remote Sensing, and Statistical Modeling

Current Research Projects:

Development of new methods for detecting and assessing pelagic fishes (sardines and albacore; Office of Naval Research, NOPP program)

Relevant Publications

Brown, E.D. 2002. Life history, distribution and size structure of Pacific capelin in Prince William Sound and the Northern Gulf of Alaska. ICES Journal of Marine Science, 59:983-996.

Brown, E.D., Seitz, J., B. L. Norcross, and H. P. Huntington. 2002. Ecology of Herring and Other Forage Fish as Recorded by Resource Users of Prince William Sound and the Outer Kenai, Alaska. Alaska Fishery Research Bulletin 9(2): 75-101.

Brown, E.D. and B.L. Norcross. 2001. Effect of herring egg distribution and ecology on year-class strength and adult distribution: preliminary results. Herring 2000, Alaska Sea Grant College Program, AK-SG-01-04: 335-345.

Ford, R.G., D.G. Ainley, E.D. Brown, R.M. Suryan, and D.B. Irons. In Press. The foraging of black-legged kittiwakes in Prince William Sound, Alaska: a model optimizing success as a function of colony size and location. Ecological Monographs.

Suryan, R.M., D.B. Irons, M. Kaufman, J. Benson, P.G.R. Jodice, D.D. Roby, and E.D. Brown. 2002. Short-term fluctuations in forage fish availability and the effect on prey selection and brood-rearing in the black-legged kittiwake *(Rissa tridactyla*). Mar. Ecol. Progr. Ser. 236: 273-287.

Purcell, J.E., E.D. Brown, K.D.E. Stokesbury, L.H. Haldorson, and T.C. Shirley. 2000. Aggregations of the jellyfish *Aurelia labiata*: abundance, distribution, association with age-0 walleye Pollock, and behaviors promoting aggregation in Prince William Sound, Alaska, USA. Mar. Ecol. Prog. Ser. 195: 145-158.

Research and Publication Collaborations

Rick Broduer, NMFS, NW Science Center
Mark Benfield, LSU, Baton Rouge
James Churnside, NOAA Environmental Technology Laboratory
Robert Foy, UAF SFOS FITC
John Horne, University of Washington, School of Fisheries
David Irons, USFWS, Migratory Bird Unit
Martín Montes Hugo, UAF SFOS IMS
Brenda Norcross, UAF SFOS IMS
Jennifer Purcell, University of Maryland
Mike Sigler, NMFS Auke Bay Lab
Robert Suryan, Oregon State University
Chris Wilson, NOAA NMFS Alaska Fisheries Science Center, Seattle

Ewen McLean
Professor of Fisheries and Director, VPISU Aquaculture Center

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Education:

1987: Ph.D. Fish Physiology, University of Bradford, School of Biomedical Sciences, Richmond Road, Bradford, West Yorkshire, BD7 1DP, U.K. Dissertation: *Intact Protein Absorption in Teleosts*. i-ix, 235pp.

1982: M.Sc. (CNAA) Applied Fish Biology, University of Plymouth, Department of Biological Sciences, Drake Circus, Plymouth, Devon, PL4 8AA, U.K. Thesis: *Sub-lethal toxicity of potassium dichromate upon the thick-lipped gray mullet Chelon labrosus (Risso).*

1981: B.Sc. (Hons.) Fish Science, University of East London (NELP), School of Independent Study, Stratford, London, E15 2LL, U.K. Dissertation: *Sub-lethal chromium toxicity to Paleomonetes varians*

Employment history:

2001-date: Virginia Polytechnic Institute and State University (VPISU), College of Natural Resources, Department of Fisheries and Wildlife Sciences, 100 Cheatham Hall, Blacksburg, VA 24061-0321
Position: Professor of Fisheries and Director, VPISU Aquaculture Center

1999-2001: Sultan Qaboos University, College of Agriculture, Department of Marine Science & Fisheries, P.O. Box 34, Al-Khoud, Postal Code 123, Muscat, Sultanate of Oman.
Position: Head of Department

1993-1999: University of Aalborg, Department of Civil Engineering, Biotechnology Laboratory and Laboratory for Aquatic Biotechnology, Sohngaardsholmsvej 57, DK-9000 Aalborg, Denmark.
Positions: Research Leader/Senior Lecturer/Laboratory Head.

1987-1993: DFO, Aquaculture & Biotechnology Sections, West Vancouver Lab., 4160 Marine Drive, West Vancouver, B.C., V7V 1N6.
Positions: Postdoctoral Research Fellow (DFO-NSERC)
Industrial Research Associate (Amgen)
Research Associate (Monsanto)

1984-1987: University of Bradford, Dept. Biomed. Sci., Richmond Road, W. Yorks., U.K.
Position: Ph.D./Postdoc. Research Assistant.

1983-1984: Thameside Park Association, Thames Road, Barking, Essex, U.K.
Position: Program Head, Ecology Unit.

Example consultancies:

Since 1990, I have been engaged as a consultant in various fields, covering institutional and curriculum development, project review, extension training, feasibility studies and field trials, proposal structuring, short-course design and execution totaling approximately 3 person years. Some specific examples of consultancies undertaken, together with client identification are noted below:

- McLean, E. (1990). Damsa, Nolalu, Ontario, Canada. Put-and-take fisheries of Northern Ontario shield lakes. Surgical field gonadectomies of various species to enhance growth and survival and production of instructional video.
- McLean, E. (1990). Laboratory for Aquaculture, "Ruder Boskovic" Institute, Zagreb, Yugoslavia. Translation/editing of Government of Yugoslavia and United Nations Development Program progress reports. Development of research initiatives in support of the Croatian aquaculture industry (freshwater and marine).
- McLean, E. (1991). Levy & Associates Research Services Ltd., West Vancouver, B.C., Canada. EIA assessment of pulp mill effluents in Kalimantan, Indonesia.

