STOMACH COLLECTION AND PRESERVATION
EBS SHELF, NORTHERN SHELF & NORTON SOUND

As part of the standard survey operations, the following stomach sampling will be conducted during RACE's 2010 summer bottom trawl survey of the eastern Bering Sea shelf, including the northern extension of the survey up to the Bering Strait and into Norton Sound. Personnel trained by REFM's Resource Ecology and Ecosystem Modeling (REEM) program should be assigned to all vessel-legs of the survey. (Equipment provided by REEM for the survey vessel covering Norton Sound will be included in the Equipment List for the slope survey, but the sampling plan within Norton Sound will be directed by the instructions herein and by any approved Special Scientific Projects and Collections Applications.) Stomach samples from key fish species will be preserved at each station <u>after</u> the catch has been sorted, weighed, sexed, and measured. It is helpful to have one person assist the stomach collector after other biological specimens have been collected. The stomach collector should communicate with the deck boss and FPC if there is difficulty obtaining the target species from the catch. Stomach samples only need to be collected from "good" survey tows. Stomach samples do not need to be collected from stations added due to encountering a crab "hotspot." A sheet will be provided to record which Primary species was collected at each potential Red King Crab Re-Sampling station. During the re-sampling, the stomach sampler will target the same species for collection at each station. If clarification is needed on any part of the sampling plan, please contact Troy Buckley, Geoff Lang, Kerim Aydin, or someone else from the REEM program.

Sampling Plan:
The main species of fish to sample for stomach contents in the eastern Bering Sea (and northern Bering Sea including Norton Sound) are listed below, but additional species may require sampling depending on approval of Special Scientific Projects and Collections Applications. Generally, arrowtooth flounder, Pacific cod and walleye pollock will be sampled aboard both survey vessels, but Pacific halibut will be sampled on only one vessel (probably the vessel with IPHC personnel aboard) while large sculpins (*Myoxocephalus* spp. and *Hemilepidotus* spp.) will be collected only on the other vessel. At each station, select up to two species, one from each list for your vessel, for collection and preservation based on the major species in the catch. Try to switch among species within a list to even out the spatial coverage as much as possible. Try to collect stomach samples from every station. Species denoted with an asterisk (*) should be collected according to the size-stratification given below. When size-stratification is not required for a species, collect 10 stomach samples from as wide a size-range as possible from each haul.

<u>*Primary – both vessels</u>	<u>Secondary – vessel 1</u>	<u>Secondary – vessel 2</u>
***arrowtooth flounder**	**Pacific halibut**	**plain sculpin**
***Pacific cod**		**great sculpin**
***walleye pollock**		**warty sculpin**
		yellow Irish lord
		butterfly sculpin

*Primary species should be collected in a size-stratified manner. No more than 5 specimens per size-category should be sampled from each haul. The size-categories (cm FL) for each species are:

walleye pollock	1-24	25-39	40-54	55+
arrowtooth flounder	1-29	30-49	50+	
Pacific cod	1-29	30-59	60+	

Kamchatka flounder can be collected along with, or in place of, arrowtooth flounder. Please indicate on the Specimen Form and the Specimen label which samples are from Kamchatka flounder and which are from arrowtooth flounder.

Selecting Specimens for Stomach Sampling:
At **every** station, after the major species in the catch are evident, choose 2-3 key species, one from each list (primary, secondary, and Special Scientific Collections) which are abundant enough for stomach sampling. For rarely caught target species on the Stomach Special Collections list, please sample them whenever they are caught. Switch among species within each list to even out the spatial coverage as much as possible. With the concurrence of the field party chief, designate which specimens are to be set aside for stomach dissection after the baskets have been weighed. Set the baskets in a cool, shaded area until the rest of the catch has been processed. If limited numbers of the designated species are available and the specimens are also designated for length-frequency, gonad, liver and/or otolith samples, coordinate the sampling procedures as needed, and ask that this species be sexed and handled with extra care to avoid ejecting the stomach contents. Try to collect the samples from as wide a length-range as possible for each size-category and predator species.

