BIOLOGICAL IMPLEMENTATION PLAN

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BIOLOGICAL TASKS:

1.1.2 Biological Sampling

SECTIONS:

1. INTRODUCTION:

The overall objective of the Amchitka Science Plan is to assess, for Native Communities, the U.S. Fish & Wildlife Service and the Alaskan Department of Environmental Conservation, as well as other stakeholders, whether there are currently increased radiation health risks related to underground nuclear test shots to organisms residing around Amchitka Island and to consumers of these organisms, and to provide a baseline for future monitoring as part of long-term stewardship, prior to termination of the Department of Energy's Environmental Management responsibility for Amchitka.

The Biological Tasks in the Amchitka Science Plan involve the field sampling of biota, sample preparation, homogenization and compositing, laboratory analysis, data analysis and synthesis. While sample preparation is described in this section, the laboratory analysis is described only to the extent that it impinges on data analysis.

2. BACKGROUND

To determine for Native Communities, the U.S. Fish & Wildlife Service (USFWS) and the Alaskan Department of Environmental

Conservation (ADEC) as well as other stakeholders, whether there are increased radiation risks related to underground nuclear tests to the health of organisms residing around Amchitka Island and to consumers of these organisms, and to provide a baseline for future monitoring by DOE, it is necessary to: 1) examine radionuclide levels in marine ecosystems including plants, invertebrates, and vertebrates on and near Amchitka Island and at a reference site, 2) compare current levels with those in Amchitka organisms from the 1960s and 1970s (Crayton 2000), and with data from other parts of the Aleutians; 3) test for food chain biomagnification by sampling organisms at different trophic levels, and 4) compare the radionuclide values from the CRESP study to those of other marine organisms worldwide and to levels known to be acceptable to human and ecological health risks. The data will also provide a valuable baseline for future biomonitoring and assessment plans. With the Department of Energy planning to terminate its Environmental Management responsibility for Amchitka, these data are essential to providing peace of mind to all residents of Alaska, to the Native Communities, and to the world at large that consumes commercial seafood from the region. Further, sampling of both the marine environment and foods will be done in collaboration with people of the Aleutian/Pribilof area and this project will be coordinated with ongoing field studies under the auspices of A/PIA and the State of Alaska.

A fundamental premise of CRESP is the involvement of stakeholders in all phases of the risk assessment and management process. This has been memorialized in the diagram of the Presidential/Congressional Committee on Risk Assessment and Risk Management. Involving Native Communities and other stakeholders will improve the quality of the biological sampling, the appropriateness of the bioindicators selected, and assure that the results will be relevant to community concerns. The Long-term Stewardship Workshop held in Fairbanks (Feb 2002; CRESP 2002) identified the role of biota in the transfer of radionuclides as the highest priority for marine science in the Amchitka ecosystem, as well as for human health risk assessment (CRESP 2002). The marine science group of the Feb 2002 workshop recognized the importance of analyzing radionuclides in a number of different compartments or species groups, including 1) sedentary and sessile organisms, 2) Rockfish, 3) Atka Mackerel, and 4) dietary and subsistence foods, including birds, fish, and marine mammals. Further, identification of key pathways of radionuclide transfer in the food chain was a high priority. Such food chains include: 1)kelp-urchin-otter; 2) small fish, larger fish, seabirds and eagles, 3) small fish (such as salmon)-Pollock-Steller Sea Lion

(endangered); and 4) invertebrates -small fish-Halibut-human.

While our overall goals deal with ecosystem and human health, the objective of Task 1.1.2 is Biological Sampling, and this section relates to biological sampling and specimen handling prior to radionuclide analysis. Assessment goals are those that are of importance for the scientists, regulators, stakeholders or others, but they may not be able to be measured directly. That is, we may be interested in overall population stability of Sea Otters.

The assessment goals of the biological sampling are:

1. To determine if the foods from the Amchitka region are safe.
2. To determine if the biota of Amchitka are currently contaminated.
3. To determine if the levels of contaminants are high enough to pose harm to species themselves, to consumers, or the ecosystem.
4. To help determine which species might be useful for monitoring during long-term stewardship.

As scientists, we are interested in examining the level of radionuclides in a broad range of biota, particularly those organisms in the food chain that lead to humans. This involves measuring radionuclide levels in a range of biota. It is the usual scientific procedure to establish null hypotheses which can be tested with the data. A null hypothesis is one that predicts that there will be no difference among variables. For example, there will be do difference in cesium levels in tissue of Eiders and Guillemots. This allows us to statistically compare the levels of cesium in these two species, and arrive at a conclusion. Assessment goals can be converted into null hypotheses that relate directly to the overall project objectives:

1. Radionuclide levels in subsistence and commercial foods do not pose an increased risk to human consumers (Typically public health cancer risks are regulated to levels less than 1 in 10,000 to 1 in a million).
2 There are no differences in radionuclide levels in biota in the marine environment transects close to the Amchitka test shot cavities (Long Shot, Milrow, Cannikin) compared to the reference site.
3. There are no differences in radionuclide levels in biota in the marine environment around the Amchitka transects and background in the Aleutians (where this information is available) or from other locations.

4. Radionuclide levels in species at different trophic levels (and thus the ecosystem) in the transects from the test shots are below levels known to cause adverse effects in biota.