- Aquatic Bioscience Consultants, North Vancouver, B.C., Canada. EIA-provisions under the Govts. of British Columbia and Canada - *re:* bridge construction impact on fisheries streams and creeks, Vancouver Island, and interior British Columbia. (1991-3).
- Government of the Republic of Indonesia, Ministry of Education and Culture, Directorate of Higher Education, Jakarta, Indonesia. Marine Sciences Education Project. Mariculture and Marine Animal Physiology Specialist. (1992/3/4/5).
- Danish International Development Agency, Copenhagen, Denmark. Aquaculture Development, Bangladesh Baor Project. (1996).
- Elane Aquaculture, Zimbabwe. HACCP development for commercial tilapia producer/processor/ exporter (1998)
- Oman Fishing Company, Muscat, Sultanate of Oman. Appraisal of marine fisheries stock assessment and preliminary analyses of extant landings data. (2000).
- Ministry of Agriculture and Fisheries, Muscat, Sultanate of Oman. Evaluation of macrophyte aquaculture potential/ *A National Centre for Marine Biotechnology: A Proposal*. Development report and feasibility study. (2001).

Grants, training and extension

Since 1993, I have served on graduate degree planning and assessment committees of in excess of 40 Masters students of which I was major advisor for 25. I have trained 6 doctoral candidates. Competitive grants acquired to date exceed US$4.5 million in support of aquaculture-, and fisheries-related projects. Extension activities have included conceptualization and execution of a wide variety of workshops on best management practices in aquaculture, aquaculture production, systems design, product quality assessment, and development of fisheries extension services and management plans (shark, Spanish mackerel). I have participated as a member of: Working Committee: Hormonal Control of Growth and Development in Fishes, SeaGrant USA (1990); Scientific Council Member: Swedish National Association of Aquaculture (1995-1999); Panel Member: SEAFDEC/FAO/CIDA expert meeting on the use of chemicals in aquaculture (1996).
Group Member: GESAMP (UN/UNEP/FAO/UNESCO/IOC/WHO/WMO/ IMO/IAEA) working group on environmental impacts of coastal aquaculture (1996); Reporter to the Danish Parliamentary Commission on the Status of National Research in Aquaculture (1997); Scientific Committee: Man and coastal areas: impact of aquaculture, Fiskebockskil, Sweden (1999); Committee Member: Ministry of Agriculture & Fisheries, Fisheries Research Fund, Oman (2000-2001). I have participated in organizing or have convened 14 regional, national and international meetings relating to aquaculture and fisheries since 1992. I have acted as session chairperson at international meetings on 15 occasions since 1989.

Publications and Editorial responsibilities

Published material includes coverage in both basic and applied fields of fisheries and aquaculture science. A significant proportion of material has centered attention upon growth and quality regulation in cultured fishes with attention upon value addition to seafood products and the reduction of impact potential of production and processing systems. These have included works dealing with nutrition, feeding strategies and growth regulation, as well as fish meal and aquafeed characterization and product quality classification. Several items center attention upon mitigation of the environmental impact of aquaculture using a variety of biological and engineering strategies. A number of articles have evaluated broodstock management and controlled reproduction in cultured species. Fish vaccination, osmoregulation, and stress have also received attention. Research has also been undertaken with a traditional fisheries leaning, ranging from the effects of ghost fishing through to stock biology and assessment. Educational and extension materials have also been constructed. Experimental material employed for basic and field-based trials have included: radiata (1 spp.), molluscans (3 spp.), crustaceans (4 spp.), teleosteans (21 spp.), amphibians (2 spp.) and reptilians (1 spp,). I have published in excess of 140 scientific papers. I presently serve on the editorial boards of: *Aquaculture* (2001-date), *Aquaculture Nutrition* (Founder member, 1996-date) *Ribarstvo* (1995-date), *Bulletin of Agriculture and Fisheries Research* (2000-date), *International Journal of Recirculating Aquaculture* (2001-date), and *Bulletin of Marine Science* (1993-date).

Recent collaborations involving herring - None

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Virginia-Maryland Regional College of Veterinary Medicine
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Education:

Degree	Institution/Location	Year	Major
B.S.	Baylor University Waco, Texas	1984	Biology
M.S.	Corpus Christi State University Corpus Christi, Texas	1989	Marine Science
Ph.D.	Texas A&M University College Station, Texas	1994	Wildlife and Fisheries Sciences

Research and Professional Experience:
Associate Professor, Dept. of Large Animal Clinical Sciences, Virginia Tech
June 2003-present
Research Associate Professor, Dept. of Large Animal Clinical Sciences, Virginia Tech
2001-2003

Publications Related to the Project:

1. Craig, S.R., M.H. Schwarz and E. McLean. In press. Juvenile cobia (Rachycentron canadum) can utilize a wide range of protein and lipid levels without detrimental impacts on production characteristics. **Aquaculture.**

2. Schwarz, M.H., Mowry, D., McLean, E. and Craig, S.R. In press.. Performance of advanced juvenile cobia reared under different thermal regimes: a method for cold banking**. Journal of Applied Aquaculture**.

3. Lunger, A., S.R. Craig and McLean, E. 2006. Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. **Aquaculture** 257: 393-399.

4. Craig, S.R. and E. McLean. 2006. Sustainable aquaculture of cobia: A case study with organically certified alternate proteins In: **Nutritional Biotechnology in the Food and Feed Industry** (K. Jacques and P. Lyons, Eds.). Nottingham University Press, UK.

5. McLean, E. and S.R. Craig. 2006. Nutrigenomics in aquaculture research: a key in the 'Aquanomic" revolution In: **Nutritional Biotechnology in the Food and Feed Industry** (K. Jacques and P. Lyons, Eds.). Nottingham University Press, UK

Other Publications:

6. González, S., S.R. Craig, E. McLean, S.E. Duncan, S.F. O'Keefe, M.H. Schwarz and G.J. Flick. 2005. The Dietary protein requirement of southern flounder (*Paralichthys lethostigma*). **Journal of Applied Aquaculture** 17(3), 37-50.