Individual fish should be chosen randomly and should be checked for signs of regurgitation and net-feeding. Sometimes you will need to go through several fish before finding an acceptable specimen to collect. Regurgitation is indicated by digested food items in the mouth or gills and/or a flaccid stomach. If the fish is determined to have regurgitated, discard it and select a replacement fish. If the replacement fish shows signs of regurgitation or has a naturally empty stomach, discard it and select another fish; **the idea is to replace a sample rejected due to regurgitation with a sample from a fish that was also feeding.** Net-feeding may initially be suspected when a fish tail or crab legs are visible in the throat of the predator. Examine this prey for any signs of digestion. Prey movement, or a fish with clear eyes (without any cloudiness or whiteness on the surface of the eye) are indications that net-feeding occurred. If you make a determination of net-feeding, discard this sample and replace it with another randomly selected sample; **the idea is to replace a sample rejected due to net-feeding with a randomly selected sample.** It is okay to collect a naturally empty stomach if it isn't a replacement for a fish that regurgitated.

Predator information (species, length, sex, spawning condition, sample number) and haul information (vessel, cruise, haul number) for each sampled stomach should be recorded on a Specimen Form for each species from each haul. The same information should be recorded on a Specimen Label which is put into the bag with the stomach. It is best if specimen numbers begin with “1” for each species and count upward through the leg or through the survey. Avoid starting at “1” at each station.

Red King Crab Re-Sampling Stations – If you are aboard a vessel that is re-towing red king crab stations in Bristol Bay, the Primary species that was sampled during the regular survey should be re-sampled from the same locations. A sheet will be provided to record the Primary species that was collected at each station that may be re-sampled later in the survey. The information will need to be shared and recorded by both stomach collectors on leg 1 so that both vessels have a complete accounting of which Primary species was collected at each station. This sheet will be used as a reference by the stomach collector aboard leg 2 or leg 3 when the re-sampling may be performed.

Zooplankton tows – If you are aboard the vessel doing a zooplankton tow at the end of the day, be sure to sample walleye pollock (or arrowtooth flounder or Arctic cod) from the last tow of the day. Other species can also be sampled in addition to walleye pollock (or arrowtooth flounder or Arctic cod). This project was submitted as part of the Special Scientific Projects and Collections Application, Supplemental Sampling for Trophic Modeling of Eastern Bering Sea Shelf Fishes.

Formalin Solution:
The 10% buffered formalin solution should be mixed in the 5-gallon buckets using proper safety procedures for handling formalin. Fill the bucket half-full with sea water. Add one heaping scoop (1/8th cup) of baking soda to a bucket. Add 1 liter of formalin. Keep the cut (removable) lids on the buckets until the level of the solution and samples is about 2-3" from the rim.

Dispose of the empty formalin jugs properly. Empty formalin jugs must be double rinsed prior to disposal or recycling. Do so by filling the bottle with salt water until full, drain the water into the scupper, and repeat. Remove the labeling from the bottle to the best of your abilities and carefully poke several holes in the bottle to prevent the bottle from being re-used. The bottle can then be disposed of or recycled (as allowed by vendor).

When Buckets are Full:
Continue placing collected samples into the buckets until the level of the formalin solution is about 2-3" from the rim. Replace the cut lid with a properly labeled, uncut lid to seal it for storage and transport. Use a permanent marker to label the uncut lids. It is easiest to label lids when they are dry and before they are sealed on the buckets. The uncut lid should indicate the predator species, vessel name, leg number, collector’s name, year, season (summer), and “10% formalin.”

At the End of Each Leg:
At the end of each leg, buckets more than half-filled with samples should be sealed with uncut lids. All sealed buckets should be further labeled with luggage tags attached to the handles and with chemical property stickers for 10% formalin stuck to the side of the

buckets. The luggage tag should indicate predator species, year, vessel name, and collector's name. **Bring your specimen forms back to Seattle at the end of each leg.**

All of the properly sealed and labeled buckets should be taken to OSI at the end of each leg for storage. Coordinate this delivery with your FPC. The OSI account is under the name, "Geoff Lang" or "NOAA Food Habits", and shipping will be arranged at a future date. Telephone Geoff Lang with any questions; 206-526-4196 or 425-343-4181. Take 2 copies of the 10% formalin MSDS with the buckets to OSI – OSI might want to file one and the other one is to be placed in a visible spot on the pallet of buckets. **Keep a record of the number of buckets you deliver to OSI and give it to Geoff Lang when you return to Seattle.**

Prepare the sampling equipment and buckets for the next stomach collector. They may have very little time to find equipment and get things set up for themselves. Leave them a detailed note about where equipment is stored, how you have things set up for them (including the number of buckets that are set up), and how much you have accomplished on each project.