5. There are no differences in radionuclide levels among species.

6. There are no differences in concentration factors for organisms above primary producers (plants).

These null hypotheses will form the basis of CRESP reports to the regulators, the Aleut/Pribilof Islanders, natural resource trustees (USFWS, ADEC), and to other stakeholders. The specific measurement objectives for the biological sampling and sample preparation (Task 1.1.2) follow:

3. MEASUREMENT OBJECTIVES for Task 1.1.2:

3.1. Measurement Objectives

Measurement objectives are those that can be measured in the field or laboratory (rather than derived features, such as health or safety). For example, although we are interested overall in the health and well-being of the Amchitka ecosystem, and the safety of the foods for human consumption with respect to radionuclides that might derive from the Amchitka test shots, we must select characteristics that can be measured or quantified that relate to our overall goals. The measurement objectives for this section are:

1. To collect marine organisms from around and on Amchitka Island and at reference site.

2. To collect organisms (or their ecological equivalents) that represent different trophic levels and lifestyles (migratory, sedentary).

3. To process these organisms to reduce mass, where possible by dissection, and to set up the composites of samples (time permitting) while on the ship.

4. To maintain laboratory notebooks for sample tracking.

5. To maintain adequate QA/QC for samples collected and for sample preparation.

6. To transport specimens, under appropriate Chain of Custody, to the Rutgers laboratory for homogenization and blending of composites.

7. To transport specimens under appropriate Chain of Custody to the analytical laboratories.

8. To receive the analytic data and integrate it into the

CRESP-Amchitka Data Base.

3.1. The products derive from the measurement objectives, and those listed below by number correspond to the measurement objectives given above. We also list the primary responsible parties):

1. A printout of the samples collected on the boat will be completed by 3 weeks following return to Rutgers (Burger, Gochfeld, Jewett, Volz, Vyas).
2. A table of organisms collected (by trophic level and frequency) by the main sampling team will be completed by 6 weeks following return to Rutgers (Burger, Gochfeld, Jewett). Data from the NOAA trawl will be integrated by 8 weeks following return to Rutgers (Burger, Gochfeld, Jewett, Volz, Weston, Vyas).
3. A table of the mean relative size of organisms, with sample weights collected will be completed 3 months after return to Rutgers (Burger, Gochfeld, Burke).
4. Laboratory notebooks will be available for inspection upon return to Rutgers, and periodically as samples are prepared (Burger/Gochfeld, Burke).
5. A list of voucher specimens and still photographs will be available 2 months after return to Rutgers (Burger, Gochfeld, Volz), and a list of intertidal/subtidal videos will be available 2 months after return to UAF (Jewett).
6. Chain of Custody forms attached to samples sent to Rutgers will be available for inspection as soon as specimens are in adequate freezer conditions (entire team).
7. Laboratory specimen preparation can be assessed by examining the laboratory notebooks regularly, and by accessing the data base (maintained by V. Vyas).

In order to accomplish these seven objectives, we have developed a two-prong approach. 1) A research vessel that will incorporate traditional sampling and collection methods as well as Aleut hunting and fishing methods and 2) A NOAA trawl that is fishing according to normal commercial fishery practices. Because we will be able to obtain samples from that trawl that include benthic organisms, using this approach will allow us to obtain and make available for analysis organisms that both represent not only the marine-Aleut subsistence food web but the commercial fishery take as well. This represents a unique approach for collecting samples in terms of both methodology and the diversity of ways the marine environment functions and is used. A further benefit of the NOAA trawl is that, if there proves to be definable data comparability

between the several approaches, then the fact that the NOAA trawling mission is repeated every two years may open up an especially efficient way of regularly monitoring the Amchitka marine environment.

4. LOGISTICS

4.1. Disclaimer

The biological sampling is under the direction of J. Burger and S. Jewett. The original biological sampling time schedule was planned with sufficient time to obtain the necessary specimens, including contingency days for adverse weather which can preclude both diving and land-based operations. The current schedule may not provide adequate time for the complete original sampling scheme in the case of inclement weather. While the current ship schedule was constrained by a number of factors, we expect it will be sufficient to conduct the necessary sampling. Naturally, we cannot control the weather, and hope that our contingency bad-weather days are sufficient. Moreover, the timing of our sampling cannot be ideal for all species, thus both the samples collected (adult birds versus eggs) and the species collected (due to inherent seasonalities) may require ecological equivalents.

4.2. Responsibilities of Rutgers/UAF

The main responsibility for biological sampling rests with personnel from Rutgers, UAF and A/PIA (with the above constraints), with additional help from the Project Director.

The main responsibility for sample preparation on the boat rests with the entire biological team. Responsibility for tracking the samples at Rutgers rests with Burger and Gochfeld, assuming that the necessary freezers and storage, and equipment are available. Responsibility for data management lies with Vyas.

4.3. Time Line

4.3.1. March to 15 June - assemble field equipment, write implementation plans, and develop QA/QC procedures.

4.3.2. 15 June-27 June - mobilization on Anchorage and Adak, acquire additional field gear and equipment, collect samples on Adak to test equipment and protocols. Samples archived and handled according to overall protocol.

4.3.3. 24-27 June - meet with the personnel from the first cruise to determine whether any data gathered can help refine our sampling scheme. Refine sampling scheme.

4.3.4. 27 June - 22+ July - Conduct biological field sampling

around Amchitka and at reference site - Kiska-Buldir. Additional sampling at Adak will be undertaken only if Kiska-Buldir sampling is deficient for key biota.