7. Rasmussen, M.R., J. Laursen, S.R. Craig, and E. McLean. 2005. Do fish enhance tank mixing? **Aquaculture**, 250(1-2): 162-174.

8. Neill, W.H., E.L. Oborny, Jr., S.R. Craig, M.D. Matlock and D.M. Gatlin, III. 2004. Estimating metabolism of fish in aquacultural production systems. **International Journal of Recirculating Aquaculture**. 4: 25-32.

9. Neill, W.H., T.S Brandes, B.J. Burke, S.R. Craig, L.V. DiMichele, K. Duchon, R.E. Edwards, L.P. Fontaine, D.M. Gatlin, III, C. Hutchins, J.M. Miller, B.J. Ponwith, C.J. Stahl, J.R. Tomasso, and R.R. Vega. 2004. Ecophys.Fish: a simulation model of fish growth in time-varying environmental regimes. **Reviews in Fisheries Sciences**. 12: 233-288.

10. McLean, E. and S.R. Craig. 2003. Overcoming barriers to the oral delivery of peptide and protein therapeutics to aquacultured organisms. In: **Nutritional Biotechnology in the Food and Feed Industry** (K. Jacques and P. Lyons, Eds.). Nottingham University Press. United Kingdom.

Recent collaboration involving Pacific herring – None

Michael H. Schwarz
Virginia Polytechnic Institute and State University College of Agriculture and Life Sciences
Department: Fisheries and Wildlife Sciences
Academic Rank: Professional Lecturer

Education:

2001 – 2005	Virginia Tech. Ph.D. Candidate, completed fall, 2005
1989 – 1991	Texas A&M University. Masters of Agriculture/Aquaculture Option.
1985 – 1989	Texas A&M University. Bachelors of Science/Wildlife and Fisheries Sciences.
1983 – 1985	Brevard Community College. Associates Degree.

Experience:

2005 – Present	Founding member: IISBA (International Initiative for Sustainable and Biosecure Aquafarming)
2004 – Present	Board member: OAI (Organic Aquaculture Institute)
1997 – Present	Aquaculture Specialist, Virginia Tech.
1996 – Present	President/CEO of Quantum Tides, Inc. Texas-based aquaculture consulting firm.
1995 – 1996	Operations Manager, HarvestFresh Seafoods, Inc., a multimillion dollar marine foodfish production facility in Bacliff, Texas.
1992 – 1994	Biologist/Hatchery Manager, HarvestFresh Seafoods, Inc.
1991	Masters Research performed at Redfish Unlimited, Bacliff, Texas.
1986 – 1990	Worked at various research and industrial production facilities financing education.

Boards, Committees, Chair:

2004 – Present	VAAEA & NACAA
2004 – Present	Southern Region Aquaculture Center: Technical Committee Member
2003 – Present	Extension Leadership Council: Hampton, Virginia.
2003 – Present	Virginia Aquaculture Association
2000 – Present	Virginia Academy of Science (VAS) VAS Section secretary (2000) VAS Section Chair (since 2001)
2000 – Present	Editorial Board: International Journal of Recirculating Aquaculture.
1999 – Present	Virginia Trout Growers Association
1997 – Present	Committee Member: Commercial Fisheries and Shellfish Technologies.
1989 – Present	World Aquaculture Society, WAS & WAS US Chapter: WAS Program Committee for AQUA 2006, Florence, Italy. WAS Conference Quality Committee (since 2003 WAS Education and Curriculum committee (since 2004)

Honors Societies:

2002 – Present:	Omicron Tau Theta

Recent Publications Related to the Project:

Craig, S.R., M.H. Schwarz, E. McLean (2005). Nutrition Research with Cobia. **Global Aquaculture Advocate** 8(1): 76-78.

Schwarz, M.H. (2004). Fingerling Production Still Bottleneck for Cobia Culture. **Global Aquaculture Advocate** 7(1):40-41.

King, N., M.H. Schwarz, D. Mowry, J. Zimmerman. (2004). Intensive Work on Cobia Larvae. **Fish Farming International**. October, 2004.

Mowry, D.E., M.H. Schwarz, K.P. Hughes, M.L. Jahncke, S.A. Smith. (2004). Efficacy of Hydrogen Peroxide in Marine Recirculating Aquaculture Systems holding Summer Flounder (*Paralichthys dentatus*). **Journal of Applied Aquaculture**. (In Press).

Mowry, D.E., M.H. Schwarz, S. Craig, J. Holt, J. Kaiser, O. Stevens, B. O'Hanlon. (2004) The Cobia Connection: Research Institutions and Private Sector Continue Collaboration to Develop Culture Protocols. **Hatchery International.** 5(6): 12-15.

Other Recent Publications

Gaylord, T. G., M.H. Schwarz, G.M. Davitt, R.W. Cool, M.L. Jahncke and S.R. Craig. 2003. Thermal optima for the culture of juvenile summer flounder (*Paralichthys dentatus*). **Journal of Applied Aquaculture**. 14 (3-4).

Gaylord, T. G., M.H. Schwarz, G.M. Davitt, R.W. Cool, M.L. Jahncke and S.R. Craig. 2003. Dietary Lipid Utilization by Juvenile Summer Flounder (*Paralichthys dentatus*). **Journal of the World Aquaculture Society**. 34(2): 229-235.

Flatfish Culture II. 2003. (Michael H. Schwarz, Guest Editor). **Journal of Applied Aquaculture** (Volume 14, No.3/4). Hayworth Press, Inc. 10 Alice Street, Binghamton, New York 13904-1580 USA.

Schwarz, M.H. 2003. Marine Finfish Larviculture. **Aquaculture Magazine**. 29(6): 14-19.

Cool, R.W., D.E. Mowry, M.H. Schwarz, M.L. Jahncke and D.E. Kauffman. 2003. Status of Aquaculture at the VSAREC. Virginia Aquaculture Association Newsletter. June, 2003.