Equipment List per Vessel (Furnished by REEM):

7000 specimen labels	1 clipboard	4 permanent markers
700 specimen forms	4 knives	4 pair forceps
60 five-gallon buckets	8 cut lids	3 pair trauma shears
60 uncut lids	60 luggage tags	2 hemostats
16 gallons Formalin	9 cups baking soda	2 measuring scoops
6 MSDS for 10% Formalin	2 MSDS for 100% Formalin	
2 MSDS for Arm & Hammer baking soda		
1 pair safety goggles	1 rubber mallet	1 3-ring binder
60 10% Formalin (chemical properties) stickers		
7000 stomach bags (500 large, 2500 medium, 4000 small)		

Formalin Handling Procedures

Formalin Handling Protocol:

• Formalin is a relatively hazardous chemical and must be handled appropriately to ensure your safety. You are dealing with a small quantity of formalin and if these guidelines are followed your exposure will be well below established safe exposure levels.
• Read the Material Safety Data Sheet (MSDS) before using formalin to understand its properties.
• ALWAYS wear gloves, rain gear, and goggles/safety glasses when directly using formalin.
• ALWAYS use formalin on an open deck -- DO NOT use below decks or in your cabin.
• Inform captain and crew that you have formalin onboard, where it is stored, location of Material Safety Data Sheets (MSDS), potential hazards, and what to do in case of spill.
• IF spilled -- this is a small enough quantity to dilute with water and wash overboard.
• Add formalin to bucket that is already half full with seawater, rather than adding seawater to the formalin. This will ensure that the formalin is quickly diluted, and will lessen the chance of getting formalin splashed on you.
• Use extreme caution when adding formalin to bucket with seawater, hold the bucket lid over as much of the bucket as possible while pouring the formalin, creating a 'shield'.
• IF formalin comes into contact with your skin or eyes—rinse immediately, and thoroughly, with water for 15 minutes as per MSDS.
• IF ingested -- consume large quantities of water, do not induce vomiting, and seek medical attention as soon as possible as per MSDS.
• IF overcome by fumes -- move to fresh air, administer oxygen if necessary and available as per MSDS.

Where to Keep Formalin Onboard?

• Formalin should be stored in a well ventilated space. 100% formalin should be stored at or above 50°F, below this temperature it will lose its potency as a component of the formalin precipitates from the solution. Pure formalin is considered a flammable material and should be stored in an appropriate flammable storage area until it has been diluted as described above. Once diluted to a 10% solution, formalin can, and should be stored on a weather deck if possible. It won’t freeze.
• It is best to store your bucket securely tied to an immobile object. Leave the bucket in place and carry samples to the bucket after you are done with your sampling. This will avoid the potential of spilling formalin in the factory and will keep the formalin away from fish processing operations.
• Do not to submerge your gloves in the formalin when you add samples to the bucket. If the samples float, use a pair of forceps or some other sampling tool to submerge the samples. Rinse with water after formalin contact.
• Anytime formalin gets spilled and/or inadvertently comes into contact with any object other than your samples, flush the object or area with plenty of water.

IPHC Collection Permit, 2006-2010

CLIFFORD ATLEO
PORT ALBERNI, B.C.
JAMES W. BALSIGER
JUNEAU, AK
RICHARD J. BEAMISH
NANAIMO, B.C.
RALPH G. HOARD
SEATTLE, WA
PHILLIP LESTENKOF
ST. PAUL, AK
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INTERNATIONAL PACIFIC HALIBUT COMMISSION

ESTABLISHED BY A CONVENTION BETWEEN CANADA

AND THE UNITED STATES OF AMERICA

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April 25, 2005

To Whom It May Concern:

The Alaska Fisheries Science Center, Resource Ecology and Fisheries Management Division (National Marine Fisheries Service) is authorized to capture and retain for scientific purposes up to 600 halibut (*Hippoglossus stenolepis*) per year in each of three areas, using research trawls, handlines, or longlines, during the calendar years 2006 through 2010. The place of and manner of capture will be determined in accordance with the Center's research plans concerning food habits and will include the areas of the eastern Bering Sea, Gulf of Alaska, and Aleutian Islands. Collection may be from research vessels or by research staff aboard domestic commercial fishing vessels. The principal investigator for this study is Dr. Kerim Aydin.

Halibut obtained under this permit may not be retained for purposes other than scientific research and may not be sold. Halibut captured in excess of the limits specified in this permit must be returned to the sea with a minimum of delay and injury.

B M Leaman

Bruce M. Leaman
Executive Director