4.3.5 22 July - 26 July - Transport specimens to Anchorage from Adak, and to Rutgers, oversight by D. Volz.

4.3.6. 14 July-12 August. Deployment of Jim Weston from Memphis to Adak, check equipment, conduct sampling on the NOAA trawl vessel, transport specimens to Anchorage, for shipment by Volz to Rutgers.

4.3.7. Early August - 2 day meeting at CRESP headquarters of Burger, Gochfeld, Kosson, Powers, Vyas and Volz to consider final screening analysis design. A later meeting will be scheduled to design further radionuclide analysis following the results of the initial screening.

4.3.8. 27 July to 30 November - specimen handling and data entry. Specimen handling includes any dissection remaining (from either ship), compositing, homogenization, blending, and drying to be done at Rutgers.

4.3.9. 10 August to 30 November - specimen shipment to analysis laboratories (handled by CRESP).

This plan only runs to 30 November 2004 because of CRESP budget constraints.

5. BIOLOGICAL SAMPLING ON THE SHIP

The daily biological sampling will depend upon location (Amchitka, reference site), weather and safety, and sampling needs. To address these issues, the biological team, in conjunction with the PI, agreed to teams, task priorities, and operational procedures (CRESP HQ meeting, 12-13 April 2004):

5.1. Teams and task priorities. At a meeting of all biological sampling personnel (12-13 March 2004), the following teams and priorities were agreed upon:

5.1.1. Burger/Gochfeld/Volz (+ 2 technicians and 1 sample preparator)

a. Collect bird eggs and rats.
b. Maintain Chain of Custody.
c. Oversee sample preparation
d. Oversee Aleut sampling
e. assist in collecting samples in intertidal
f. Data management/chain of custody and data entry
g. Help where needed with other activities

5.1.2. Jewett (+ 3 divers)

a. Collect invertebrates by diving in subtidal and

intertidal.
b. Maintain Chain of custody for specimens.
c. Enter their collection data where possible.
c. Help with sample preparation
d. Help where needed with other activities.

5.1.3. Patrick (+ 2 Aleut hunters/fishers) (oversight: Burger, Volz)
a. Collect subsistence organisms, including marine mammals, fish and invertebrates
b. Chain of Custody for their samples.
c. Enter data from these samples where possible
d. Help with sample preparation
e. Help where needed with other projects.

5.1.4. Swing team (Volz).
a. Safety and health (Volz/Gochfeld)
b. Data management. Chain of Custody and data entry for any samples collected.
c. Specimen collection
d. Help where needed with other projects.
e. Transportation of specimens to Anchorage.

5.1.5. Jim Weston is in total charge on the NOAA trawl.
a. Collect necessary samples
b. Maintain Chain of Custody.
c. Preparation of specimens.
d. Data recording and management
e. Transportation of specimens to Anchorage.

5.2. Operational Rules and Responsibilities

5.2.1. Volz is in charge of operational logistics for the CRESP expedition, Burger of the overall biological sampling, Gochfeld of health/safety, and Jewett of divers. Burger/Volz are in charge of Shipboard data management/data entry. Any disagreements about sampling/boats/logistics will be mediated by Volz/Burger. Responsibility for any expedition abort decision will be made by Volz/Burger/Gochfeld.

5.2.2 The ship captain makes all decisions about the routes and safety of crew and the Ocean Explorer.

5.2.3. Health and safety ALWAYS come first. Divers, boat activities, and land-based operations will not be allowed under conditions that jeopardize health and safety.

5.2.4 Chain of custody, safe handling of specimens, and appropriate labeling come next. If samples are not adequately labeled and do not have a Chain of Custody they will be unusable. Chain of Custody must be filled out as samples are collected and

handled by the person doing the collecting.

5.2.5. It is expected that people will be working to capacity, given the constraints of individual tasks (e.g. diving) while on the ship.

5.2.6. Everyone will pitch in with all tasks where possible, in a triage manner. For example, if the Aleuts have a marine mammal that requires attention, personnel will be organized to do so.

5.2.7. It is expected that everyone will help with sample preparation whenever possible. For example, if the seas are rough and we are docked, everyone can help with this task.

5.2.8. All collecting will reflect our federal and state permits.

5.2.9. All information relative to samples will be entered first in the laboratory notebooks, and then in a computer spreadsheet for necessary redundancy. The notebooks represent the official record.

5.3. Decision Logic for Field Sampling

We all recognize that there will be a number of difficulties concerning sampling that include 1) weather constraints, 2) difficulty of obtaining some species because of logistics, presence or abundance, 3) personnel triage to obtain samples, 4) life cycle stage (eggs vs chicks), and seasonality (which species can be found). Decisions on sampling on board will be made by Burger in consultation with Jewett and Gochfeld.

5.3.1. Decision Logic for Field Sampling

5.3.1.1. Take adequate sample for analysis of one of each trophic level representatives first. In other words, we must have at least one representative of each trophic level for analysis. A preliminary list follows:

Kelp/Ulva
Chiton/Sea Urchin/Barnacle
Blue mussel/Basket Star/Rock Jingle
Octopus
Red king Crab/Brown Crab
Ocean Perch
Dolly Varden
Atka Mackerel
Halibut
Cod/Pollock
Eagle
Gull/Puffin

Eider
Pigeon Guillemot
Harbor Seal/Sea Otter

5.3.1.2. For some species, different age classes (eggs, chicks, adults) will be collected depending upon logistics and the stage of reproduction encountered.