Recent collaborations involving Pacific herring - None

Brendan Cameron Delbos

Education

Master of Science in Aquaculture and Fisheries, May 2001 — Louisiana State University
Bachelor of Science in Marine Biology, December 1997 — Southampton College

Work Experience

Marine Fish Hatchery Manager: Virginia Seafood AREC
2005-Present
Zebrafish Facility Manager: University of Massachusetts at Amherst
2003-2004
Hatchery Manager: Mass Fin Tech (formerly Fins Technology), Turners Falls, MA
2002-2003
Hatchery/Live Feed Manager: Fins Technology, Turners Falls, MA
2000- 2002
Assistant Farm Manager: Eden Brook Trout Hatchery, Forestburgh, N.Y.
1997-1998

Publications

Lindell, S.R., **B. Delbos**, R. Perham, J.Goldman, E.M. Hallerman, T.O. Brenden. 2004. Hatchery and growout performance of sunshine bass and backcross hybrid striped bass in recirculating aquaculture systems. International Journal of Recirculating Aquaculture. 5, 43-54.

Weirich, CR, CC O'Neal, **BC Delbos**, and CG Lutz. 2001. Effects of stocking sac-fry and hatchery-fed fry on production of fingerling channel catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society. 32(1):112-116.

Fuiman, L.A. and **BC Delbos**. 1998. Developmental changes in visual sensitivity of red drum, *Sciaenops ocellatus*. Copeia. 4: 936-943.

Selected Presentations

Delbos, BC. 2006. Applied Finfish Hatchery Management. Aquaculture America 2006. Annual Meeting of the US Chapter of the World Aquaculture Society, Las Vegas, NV

Delbos, BC, MH Schwarz, S Craig, E Mclean. 2005. Cobia express growth variance following changes in rearing temperature. 2nd International Sustainable Marine Fish Culture Conference. Ft. Pierce, Fl.

Delbos, BC. 2004. Biological Filtration. Aquaculture America 2004. Meeting of the US Chapter of the World Aquaculture Society, New Orleans, LA.

Delbos, BC and CR Weirich. 2001. The effect of feeding salmon starter to channel catfish fry on subsequent production of fingerlings. Aquaculture 2001, The Annual International Triennial Conference and Exhibition of the World Aquaculture Society, Orlando, FL.

Delbos, BC, CR Weirich, D Fernandez, and RL Thune. 2001. Evaluation of a live attenuated vaccine for the control of enteric septicemia of catfish under simulated production conditions. Aquaculture 2001, The Annual International Triennial Conference and Exhibition of the World Aquaculture Society, Orlando, FL.

Delbos, BC, CR Weirich, CC O'Neal, and CG Lutz. 2000. The effect of age at stocking on production of fingerling catfish. Annual Meeting of the Catfish Farmers of America, Albuquerque, NM.

Delbos, BC, DE Ashe and CR Weirich. 2000. A comparison between volumetric and gravimetric methods for enumeration of channel catfish *Ictalurus punctatus* fry. Aquaculture America 2000, Annual Meeting of the US Chapter of the World Aquaculture Society, New Orleans, LA.

Recent collaborations involving Pacific herring - None

BUDGET JUSTIFICATION – Funding has been approved for FY 07 only; PI will need to resubmit FY 08-FY10 for consideration in FY 08.

We are requesting a total of **$1,284,600** (plus 9% G&A of $115,600, for a total project cost of **$1,400,200**) in EVOS Trustee Council funding over four years to complete this project. The project will utilize the existing infrastructure of the Alaska SeaLife Center (ASLC) and the Seward Shellfish Hatchery (SSH). Both facilities are equipped with flowing seawater and freshwater as well as appropriate temperature control, lighting, algal cultures and miscellaneous culture equipment needed to conduct the studies described in this proposal. The project will also utilize MariCal's significant investment (~$600,000) in its molecular laboratory facilities and analytical equipment, and the extensive scientific expertise of researchers in the fields of molecular biology, fish culture, nutrition and physiology, and fisheries ecology.

Alaska SeaLife Center Component Justification

Personnel: ASLC personnel include: **Howard Ferren**, Restoration Program Manager, will serve as co-P.I. and will devote 0.5 months of his time in Year 1 and 1.5 months of his time annually in Years 2-4 to the project coordinating facilities, working with stakeholders, participating with EVOS TC on herring planning and project integration, and coordinating with resource management agencies. **Brendan Smith**, Research Education Coordinator, will spend approximately 2 weeks per year preparing the Cordova stakeholders' meetings and other public outreach activities including website content development for the project. **Dana Sitzler**, Education Director (2 weeks per year in Years 2-4), and **Rachel Simon**, Distance Education Specialist (2 months per year in Years 2-4), will develop an ecosystem-based education program based on herring for school-aged children.

Travel: We are requesting $1.5k in Year 1 and $3k annually in Years 2-4 in travel for Ferren to attend the annual EVOS meeting and meetings that involve herring restoration planning and community participation and outreach and for Sitzler and Smith to attend the Cordova stakeholders' meetings as required.

Contractual: A total of $663.6k of the requested EVOS funding is for a major contract to **MariCal,** the key collaborating partner. See detail in separate justification section below. Funds are requested for providing space for rearing herring both at ASLC (3 months per year in Years 2-4) and SSH (2 months in Year 1; 3 months per year in Years 2-4). Vessel charter funds are requested each year to collect sexually mature herring by gill net from PWS spawning stock. Data management costs will provide electronic data storage in the ASLC data warehouse and technical expertise with computing, data manipulation and analysis in Years 2-4.

Commodities: We are requesting $92.1k over three years for commodities including chemical reagents and feed for herring culture and laboratory analysis of samples collected to characterize growth and development. Funds are requested in Years 1 and 2 to purchase tanks for larval herring and live feed at the SSH. Funds are also requested for expenses relating to public outreach, especially the Cordova stakeholders' meetings and exhibitry or public display.

