**Eastern Bering Sea Slope**

**Title:** Bitter Crab Syndrome in the North Pacific

**General Description and Justification:** Please provide a succinct (500 words maximum) description and justification for your proposed project or collection. Please be sure to detail the goals of the project, how the data or specimens will be used, and why it is important for this project to take place at this time (opposed to during future surveys, other available research platforms, etc.).

Bitter crab syndrome (BCS) is a potentially fatal disease of commercially important *Chionoecetes* spp. in the North Pacific and is emerging worldwide in other decapod hosts. Efforts to monitor disease incidences and distribution in the Bering Sea over the last 25 years have generated data to track possible trends in the North Pacific and may provide insight as to how climate conditions influence disease patterns.

Recently, the causative agent of BCS was identified in king crab from Russian waters demonstrating susceptibility of these hosts. BCS has not been identified in king crabs from Alaskan waters, but considering the commercial importance of king crab, monitoring these species for the disease is of added importance. Additional sampling efforts targeting deep water crabs to determine incidence and distribution of BCS may lead to information on BCS associated with changes in abiotic factors, particularly depth and temperature.

The primary goal of this project is to survey EBS slope *Chionoecetes bairdi* and *C. opilio* for BCS using molecular methods. Additionally, visually infected *Lithodes aequispina* will be sampled for BCS. This as a proactive approach to disease monitoring and estimating that portion of natural mortality associated with BCS in North Pacific commercial crab species.

**Name of Requestor / Point of Contact:** Pam Jensen

**Email:** Pam.Jensen@NOAA.gov

**Daytime Telephone:** 206-526-4122

**Affiliation of Requestor:** AFSC – Kodiak

**Data Types:** Biological specimen

**Detailed collection procedure:** Provide a detailed description of collection procedures including the use of any special equipment and forms. Be as specific as possible and include the desired sampling location(s). If your protocol is graphical and/or cannot be described in text, e-mail the file, including your project title, to RACE.Surveycollections@noaa.gov. For accepted projects, the applicant will be asked to provide an updated full draft of the protocols for inclusion in the At-Sea Operations Manual and a ‘cheat sheet’ for deck operations along with an example of any special data forms.If e-mailing a file, please reference the title of your project in the filename and list below in the provided box.

Sampling will be carried out by Kodiak personnel (Pam Jensen). *Chionoecetes bairdi* and *C. opilio* will be sampled as they are caught, with no more than 50 samples per station. Only visually BCS-positive looking *Lithodes aequispina* will be sampled. For each crab, record species, size, sex, shell condition, haul number and visual BCS status on the provided 96-well format datasheet. Using a clean syringe, extract >0.2mL hemolymph, and remove air from syringe. Drip one drop of hemolymph onto a clean labeled slide (use pencil to write Plate##, Well Alpha# on slide) and smear the drop using another clean slide (allow slides to air-dry inside). Then, insert the remaining 0.2mL hemolymph into the corresponding well in the 96-well collection plate of 100% ethanol. Invert plate to mix, dispose of sharps and biological waste. If time is short, or crabs are too small for successful hemolymph withdrawl, place a leg from large crab, or place entire small crab, in a plastic bag with label and freeze instead of drawing hemolymph.

**Geographic Region of Collection** Can your collection be distributed throughout the entire survey area? If not and the sampling area is more restricted than the general survey terms of Gulf of Alaska or Eastern Bering Sea Shelf then please provide bounding coordinates and name of region (if known) in the fields provided below.

Place keywords: Example: Bristol Bay, Kodiak, Shumagin,Yakutat

Opportunistic sampling

**Fish Crab or Invertebrate?**

Crab

**Species to be collected:** List species by scientific names

*Chionoecetes bairdi*

*Chionoecetes opilio*

*Lithodes aequispinus*

**Estimated Time:** In general, how much time does it take to set up, collect, record, and preserve each sample ?

Approximately 2-3 minutes per sample

**Sampling Design**\*Please select a sampling method that best describes your project. DO NOT SUBSAMPLE = collect every one that comes up in the net; SUBSAMPLE RANDOMLY = the specimens do not need to meet ANY set criteria and therefore will be collected at random across the collection area; SUBSAMPLE SELECTIVELY = Collect only those specimens that meet the criteria set by you in the next question. SUBSAMPLE RANDOM STRATIFIED = specimens will be collected randomly from each of the strata you designate in the next question.