5.3.1.3. Our diving and collecting time in each zone (intertidal/subtidal) should reflect the need to fill each trophic level.

5.3.1.4. Within teams, there are priorities, however, availability cannot be predicted until we are on site:

5.3.1.4.1. Land-based - Priorities are gull, eider, guillemots, eagles, rats

5.3.1.4.2. Divers - Priorities are Kelp, mussel/basket star, octopus/ chiton/others

5.3.1.4.3. Aleuts - Priorities are mammals, predatory fish, others.

5.3.1.5. Collecting takes priority over sample preparation, since this COULD be done on land later and we have one opportunity and limited field time in 2004.

6. DAILY ACTIVITIES:

6.1. Background

We discussed sampling at each location (Long Shot, Milrow, Cannikin, Reference Site). This will require a reasonable amount of good weather. Sometimes it is possible to work for part of a bad-weather day, but sometimes there are two or three bad days in a row. There is a trade-off between sampling and sample preparation: fewer total days on the boat means less sample preparation time since the field sampling has to get done.

The NNSA-funded components of Amchitka Science Plan has as its basic element biological sampling including sample preparation, and radionuclide analysis. We designed the sampling to do three things: 1) assess the current risk to the organisms in the marine ecosystem (from Kelp to eagles, seabirds, mammals, and top-predatory fish), and 2) assess current risks to humans. 3) This information will then be used to design long-term stewardship focusing on informative bioindicator species which can be sustained in a reasonable and cost-effective biomonitoring plan. The overall Science Plan is answerable to all four signatories and has a broader base than the NNSA-funded component.

The biological sampling therefore represents different nodes on the food web selected in conjunction with the 4 initial

signatories (and stakeholders). The sampling plan is comprehensive, and recognizes that top-trophic level species are indicators of bioaccumulative contaminants (e.g. strontium, cesium). Hence the focus on top-level birds, mammals and fish. Further, it is largely the top-trophic level organisms that the people eat (all the eggs and birds EXCEPT eagles, fish, marine mammals). Each of the species groups is important, and tells us something different about the food web, potential radionuclide distribution in the marine ecosystem, and potential human exposure.

To some extent our daily schedules will be influenced by the task priorities agreed upon. A large part of Burger's responsibility each day will be in discussion with Jewett, Gochfeld, Patrick and Volz to determine what organisms to target where. This involves triage decisions that must begin when we get on site and be updated each day depending on field success and weather.

Of the species targeted we will have to determine:

a) Which target species are abundant and accessible and can be collected easily in adequate quantity,

b) Which target species are rare or absent and cannot be targeted, in which case an ecological alternative will be designated by Burger in consultation with Jewett, Patrick and Gochfeld, with an emphasis on representatives from each trophic level.

c) Which target species are present but in limited quantity, which will demand intensive effort to obtain adequate samples.

While we have ecological equivalents, we will try to get the samples we initially targeted in order to facilitate communication with the stakeholders. Some species may be more or less common than we expected, some invertebrates may be smaller than we expected (needing more individuals per sample). Deployment of teams will depend on how we are coming with the overall sampling scheme. These decisions must be made on site by the team leaders.

Our optimal sampling priority will be Long Shot and Cannikan, Milrow, and Kiska. However this priority will be influenced by weather and how our sampling is proceeding. After sampling on Long Shot and Cannikan, Burger, in consultation with Volz, Jewett and Gochfeld will decide whether to proceed immediately to Kiska, or to go to Milrow. It is imperative to have samples from the reference site (Kiska-Buldir). Should key biota deficiencies be noted in the Kiska sampling, samples taken at Adak could supplement the data.

6.2. Types of Collecting Days

There are five collection types (which will be mixed and

matched, depending upon the weather as well as on the accessibility of target organisms or their ecologic equivalents):

6.2.1. Collecting bird samples

6.2.2. Collecting rat samples

6.2.3. Collecting invertebrates in intertidal

6.2.4. Collecting invertebrates in the subtidal

6.2.5. Collecting Aleut foods, including mammals, inshore fish and other organisms.

On bad weather days it may be possible to do some limited field work (depending on wind and rain), while on other days work will be confined to the boat and will focus on sample preparation and volume reduction.

6.3. Collecting Bird samples

6.3.1. The objective of the bird collection is to obtain samples at high trophic levels, species of interest to Fish & Wildlife Service, and species that are eaten by Aleuts, based on public meeting in the Aleutian communities. The actual stage to be collected depends upon temporal patterns of nesting. There are sampling goals: 1) collecting gull and eider eggs; 2) collecting Eagle eggs (or chicks); 3) collecting samples (eggs, dead chicks or adults) from the seabird colonies (puffins, guillemots, cormorants).

There will be a safety/activity briefing prior to each field day. Field parties whether on land or water will notify Volz of their locations and objectives. There will be review of locations and appropriate vehicles, field equipment and safety gear. Field teams should have two forms of communication in case of emergency.

All field activities will involve a buddy system.