Equipment: An experimental net pen is requested for field studies in the fourth year of the project. The estimated cost for the system including nets is $60k.

Indirect: The ASLC indirect rate is calculated as 27.08% of modified total direct costs (excluding equipment greater than $5,000).

MariCal, Inc. Component Justification (major contractor)

Personnel: MariCal personnel include: **Tim Linley Ph.D**., Senior Research Scientist will serve as the Principal Investigator for the project and coordinate all aspects of the studies, including data collection, analysis and reporting. Dr. Linley will devote 2 months of his time in Year 1 and 50% of his time annually in Years 2-4 to the project, of which approximately 2 months per year will be spent in Alaska at Seward or in PWS. **Marlies Betka Ph.D.**, Vice President of Research, will serve as co-P.I. for the project. Dr. Betka will establish and supervise protocols for immunocytochemistry, protein expression and Na+K+ATPase activity assays and collaborate with the P.I. and consultants in data analysis and reporting. Dr. Betka will devote 0.5 months of her time in Year 1 and 33% of her time annually in Years 2-4 to the project. **Caroline Cherry, M.S.,** Senior Research Associate for MariCal, is based at the ASLC where she manages the husbandry and research activities for the joint ASLC–MariCal salmon project. Ms. Cherry will assist in sample collection and conduct the molecular assays for the project at the ASLC in conjunction with her duties there. Her contribution (1.5 months annually) to the project is in-kind. We are also requesting funds for a **full time Research Associate** dedicated to herring project activities in Seward and Prince William Sound in Years 2-4. This person will have responsibility for day to day operation of the project, sampling, data collection, and will assist in analysis and report writing. The person in this position will have post-graduate education in marine biology, fisheries or aquaculture, with at least 5 years of experience in marine fish culture, including live feeds, and preferably stock enhancement of marine fish. In addition to their education and experience, we will also provide training and additional hands on experience for this individual through the Virginia Tech Aquaculture Consulting Group. This individual will be under the direct supervision of the P.I.

Travel: We are requesting $12.4k in Year 1 and $17.7k annually in Years 2-4 in travel for Linley, Betka and the to-be-named Research Associate to attend several professional meetings and for sample collections and travel to PWS. Travel will include the annual Marine Science Symposium in Anchorage, American Fisheries Society annual meeting, and meetings that involve herring restoration planning, community participation and outreach. The total includes 10 round trip inter- and intra state airfares and per diem at $200 per day.

Contractual: A total of $158k is requested over 4 years (75 days annually in Years 2-4) for professional service by the **Virginia Tech Consulting Group** (CVs included) to establish protocols, design and assist in operation of the larval herring and live feed culture system at the SSH. Approximately half of the Group's time will be engaged in training the MariCal Research Associate in methods for larval fish and live feed at Virginia Tech Hampton Facility as well as consultation with the P.I. to plan experiments, provide advice during larval culture, and participate in data analysis. The remaining time will be spent at ASLC participating in larval culture efforts. Travel is included in this contractual request for 2 members to attend the EVOS annual meeting and for travel to Seward and residence while there (1 month annually) to conduct work at the SSH and ASLC. We are also requesting funds in the amount of $4,700 annually in Year 2-4 (0.5 months) for **Dr. Evelyn Brown**. Dr. Brown will coordinate integration of the stock enhancement research activities with related projects in ecosystem modeling and the experimental nursery bay in Prince William Sound.

Commodities: The only requested commodities item is shipping costs for sending samples back and forth between MariCal's Maine headquarters and the project location in Alaska.

Data Management and Quality Assurance/Quality Control Statement

1. This experimental study is a factorial design (fixed effects) to analyze species-specific data. The dependent variables to be studied include: survival, length, weight, Na^+K^+ATPase activity, whole body energy content, blood ion concentration, and calcium receptor expression and concentration. Data types include frequency as well as continuous variables. Survival will be tested via likelihood ratio (G-test). All other data collected will be subjected to repeated measures ANOVA.
2. Power analyses were conducted for sampling regimes at each stage of the study to ensure adequate sample sizes for statistical analyses.
3.
    a.) Export from file follows.
    b.) Fields associated with dataset:
        i. Length (mm)
        ii. Weight (g)
        iii. Survival (#)
        iv. Whole body energy content (kJ/g)
        v. Blood ion concentration (mmol/L)
        vi. Na^+K^+ATPase activity (µMol ADP•hr^{-1}•mg protein)
        vii. Calcium receptor expression
4. Not applicable.
5. All samples will be collected, appropriately labeled, and frozen by personnel from MariCal, Inc. or the Alaska SeaLife Center until analyzed. Samples will be stored in regular or ultralow temperature freezers, as appropriate, at the Alaska SeaLife Center and catalogued in Freezerworks software to facilitate tracking and distribution.
6. Calibration of analytical equipment will be performed by methods supported by the manufacturer of the equipment.
7. Information collected from laboratory analyses is entered into Microsoft Access and stored jointly on servers at ASLC and at MariCal. Statistical methods will be confined to ANOVA and are described above and in the Project Plan. All results from studies will appear in Annual Reports and peer reviewed publications. Statistical software will include SYSTAT and SigmaStat.
8. The data collected by this project will be integrated with the Alaska SeaLife Center's Scientific Data Warehouse. Centralization of this data, into a data warehouse will ensure security and facilitating analysis and dissemination of this valuable information. Inclusion into this data warehouse allows the attachment of metadata as well as the implementation of standards and quality assurance/control. Data input forms check the data so typing or other mistakes do not put incorrect data in the database. This and implementation of additional QA/QC procedures assure the highest quality datasets. The design of this system incorporates open standards, including XML, SOAP, TCP/IP and HTML to facilitate integration with other systems and organizations. Collaboration with other institutes and scientists will greatly enhance the value of the datasets collected. This scientific data warehouse will provide the framework and tools for efficient analysis of the data.