Subsample Selectively

**Selective and Randomly Stratified Subsamples ONLY:** Specify The Criteria. For Choosing Specimens Examples: selective criteria could be specific size ranges, photogenic, specific depths, etc; random stratified could be specimens collected by sex/size/area;

From *Chionoecetes bairdi* and *C. opilio*, sample up to 50 crabs per station, without regard to location or depth. 30 crab per length bin will be sampled. As catches of *C. bairdi* and *C. opilio* on the slope are typically low, catches of 50 or more crabs are expected infrequently; no more than 4 such hauls will be sampled.

From *Lithodes aequispina*, collect hemolymph only from visually positive crabs, without regard to location, depth, size, sex or carapace width.

**Target Quantity:** How many specimens do you want collected?

Up to 500.

**If the requested amount or frequency of specimens is not achieved, will the request still be useful?**

Yes

**Specimen Type**\* The typical specimen types to be collected on the surveys are listed below. Please select the type that best describes your collection. If none meet your collection's description, select 'Other' and provide your own

Blood smear

Pathological Tissue

Muscle tissue

Whole animal collection

**Do you require individual specimen-level data to be collected?** yes = each specimen sample will be given a unique specimen number.

Yes (crab width, sex, shell co)

**Do you need to be able to link your project to haul data?\***If you need haul data (e.g. latitude and longitude, depth, temperature, etc) for each of your specimens, it is mandatory that the CruiseNumber, VesselNumber, and Haul numbers be recorded every time we collect your samples; any forms provided by you must contain those fields. Copies of haul data will be e-mailed to the the requestor when survey data are finalized after the end of the field season (usually by October of the same calendar year)

Yes – please link my collection with haul data

**Supplies provided by the AFSC**\*Small quantities of some supplies, such as sample bags, freezer boxes, and standard chemical like ethanol and formaldehyde may be available, but applicants must arrange this specifically with survey contact(s) prior to the start of the survey. If asking for large specimens, then you must supply containers. Select supplies and equipment needed for your project or collection that AFSC will supply

Freezer box

(CLang Note: other choices: specimen labels, ethanol, formalin, none)

**Supplies provided by the requestor**\*List all remaining supplies and equipment needed for your project or collection that you will be providing

500 1mL Syringes, 5 96-well collection plates filled with 100% ethanol (<1mL/ well), glass microscope slides and boxes, plastic bags, sampling trays and caddies, calipers, sharps container, waste bags, datasheets, shipping materials

**Chemical Hygiene**\*Select or type in all chemicals and hazardous materials in your project. Please e-mail MSDS's for all chemicals other than formaldehyde, ethanol, and glycerol/thymol to RACE.Surveycollections@noaa.gov. Special haz mats will not be allowed. They must conform to those that we are already using. FAILURE to disclose chemicals and hazardous materials will terminate your project.

Ethanol

(CLang Note: other choices: ethanol, formalin, other: blank)

**PERMITS**

It is the responsibility of the person making the request to obtain all the necessary permits required for the collection and shipment of specimens, and the RACE Division must have copies of the permits no later than 20 April 2014. Note that RACE Division survey efforts are currently covered under ADF&G Fish Resource Permit CF-10-038 for expected levels of whole specimens or samples taken. NO LIVE ORGANISMS, TISSUES, OR VIABLE GAMETES are currently covered by this permit and will need separate permit. Please direct any questions regarding permit coverage to a particular project to RACE Deputy Director, Frank Morado (206-526-6572).

**Permits issued or pending:**

ADF&G Fish Resource Permit CF-10-038

**SHIPPING INSTRUCTIONS**

In 2014: The EBS shelf, EBS slope, and AI surveys originate and terminate in Dutch Harbor, Alaska. Equipment and sample collections will be shipped to the requester from Seattle. We typically use FEDEX or Alaska AirCargo. Contacts and phone numbers of someone available 24/7 to discuss logistics and problems or authorize billings at the anticipated time of shipment (i.e., end of leg and end of survey). The person making the request will pay for all charges associated with shipping and storage including the following: 1) completed shipping company forms with the shipping account number 2) any additional packing materials such as zip lock bags 3) if samples are frozen, coolers or commercial waxed boxes must be provided.

**24/7 Contact Name**:\* Christie Lang

**24/7 Contact Phone Number**:\* 206-554-1755

**Detailed shipping instructions:** [NOTE: FedEx next-day shipping imposes some complications. If you are requesting this method of shipping, please make sure to plan carefully with the Survey Coordinator.]

Ship with RACE survey supplies