6.3.2. Procedures

6.3.2.1 Gulls and Eiders

These species nest in similar habitats, more or less together in the grass in small "colonies" on the island surface in the vicinity of lakes. We will locate gull colonies on our trip up the island. We will collect eggs (or chicks) on our trip back to the ship, or in a targeted sampling trip. The gulls are attentive and by patiently and carefully watching their behavior, even well-hidden nests can be discovered.

6.3.2.2 Eagles

Eagles eat quite large fish as well as rats, and as top-level predators are of particular interest because of bioaccumulation. The USFWS specifically requested the addition of Eagles to the Science Plan. For Eagles, we will take the enclosed vehicles and go to the known nest sites obtained from R. Anthony,

in an attempt to take one egg from each nest. In most cases the nests are within 2 km of a vehicle track. So we will park and hike to the nests which are scattered along the shoreline. Some of the nests will Not be reachable because of height or other safety considerations. Collection at other nests will involve teams of two people at least. Eagles do not like to have their eggs taken, so at each accessible nest we will distract the adult, and one person will attempt to keep the adult at bay, while the second person removes an egg (we will wear hard hats and protective vests and gloves. Eggs will be placed in rigid containers, and carried in a collecting box for transportation back to the ship. If any dead chicks are found, they will be salvaged (put in plastic bags in an ice chest for transport back to the ship).

For Eagles and all other samples, the exact location of each collection site will be noted on clipboards and Chain of Custody forms, using GPS. Fish & Wildlife Service is quite interested in knowing which nests we sample, and in the radionuclide results for the eagles. With two teams and good luck we should be able to obtain our samples in two days. Some nests may require more than one visit, if special gear or additional personnel are required.

6.3.2.3. Colonial seabirds

Colonial seabirds may be located on Amchitka itself, small offshore islands, or under the dock in Constantine Harbor. Preliminary information on location will be provided by USFWS, but it will be necessary to reconnoiter the island by vehicle and possibly to circumnavigate by ship. There are two options for the species on Amchitka.: 1) taking the vehicles up the Infantry road over the mountain pass to the north end with our equipment and camping overnight 2) taking the ship to the north end and hiking up to nesting areas if we can find a clear path. There are several seabird colonies along the Amchitka coast. They are not necessarily in the same location from year to year. When we reach a colony we will walk over the ground searching for nests and take eggs if present or chicks that we find. Nests are scattered in this habitat, and the timing may make finding nests difficult. Puffin nests will have to be dug out. Eggs will be collected and placed in egg cartons, numbered, and will have a Chain of Custody form including the GPS location. If only chicks are available they will be picked up, euthanized, and placed in plastic bags in ice chests. If the birds are concentrated in accessible areas, this aspect should take 2-person days, depending upon the phenology of the birds. Since USFWS is dubious about accessibility from the sea, we will plan to make an exploratory trip by vehicle when we arrive.

We have been told to plan on a minimum of four hours (by truck) to get to the north end.

We will attempt to complete the seabird sample because they are fish-eaters at the top of their food chain, and the birds and their eggs are consumed by islanders. Seabirds feed mostly inshore, eating fish from the surrounding waters. These seabirds are traditional Aleut foods (and the Fish & Wildlife Service was interested in them because they intersect their Wildlife Refuge responsibilities and marine foods). At all times we will be looking to salvage specimens, and will collect by shooting if necessary (recommended by U.S. Fish & Wildlife Service).

6.3.2.4. The analysis protocols for avian tissues are the same as for marine mammals. The bird collecting will be by Burger/Gochfeld/assistants, and by Volz when he has the opportunity.

6.4. Collecting Rat samples.

Rats are being collected because U.S. Fish & Wildlife Service and ADEC were interested in them, and to eliminate them as a potential source of radionuclides for Eagles. Rats make up a significant portion of the diets of some of the Amchitka Eagle population.

Rats will be collected on Amchitka itself. If we are at the dock we will take vehicles, otherwise we will take a small skiff to the land. We will set a series of ordinary rat traps (snap-type) in likely places (recording GPS locations). Traps will be baited with peanut butter or dead fish, whichever we find they are attracted to. Setting a trap line usually takes 4 hours or so. Workers must be very careful setting the traps to avoid injury. Gloves must be worn. Traps will be set in the afternoon and checked the following morning.

We will dissect rats for liver, muscle and bone. Rat tails will be collected for the USFWS. Burger will participate in and supervise the preparations.

6.5. Marine Sampling

6.5.1. Background

Our plan for sequencing marine locations is: Long Shot, Cannikin, Milrow, Reference Site(s). If we get useful locational information on freshwater seeps from Mark Johnson, that will be used to localize sampling in one or more of the Amchitka sites. Otherwise we are planning that the Long Shot sampling will be targeted between Crown Reefer Point and Cyril Cove at Square Bay and the Milrow sampling between Duck Cove and Rifle Range Point (both places which the "Green book" people studied 30+ years ago).

The Cannikin site will be chosen based on known faults between Petrel Point and immediately south of Banjo Point, inclusive of White Alice Creek. One reference site will be along the eastern side of Kiska Island, including Kiska Harbor and/or Vega Bay. Preliminary information indicates that Kiska will have the same marine plants and animals that will be sampled on Amchitka, and if necessary, nearby Buldir which offers the seabird diversity comparable to Amchitka.

We anticipate that 5-7 days sampling should be sufficient for each site, including time to move from site to site.