EXPORT FROM METALITE METADATA FILE

Development of Technology to Support Restoration of Herring in Prince William Sound: Use of In Vitro Studies to Validate and Optimize Restoration Actions

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What does this data set describe?

Title:
Development of Technology to Support Restoration of Herring in Prince William Sound: Use of In Vitro Studies to Validate and Optimize Restoration Actions
Abstract:
Data contained in this study include gross length and weight of herring through their juvenile life history; changes in molecular traits that are involved in osmoregulation, nutrition and immune function; bioenergetic status at various juvenile life stages.

1. **How should this data set be cited?**

   Linley, T, 20071231, Development of Technology to Support Restoration of Herring in Prince William Sound: Use of In Vitro Studies to Validate and Optimize Restoration Actions: Aquaculture Research, London, England.

   Online Links:

   - <http://www.blackwell.synergy.com/>

2. **What geographic area does the data set cover?**

   West_Bounding_Coordinate: 149.7322
   East_Bounding_Coordinate: 145.5708
   North_Bounding_Coordinate: 61.2667
   South_Bounding_Coordinate: 59.8075

3. **What does it look like?**
4. **Does the data set describe conditions during a particular time period?**

   Beginning_Date: 01-Mar-2007
   Ending_Date: 03-Mar-2009
   Currentness_Reference: ground condition

5. **What is the general form of this data set?**

   Geospatial_Data_Presentation_Form: diagram

6. **How does the data set represent geographic features?**
    a. **How are geographic features stored in the data set?**

        This is a Point data set.

    b. **What coordinate system is used to represent geographic features?**
7. **How does the data set describe geographic features?**

Who produced the data set?

1. **Who are the originators of the data set?** (may include formal authors, digital compilers, and editors)
    - Linley, T
2. **Who also contributed to the data set?**
3. **To whom should users address questions about the data?**

Why was the data set created?

These data are needed to characterize important factors for culture and stock enhancement in herring.

How was the data set created?

1. **Where did the data come from?**
2. **How were the data processed and modified?**

How reliable are the data; what problems remain in the data set?

How can someone get a copy of the data set?

Are there legal restrictions on access or use of the data?

Access_Constraints: None
Use_Constraints: Not to be used for publication or citation without permission

1. **Who distributes the data set?** (Distributor 1 of 1)

    T. Linley
    MariCal, Inc.
    400 Commercial Street
    Portland, ME 04101

Portland, ME 04101
USA

207-773-2500 (voice)
207-773-2522 (FAX)
tlinley@marical.biz

2. **What's the catalog number I need to order this data set?**
3. **What legal disclaimers am I supposed to read?**

   Distributor assumes no liability for use of data by third parties

4. **How can I download or order the data?**

Who wrote the metadata?

Dates:

Last modified: 03-Aug-2006

Metadata author:

T. Linley
MariCal, Inc.
400 Commercial Street
Portland, ME 04101
Portland, ME 04101
USA

207-773-2500 (voice)
207-773-2522 (FAX)
tlinley@marical.biz

Metadata standard:

FGDC Content Standards for Digital Geospatial Metadata (FGDC-STD-001-1998)

Generated by mp version 2.6.0 on Fri Aug 04 11:28:24 2006

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Budget Category:	Approved FY 07	Proposed FY 08	Proposed FY 09	Proposed FY 10	TOTAL PROPOSED
Personnel	$4.9	$23.3	$24.6	$25.7	$78.5
Travel	$1.5	$3.0	$3.0	$3.0	$10.5
Contractual	$50.5	$218.4	$249.7	$264.0	$782.6
Commodities	$10.0	$24.5	$23.8	$33.8	$92.1
Equipment	$0.0	$0.0	$0.0	$60.0	$60.0
Subtotal	$66.9	$269.2	$301.1	$386.5	$1,023.7
ASLC - Indirect	$18.1	$72.9	$81.5	$88.4	$260.9
Total w/o G&A	$85.0	$342.1	$382.6	$474.9	$1,284.6
General Administration (9% of subtotal)	$7.7	$30.8	$34.4	$42.7	$115.6
Project Total w/G&A	$92.7	$372.9	$417.0	$517.6	$1,400.2
FTE	0.3	2.2	2.2	2.2	
Other Resources: (Cost Shares)					

Comments:
Trustee Council approved the FY 07 funding only; PI will need to resubmit the proposal request for FY 08 - FY 10.

In this box, identify non-EVOS funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

FY 07-10

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

Date Prepared:

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Description	Step	Budgeted	Costs	Overtime	Sum
Howard Ferren	Co-PI		0.5	7.5	0.0	3.8
Brendan Smith	Research Education Coordinator		0.3	3.8	0.0	1.1
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.8	11.3	0.0	
					Personnel Total	$4.9

Travel Costs:		Ticket	Round	Total	Daily	Travel
Description		Price	Trips	Days	Per Diem	Sum
Annual Marine Science Symposium	("ticket" = mileage)	0.1	1	2	0.2	0.5
Herring Research/Planning meetings	("ticket" = mileage)	0.1	1	1	0.2	0.3
Cordova Stakeholders meetings		0.5	1	1	0.2	0.7
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$1.5

FY 07

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Contractual Costs:			Contractual
Description			Sum
4A Linkage	MariCal, Inc.		42.9
Seward Shellfish Hatchery facility lease		2 months @ $3.8k/mo	7.6
If a component of the project will be performed under contract, the 4A and 4B forms are required.		**Contractual Total**	$50.5

Commodities Costs:		Commodities
Description		Sum
Rearing tanks for shellfish hatchery	8 @ $300 each	2.4
Misc plumbing fittings		0.5
Chemicals, reagents, feed		6.5
Freight/postage		0.5
Stakeholders' meeting supplies and handouts		0.1
	Commodities Total	$10.0

FY 07

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

New Equipment Purchases:	Number	Unit	Equipment
Description	of Units	Price	Sum
			0.0
none			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency

