October 30, 2007

Mr. Michael Baffrey
Executive Director
Exxon Valdez Trustee Council
441 West 5th Avenue, Suite 500
Anchorage, AK 99501-2340

Dear Mr. Baffrey:

We are writing to you in response to the decision made by the EVOS Trustee Council at the October 12, 2007 meeting that we participated in regarding the partial funding of our FY 2008 proposal, *Development of Culture Technology to Support Restoration of Herring in Prince William Sound: Use of In Vitro Studies to Validate and Optimize Restoration Actions.* As you recall, discussions by the Trustee Council emphasized the importance of maintaining our established collaboration with the Japanese scientists because of their expertise in the field of herring culture techniques. According to our understanding of the directive provided by the Trustee Council at the meeting, you, as the Director, have been given the discretion to develop and fund a 1 yr modified proposal with MariCal and the Alaska SeaLife Center to further this collaboration with these Japanese researchers and provide EVOS the option to expand this collaboration should herring culture techniques be pursued to help restore herring in Prince William Sound.

In lieu of the Trustee Council's decision to defer funding on the specific aspects of our project involving herring culture techniques until completion of a herring restoration plan, we are respectfully submitting the following amended proposal that is specifically designed to maintain our previously established collaboration with the Japanese herring researchers and foster their further involvement to address critical questions regarding factors that may be limiting recovery of herring in Prince William Sound. Please note that the two Objectives we have proposed are focused on scientific topics that were encompassed by our original proposal and thus should not require any additional scientific or technical review.

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Objective 1: Plan and coordinate travel for Dr. Takahiro Matsubara and an associate or designee to travel to Alaska to attend the Marine Science Symposium in January 2008, participate in an EVOS Trustee Council sponsored workshop on herring stock restoration, visit the fish culture facilities at the ASLC, the Seward Shellfish Hatchery and USGS Marrowstone Field Lab (Nordland, WA), tour potential stock restoration rearing and release sites in PWS (e.g. Tatitlik), and meet with scientists and interested parties involved in the Prince William Sound herring restoration effort. Dr. Matsubara is an internationally recognized expert in the field of fish endocrinology with a specific focus in the area of reproduction. He had authored over 50 peer reviewed papers and has collaborated on multiple occasions with fish physiology researchers in the U.S. As the Section Chief for the Resources Enhancement Section of the Hokkaido National Fisheries Research Institute, he directs all aspects of fish culture investigations at the Akkeshi Field Station, including those for Pacific herring.

For Objective 1, we propose to have Dr. Matsubara and an associate make a presentation at and EVOS TC sponsored herring restoration workshop to be held in conjunction with the Marine Science Symposium in January 2008. The goal of the workshop will be to provide a forum for the exchange of information related to past, present and future efforts to restore herring stocks in Japan and PWS. Dr. Matsubara and his colleague(s) will describe the research, development and implementation of herring culture techniques for stock supplementation that has taken place in Japan during the last 20 years. The presentation will focus on the technical problems encountered during the early phases of the program, the economic bottlenecks in scaling up production from the laboratory to large-scale releases for stock supplementation, and post-release evaluation to determine thee contribution to recruitment.

In conjunction with this trip, we will arrange for Dr. Matsubara and his colleague(s) to travel to Seward to tour the ASLC and Seward Shellfish Hatcheries where they will be able to view and assess these facilities as to their potential use for conducting culture related research for herring. This visit will include a review of the ASLC – MariCal program progress to date and discussions to guide future research efforts and topics. We will also attempt to include in these collective discussions with Dr. Matsubara a preliminary assessment of the likely costs of scaling a hatchery-based herring culture effort into a larger pilot-study project for releasing juvenile herring in PWS. As part of this workshop, we are also planning a meeting between Dr. Matsubara and Dr. Shannon Atkinson, UAF faculty member and director of the UAF Endocrinology Laboratory which is located at the ASLC. The purpose of this meeting and laboratory tour will be to foster further linkages between Alaska based endocrinology researchers and determine the availability and adequacy of the laboratory to conduct specific analyses for the research described below.