6.5.2. Collecting Invertebrate Samples in intertidal

This will be a combined effort of Jewett's team and Burger's team (time permitting). Jewett's team will concentrate first on subtidal organisms at each site and then on intertidal by SCUBA. Burger's team will work in the intertidal when possible. Walking over kelp-covered boulders can be a dangerous task, and personnel safety will not be compromised if there is tidal surge. Weather and tidal conditions will also influence Jewett's choice of sampling location. After examining the tide tables for July, there are few minus tides to allow intertidal sampling; the lowest is only –1.9 ft. So, based on limited access to this region and the dense kelp cover over exposed rocks, much of the sampling in the low intertidal region will be accomplished via divers when the intertidal region is flooded.

6.5.3. Collecting Invertebrate Samples in subtidal

This will be done entirely by Jewett's team and will be their first priority. Depending on how quickly goals can be achieved, his team will move into the intertidal. Subtidal sampling will obtain representative samples along fixed transects close to identified faults adjacent to the three shot sites. The number of faults at Milrow, Long Shot, and Cannikin are 2, 5, and 4, respectively. Additional sampling may occur at sites identified by Mark Johnson's CTD data. Highest priority for sampling will be given to Milrow and Long Shot sites and will depend on the sea state and weather. Sampling along each fault transect will occur within 2 or 3 depth zones, i.e., 0-10 m, 10-20 m, and 20-25 m as well as intertidal. A GPS position will be taken at the midpoint of each depth zone for each transect. Two 2-person dive teams (from 1 large or 2 small skiffs) will target specified plants and invertebrates to be placed in separately-labeled mesh collection bags. One diver will remain in a skiff to support the dive teams. One diver will make a digital video recording of the entire transect, periodically noting the depth. Videos will be taken close to the bottom (ca 40cm) to document species and higher to

document coverage. Still photographs will also be taken to document unique biota and/or geologic features. Marginal weather conditions may require a dive skiff tender. All diving operations will adhere to no-decompression limits.

Jewett's protocol will be attend daily briefing, sign out, take his safety and diving equipment in skiffs, and sample beginning at point that will be permanently marked with GPS locations of all samples. We will need to target the 1500 gram per samples that David Kosson recommended for fresh material. Many of the invertebrates are small, and large numbers will be needed for each sample. The amount of time required to adequately sample each of the three locations on Amchitka (Long Shot, Milrow, Cannikin) and the reference site(s) is estimated at 5-7 days each. Sampling duration at the reference site will depend not only on weather, but on the density of organisms which vary from place to place and year to year.

It will not be possible to determine the actual time required until we are on site, but experience has indicated that 5-7 field collecting days per site should be sufficient.

6.6. Collecting Aleut Foods

Patrick's Aleut team will be deployed to collect specimens in their traditional manner, focusing first on marine mammals if permits are obtained, but also on fish and other organisms common in the subsistence diet. This is a very high priority for stakeholders, particularly the Aleuts and ADEC/USFWS. They will sign out with Volz each day and have a checkout of safety equipment. They will use their traditional equipment, and travel by skiff or vehicle. They may be accompanied by Volz/Burger to document activities and as a safety net. While they will fish entirely from these skiffs, when they get a seal they might obtain this on land. They would then have to go onto the land (or small island) to get the seal to bring it back to the Ocean Explorer.

Patrick and the 2 Aleut hunters will have primary responsibility for this, but Burger will need to oversee their choice of species.

6.7. Collecting on NOAA fishing trawl boat

6.7.1. Responsibility

Weston will be the sole CRESP collector on the NOAA trawl boat. As part of the conditions of his tenure on the boat he is to help as a scientific crew member the hauling operations and scientific mission of the overall Trip. His first responsibility is to obtain the specimens needed within that framework.

6.7.2. Duties

Duties include collecting fish at the Amchitka hauls and at reference hauls from Amchitka west. Target species are those fish and crabs listed in the Amchitka Science Plan (with ecological equivalents).

Primary responsibility is to collect the fish, with appropriate Chain of Custody and entering of collecting data in field notebook, followed by sample preparation (see below). Samples to be taken from fish are muscle, bone, liver. Whenever possible, at least two 25 gr samples of muscle, bone and liver will be taken from each specimen, along with the archival specimens, with appropriate labeling (indelible magic marker on the plastic bag) and Chain of Custody.

6.8. Chain of Custody.

All phases, from sample collection and preparation, to sample handling on the boat, to transfer to Rutgers or other freezers, to transfer to analysis laboratory will have appropriate Chain of Custody Forms. Forms are attached.

The overall Chain of Custody forms follow the logical progression of work from sampling through shipment to Rutgers or other freezer space in New Jersey (Fig. 1).