FY 07

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Description	Step	Budgeted	Costs	Overtime	Sum
Howard Ferren	Co-PI		1.5	7.5	0.0	11.3
Dana Sitzler	Education Director		0.5	7.0	0.0	3.5
Brendan Smith	Research Education Coordinator		0.5	3.8	0.0	1.9
Rachel Simon	Distance Education Specialist		2.0	3.3	0.0	6.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		4.5	21.6	0.0	
					Personnel Total	$23.3

Travel Costs:		Ticket	Round	Total	Daily	Travel
Description		Price	Trips	Days	Per Diem	Sum
Annual Marine Science Symposium	("ticket" = mileage)	0.1	1	4	0.2	0.9
Herring Research/Planning meetings	("ticket" = mileage)	0.1	3	3	0.2	0.9
Cordova Stakeholders meetings		0.4	2	2	0.2	1.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$3.0

FY 08

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 07 - FY 10

Contractual Costs:			Contractual
Description			Sum
4A Linkage MariCal, Inc.			191.3
ASLC facilities costs for Lab 160	3 months @ $2.9k/mo		8.7
Seward Shellfish Hatchery facility lease	3 months @ $3.8k/mo		11.4
Vessel Charter			2.0
Data management			5.0
If a component of the project will be performed under contract, the 4A and 4B forms are required.		**Contractual Total**	$218.4
Commodities Costs:			Commodities
Description			Sum
Rearing tanks for shellfish hatchery	16 @ $300 each		4.8
Misc plumbing fittings			1.5
Chemicals, reagents, feed			13.5
Freight/postage			1.5
Stakeholders' meeting supplies and handouts			0.2
Supplies for exhibitry/public display			3.0
		Commodities Total	$24.5

FY 08

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

New Equipment Purchases:	Number	Unit	Equipment
Description	of Units	Price	Sum
			0.0
none			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency

FY 08

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Description	Step	Budgeted	Costs	Overtime	Sum
Howard Ferren	Co-PI		1.5	7.9	0.0	11.9
Dana Sitzler	Education Director		0.5	7.4	0.0	3.7
Brendan Smith	Research Education Coordinator		0.5	4.0	0.0	2.0
Rachel Simon	Distance Education Specialist		2.0	3.5	0.0	7.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		4.5	22.8	0.0	
					Personnel Total	$24.6

Travel Costs:		Ticket	Round	Total	Daily	Travel
Description		Price	Trips	Days	Per Diem	Sum
Annual Marine Science Symposium	("ticket" = mileage)	0.1	1	4	0.2	0.9
Herring Research/Planning meetings	("ticket" = mileage)	0.1	3	3	0.2	0.9
Cordova Stakeholders meetings		0.4	2	2	0.2	1.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$3.0

FY 09

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Contractual Costs:			Contractual
Description			Sum
4A Linkage	MariCal, Inc.		209.6
ASLC facilities costs for Lab 160		3 months @ $2.9k/mo	9.1
Seward Shellfish Hatchery facility lease		3 months @ $3.8k/mo	12.0
Vessel Charter			2.0
Data management			17.0
If a component of the project will be performed under contract, the 4A and 4B forms are required.		**Contractual Total**	$249.7

Commodities Costs:	Commodities
Description	Sum
Misc plumbing fittings	0.5
Chemicals, reagents, feed	20.0
Freight/postage	2.0
Stakeholders' meeting supplies and handouts	0.3
Supplies for exhibitry/public display	1.0
Commodities Total	$23.8

FY 09

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

New Equipment Purchases:	Number of Units	Unit Price	Equipment Sum
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage:	Number of Units	Inventory Agency
Description		

FY 09

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Description	Step	Budgeted	Costs	Overtime	Sum
Howard Ferren	Co-PI		1.5	8.3		12.5
Dana Sitzler	Education Director		0.5	7.7		3.9
Brendan Smith	Research Education Coordinator		0.5	4.2		2.1
Rachel Simon	Distance Education Specialist		2.0	3.6		7.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		4.5	23.8	0.0	
					Personnel Total	$25.7

Travel Costs:		Ticket	Round	Total	Daily	Travel
Description		Price	Trips	Days	Per Diem	Sum
Annual Marine Science Symposium	("ticket" = mileage)	0.1	1	4	0.2	0.9
Herring Research/Planning meetings	("ticket" = mileage)	0.1	3	3	0.2	0.9
Cordova Stakeholders meetings		0.4	2	2	0.2	1.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$3.0

FY 10

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Contractual Costs:			Contractual
Description			Sum
4A Linkage	MariCal, Inc.		219.8
ASLC facilities costs for Lab 160		3 months @ $2.9k/mo	9.6
Seward Shellfish Hatchery facility lease		3 months @ $3.8k/mo	12.6
Vessel Charter			2.0
Data management			20.0
If a component of the project will be performed under contract, the 4A and 4B forms are required.		**Contractual Total**	$264.0

Commodities Costs:	Commodities
Description	Sum
Misc plumbing fittings	0.5
Chemicals, reagents, feed	25.0
Freight/postage	7.0
Stakeholders' meeting supplies and handouts	0.3
Supplies for exhibitry/public display	1.0
Commodities Total	$33.8

FY 10

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

New Equipment Purchases:	Number	Unit	Equipment
Description	of Units	Price	Sum
			0.0
Experimental net pens			60.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$60.0

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency

FY 10

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Agency: Alaska SeaLife Center via ADF&G

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Budget Category:	Proposed FY 07	Proposed FY 08	Proposed FY 09	Proposed FY 10	TOTAL PROPOSED
Personnel	$17.2	$125.4	$131.2	$138.8	$412.6
Travel	$12.4	$17.7	$17.7	$17.7	$65.5
Contractual	$13.0	$47.7	$60.2	$62.8	$183.7
Commodities	$0.3	$0.5	$0.5	$0.5	$1.8
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0
Subtotal	$42.9	$191.3	$209.6	$219.8	$663.6
Indirect (rate will vary by contractor)					
Project Total	$42.9	$191.3	$209.6	$219.8	$663.6