In addition to the proposed travel to Seward, we will also make arrangements for Dr. Matsubara and his colleague(s) to get a short (half day) tour of PWS and visit the community and a potential field site for a pilot-scale herring rearing and release project at Tatitlik. The purpose of this visit will be to broaden the interaction between scientists involved in herring stock restoration and PWS community stakeholders. Such interaction will help provide a foundation for community participation in the recovery efforts in PWS, much as they have for in Japan where community based enhancement projects play a major role in supplementing wild herring stock production.

Finally, we will coordinate with Dr. Paul Hershberger of the USGS for Dr. Matusbara and his colleague(s) to visit the Marrowstone Field Station in Nordland, WA before or after travel to Alaska. The endocrine studies proposed as part of Objective 2 are directly linked to environmental stress and disease expression in PWS herring (e.g. VHS) and this meeting will be used to develop hypotheses regarding the role of disease on reproductive function in herring, and the potential implications for stock restoration in PWS. It will also give the Japanese the opportunity to view and assess the culture techniques employed at one of major herring research facilities in the U.S.

Objective 2: Conduct an assessment (survey) of yolk proteins and products in female herring that affect gamete quality and potential larval recruitment during the spawning cycle in PWS during 2008. This second Objective is an extension of our initial sampling work in Prince William Sound and focuses on the characterization of the egg quality from spawning females and collected from roe-on-kelp sources. Data gathered from this effort will provide important insights into how spawning stress experienced by herring may affect gamete quality and larval production in the wild as well as under culture conditions. These proposed studies will focus on analysis of yolk proteins within collected eggs. Such studies will directly benefit from the collaboration of Dr. Matsubara and his colleagues as a result of his expertise in fish reproductive endocrinology.

Yolk formation in oocytes (developing eggs) of pelagic marine fish occurs through the accumulation of vitellogenin and its structural proteins, lipovitellin and phosvitin, that are synthesized by the liver in response to ovarian-derived estrogen (Hiramatsu et al. 2002). These proteins supply the amino acids necessary for embryonic development, as well as bind and deliver lipids and minerals (e.g. calcium) used for energy and structural synthesis by developing embryo. Studies have shown that stress (measured as or directly induced by cortisol) interferes with the production of vitellogenin (Berg et al. 2004), possibly through down regulation of estrogen-mediated estrogen receptor transcription (Lethimonier et al. 2000). The documented expression and incidence of viral hemorrhagic septicemia (VHS) and other pathogens in PWS herring suggest that these fish experience stress at the time of spawning which could potentially affect gamete quality and larval production. These effects may be manifest as reduced

levels of amino acids, lipids or essential minerals. Understanding time-related changes in these proteins during the spawning cycle will have direct benefit for gamete as well as roe-on-kelp collection for stock supplementation projects.

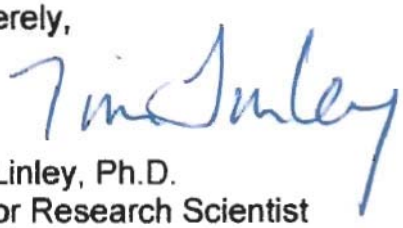
In this study, we propose to sample, measure and characterize changes in yolk proteins, amino acids and bound calcium levels in the blood and (developing) eggs of pre-spawning and spawning herring during the early, middle and late part of the spawning cycle within Matthews Bay (site of our 2007 gamete and roe-on-kelp collections), or an alternate spawning site as determined by the ADF&G. Our objective is to measure these changes in visibility healthy as well as stressed (diseased) fish identified on the basis of external symptoms (hemorrhagic tissue). Analyses of yolk proteins will be conducted at the UAF Endocrinology Lab at the ASLC and follow the method described by Ohkubo et al. (2006) and Sawaguchi et al. (2006). Analyses of amino acid content and bound calcium in eggs will be conducted at the MariCal molecular biology lab in Portland, ME. Tissue samples will be sent to the ADF&G pathology lab to determine the presence of VHS or other documented PWS herring pathogens.