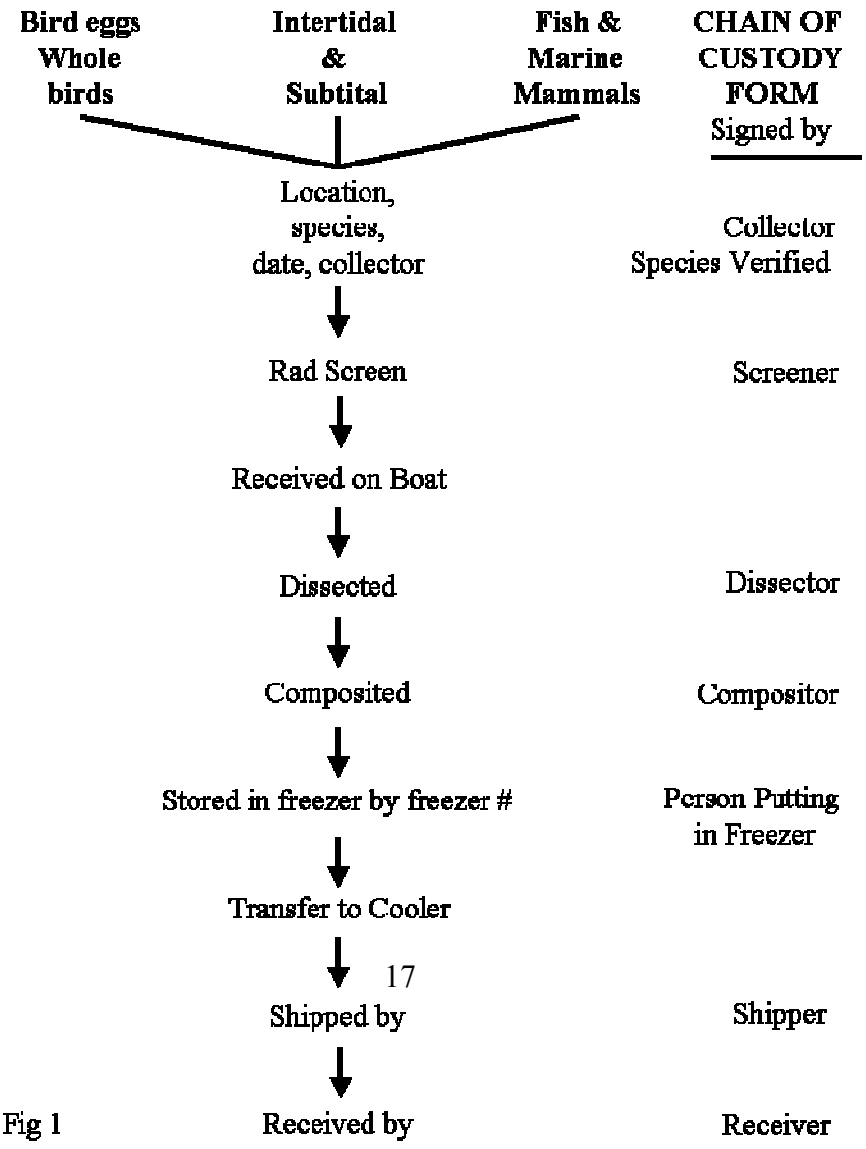


Fig 1

7. SAMPLE PREPARATION ON BOARD

All samples will be screened with hand-held instrumentation prior to being brought to the hold. Samples that are two orders of magnitude above background will be placed in leaded bags prior to preparation and after preparation (but not compositing) they will be frozen in leaded bags. Screening values will be recorded on the Chain of Custody or in the notebooks.

7.1. Sample preparation prioritization

Whenever people are not engaged in sampling, either because they are done their specific tasks for the day, or the weather does not allow field work, they will be involved in sample preparation. Sample preparation will occur during many of the days. However, most of the field are physically or physiologically grueling so that rest periods will be required. Otherwise it is anticipated that all people will be working about 12-16 hours per day/seven days a week while on ship.

7.2. Procedures

We will have 4 wet stations set up in the hold, and each will be involved in dissecting, obtaining weights on whole organisms and organ weight such as liver, putting specimens in bags, preparing labels, completing Chain of Custody forms. A major part of this task is to assemble the specimens that will be composited for analysis. A critical feature of this, meeting EPA guidance is to put together samples from one species of a similar size. Each specimen will be dissected on a fresh surface. Because the fresh-water supply on Ship is very limited, a fresh surface will be used for each sample, rather than rely on washing-down the surface. There will be cleanup between each sample to prevent cross-contamination. There will be periodic wipe-sampling measured in hand-held instruments to assure that work areas are not contamination with radionuclides. One person will be assigned as the recorder on a dry station. All data will be entered in laboratory notebooks and each page will be initialed by the data recorder. Data will then be entered on computer spread sheets.

There will be a set protocol for the order things are done in, when information is recorded, what information is recorded, what tissues and amounts are to be taken. We plan to prepare each specimen on a separate sheet of aluminum foil and change foil after each specimen to prevent cross-contamination (although all agree that this is unlikely). We will change the plastic cover on the bench frequently. As mentioned above, the supply of freshwater on a boat is limited, so we will need extra utensils so they can be washed as a group to save water.

Possible compositing of samples on board is a very critical phase because Burger will be putting specimens together only from the same sample location (GPS locations) so that if we later get a hit we have not mixed up locations even within a transect. This phase will be time-consuming.

Recording data in the laboratory notebooks is a priority, and computer data entry is secondary.

7.3. Sample preparation

7.3.1. Vertebrates

7.3.1.1. Maintain Chain of Custody. Avoid of cross-contamination

We expect to dissect vertebrates (birds, fish, mammals) on board. Gloves will be changed between each sample, and aluminum foil will be discarded between each sample. Dissection tools will be washed as a group between samples.

7.3.1.2. Tissues samples

Bird eggs - whole
Bird - Adult/chicks - muscle, bone, liver
Fish - muscle, bone, liver
Mammals - muscle, bone, liver

7.3.1.2. Samples, replicates, duplicates, archival

a = 25 g - sample to be placed in a composite for initial analysis.

b = 25 g - duplicate sample for a duplicate composite for redundancy in case there is loss or spoilage during shipping.

c = 25 g - sample to form a replicate for QC in the initial laboratory.