FY 07 - FY 10

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Personnel Costs:				Months	Monthly		Personnel
	Name	Description		Budgeted	Costs	Overtime	Sum
	Tim Linley	Principal Investigator		2.0	6.7	0.0	13.4
	Marlies Betka	Co-PI		0.5	7.5	0.0	3.8
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		2.5	14.2	0.0	
						Personnel Total	$17.2

Travel Costs:		Ticket	Round	Total	Daily	Travel
	Description	Price	Trips	Days	Per Diem	Sum
	Annual Marine Science Symposium	1.1	1	4	0.2	1.9
	Herring Research/Planning meetings	1.1	1	3	0.2	1.7
	Cordova Stakeholders meetings	0.4	1	1	0.2	0.6
	Herring culture investigations, Japan	1.1	1	31	0.2	7.3
	Collections, field work PWS	0.5	1	2	0.2	0.9
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$12.4

FY 07

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Contractual Costs:	Contractual
Description	Sum
Virginia Tech Aquaculture Center consulting contract	10.0
Communications and publications	1.0
Vessel or aircraft charter	2.0
Contractual Total	$13.0

Commodities Costs:	Commodities
Description	Sum
Freight/postage	0.3
Commodities Total	$0.3

FY 07

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

New Equipment Purchases:	Number of Units	Unit Price	Equipment Sum
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage:	Number of Units	
Description		

FY 07

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Personnel Costs:				Months	Monthly		Personnel
	Name	Description		Budgeted	Costs	Overtime	Sum
	Tim Linley	Principal Investigator		6.0	6.7	0.0	40.2
	Marlies Betka	Co-PI		4.0	7.5	0.0	30.0
	TBD	Research Associate		12.0	4.6	0.0	55.2
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		22.0	18.8	0.0	
						Personnel Total	$125.4

Travel Costs:		Ticket	Round	Total	Daily	Travel
	Description	Price	Trips	Days	Per Diem	Sum
	Annual Marine Science Symposium	1.1	3	12	0.2	5.7
	Herring Research/Planning meetings	1.1	4	12	0.2	6.8
	Cordova Stakeholders meetings	0.4	2	2	0.2	1.2
	Scientific Meeting (Linley)	0.7	1	5	0.3	2.2
	Collections, field work PWS	0.5	2	4	0.2	1.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$17.7

FY 08

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Contractual Costs:	Contractual
Description	Sum
Virgina Tech Aquaculture Center consulting contract	40.0
Evelyn Brown consulting contract	4.7
Communications and publications	3.0
Contractual Total	$47.7

Commodities Costs:	Commodities
Description	Sum
Freight/postage	0.5
Commodities Total	$0.5

FY 08

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

New Equipment Purchases:	Number of Units	Unit Price	Equipment Sum
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage:	Number of Units	
Description		

FY 08

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Personnel Costs:				Months	Monthly		Personnel
	Name	Description		Budgeted	Costs	Overtime	Sum
	Tim Linley	Principal Investigator		6.0	7.0	0.0	42.0
	Marlies Betka	Co-PI		4.0	7.9	0.0	31.6
	TBD	Research Associate		12.0	4.8	0.0	57.6
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		22.0	19.7	0.0	
						Personnel Total	$131.2

Travel Costs:		Ticket	Round	Total	Daily	Travel
	Description	Price	Trips	Days	Per Diem	Sum
	Annual Marine Science Symposium	1.1	3	12	0.2	5.7
	Herring Research/Planning meetings	1.1	4	12	0.2	6.8
	Cordova Stakeholders meetings	0.4	2	2	0.2	1.2
	Scientific Meeting (Linley)	0.7	1	5	0.3	2.2
	Collections, field work PWS	0.5	2	4	0.2	1.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$17.7

FY 09

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Contractual Costs:	Contractual
Description	Sum
Virgina Tech Aquaculture Center consulting contract	52.5
Evelyn Brown consulting contract	4.7
Communications and publications	3.0
Contractual Total	$60.2

Commodities Costs:	Commodities
Description	Sum
Freight/postage	0.5
Commodities Total	$0.5

FY 09

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

New Equipment Purchases:	Number of Units	Unit Price	Equipment Sum
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage:	Number of Units	
Description		

FY 09

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Personnel Costs:				Months	Monthly		Personnel
	Name	Description		Budgeted	Costs	Overtime	Sum
	Tim Linley	Principal Investigator		6.0	7.4	0.0	44.4
	Marlies Betka	Co-PI		4.0	8.3	0.0	33.2
	TBD	Research Associate		12.0	5.1	0.0	61.2
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		22.0	20.8	0.0	
						Personnel Total	$138.8

Travel Costs:		Ticket	Round	Total	Daily	Travel
	Description	Price	Trips	Days	Per Diem	Sum
	Annual Marine Science Symposium	1.1	3	12	0.2	5.7
	Herring Research/Planning meetings	1.1	4	12	0.2	6.8
	Cordova Stakeholders meetings	0.4	2	2	0.2	1.2
	Scientific Meeting (Linley)	0.7	1	5	0.3	2.2
	Collections, field work PWS	0.5	2	4	0.2	1.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$17.7

FY 10

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 07 - FY 10

Contractual Costs:	Contractual
Description	Sum
Virgina Tech Aquaculture Center consulting contract	55.1
Evelyn Brown consulting contract	4.7
Communications and publications	3.0
Contractual Total	$62.8

Commodities Costs:	Commodities
Description	Sum
Freight/postage	0.5
Commodities Total	$0.5

FY 10

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.

New Equipment Purchases:	Number of Units	Unit Price	Equipment Sum
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Indicate replacement equipment with an R.		**New Equipment Total**	$0.0

Existing Equipment Usage:	Number of Units	
Description		

FY 10

Project Number: 070821
Project Title: Development of Technology to Support Restoration of Herring in PWS: Use of in vitro studies...
Name of Contractor: MariCal, Inc.