Budget Request: As indicated on the attached budget, we are requesting a total of $80,600, (not including G&A), to conduct this work that will directly maintain and foster our established collaboration with the Hokkaido National Fisheries Research Institute scientists. Our request includes 2 months of personnel time for the P.I. and one month for the co-P.I. They will personally arrange for Dr. Matsubara's visit and coordinate all site visit and workshop activities. Travel in the amount of $20,000 is requested to bring Dr. Matsubara and a colleague to Alaska for 10 days each and for the P.I. and co P.I. to accompany them during their meetings, presentation and site visits. A consulting contract of $5000 is also included for Dr. Matsubara to review in detail the data obtained from our first year of effort, other data presented to him as part of any workshop proceedings, and to help establish and interact in efforts to characterize yolk protein synthesis and utilization in Pacific herring. An additional $11,000 is requested for contractual analyses of yolk and stress proteins at the ASLC, and $1,500 for supplies..

Lastly, we would like to call to your attention the need for your prompt action on our request for possible EVOS funding of our amended proposal as detailed above. We are very interested in securing a place for Dr. Matsubara as a featured speaker for the Marine Science Symposium that occurs in January 2008 as a key part of Objective #1. In order for us to proceed in this effort, we would very much like to receive a reply from your office as soon as possible. To this end, we have already secured an appointment with you this Friday, October 26th where we can address any questions or concerns that you or your staff might have after review of the proposal. We thank you for your consideration of furthering the collaboration we have established with the herring researchers from the Hokkaido National Fisheries Research Institute. We believe the

contribution of these scientists to the recovery effort for herring in PWS will be significant and that the funds requested are vital to help us ensure their continuing participation.

Sincerely,



Tim Linley, Ph.D.
Senior Research Scientist

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Herring Japanese Collaboration Budget

Single Year Budget - November 1, 2007 - October 31, 2008

				FY 08
Personnel (includes fringe)		**Mos**	**Rate/mo**	
Howard Ferren	Co-PI	1	7.5	7.5
Subtotal Personnel				**7.5**
Travel		**Quantity**	**Cost each**	
Annual EVOS Meeting (Ferren, Scientist)				
Mileage		1	0.1	0.1
Per diem		5	0.2	1.0
Subtotal Travel				**1.1**
Contractual: vessel charters, equipment rental or lease, professional services, communications, printing				
MariCal				46.4
UAF - Endocrinology Lab				
Cortisol ELISA (n=60) @ $75 per sample		60	0.07	4.2
Vitellogenin ELISA (n=60) @ $75 per sample		60	0.07	4.2
Subtotal Contractual				**54.8**
Commodities: expendable supplies w/ estimated life of less than one year and a unit value <$1,000				
Subtotal Commodities				**0.0**
Equipment: non-expendable items w/ estimated life of more than one year and a unit value >$1,000				
Subtotal Equipment				**0.0**
Subtotal Direct Costs All Categories				**63.4**
Modified Total Direct Costs (exclude Equipment)				63.4
ASLC indirect rate 27.08% of MTDC (modified total direct cost)			27.08%	17.2
TOTAL COSTS w/o G&A				**80.6**
TOTAL COSTS including G&A				**87.9**

Herring Japanese Collaboration Budget

Single Year Budget - November 1, 2007 - October 31, 2008

				FY 08
Personnel		**Mos**	**Rate/mo**	
Tim Linley	Principal Investigator	2	7.1	14.2
Subtotal Personnel				**14.2**
Travel		**Quantity**	**Cost each**	
Annual EVOS Meeting (Linley)				
Airfare		1	1.1	1.1
Per diem		10	0.2	2.0
Scientific Meeting - AFS (Linley)				
Airfare		1	0.7	0.7
Per diem		5	0.3	1.5
Japan-US travel (Matsubara)				
Airfare		2	2.6	5.2
Per diem		20	0.3	6.0
PWS site tour		1	2.5	2.5
Collections, field work PWS (TBD)				
Airfare		3	0.5	1.5
Per diem		6	0.2	1.2
Subtotal Travel				**21.7**
Contractual: vessel charters, equipment rental or lease, professional services, communications, printing				
T. Matsubara (HNFRI) consulting contract				5.0
Vessel charter - fish collection		3	1	3.0
Communications				1.0
Subtotal Contractual				**9.0**
Commodities: expendable supplies w/ estimated life of less than one year and a unit value <$1,000				
Chemicals, reagents				
HPLC (amino acids)				0.5
Spectrophotometry (calcium)				0.5
Freight/postage				0.5
Subtotal Commodities				**1.5**
Equipment: non-expendable items w/ estimated life of more than one year and a unit value >$1,000				
Subtotal All Categories				**46.4**