. d = 25 g - replicate sample for QC in reference laboratory.

e = 200 g - of sample in case we need to go to the 1000 g composite for

f = 500 g - archival. This may be used in case there is a need to measure individuals from a "hot" composite or in case DOE or stakeholders request a repeat analysis. Archived samples must be maintained frozen for at least two years following filing of the final report.

g. Larger sample taken when time does not allow for all of the above samples.

The samples to be collected generally for large organisms are shown on Fig. 2). Each individual sample will have a unique number which includes the species-location-collector -sample number-section number. That is, an individual fish will be ATKA-

LS-JB-1MA, which will mean Atka Mackerel, Long Shot- fish 1, sample A (For the composite analysis.

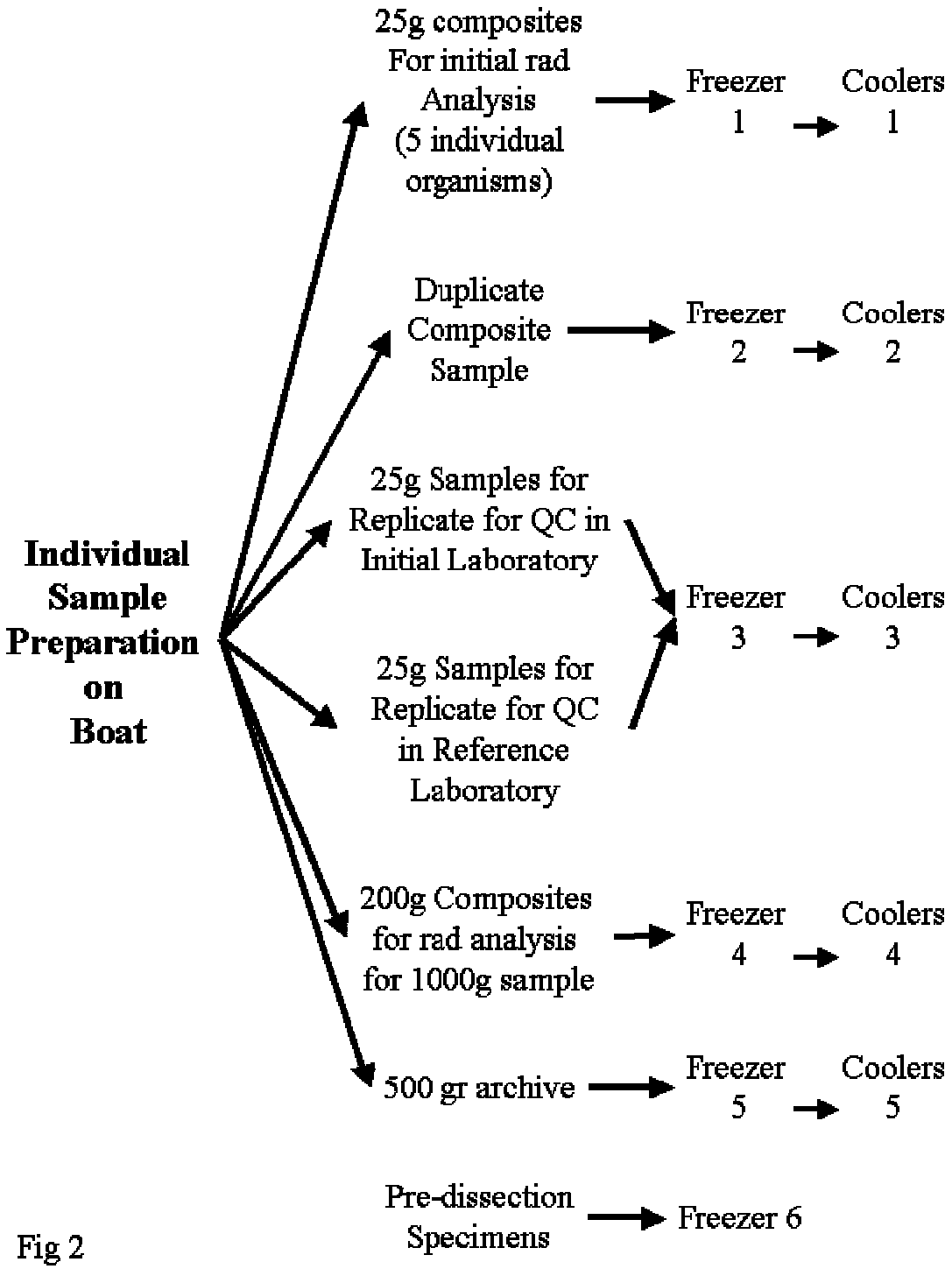


Fig 2

7.3.1.3. Compositing

Using size, weight and collecting location, we will composite samples from 5 organisms. Each individual will be labelled and bagged separately, and all five will be put together in a plastic bag. This will be put in a second bag, along with the Chain of Custody forms. There will be a second, duplicate composited sample with the same 5 organisms and a separate Chain of Custody Form. This is a duplicate sample in case there is loss or spoilage during shipping.

7.3.1.4. Single sample packaging.

All the other samples listed above will be bagged and labeled separately.

7.3.1.5. Sample storage.

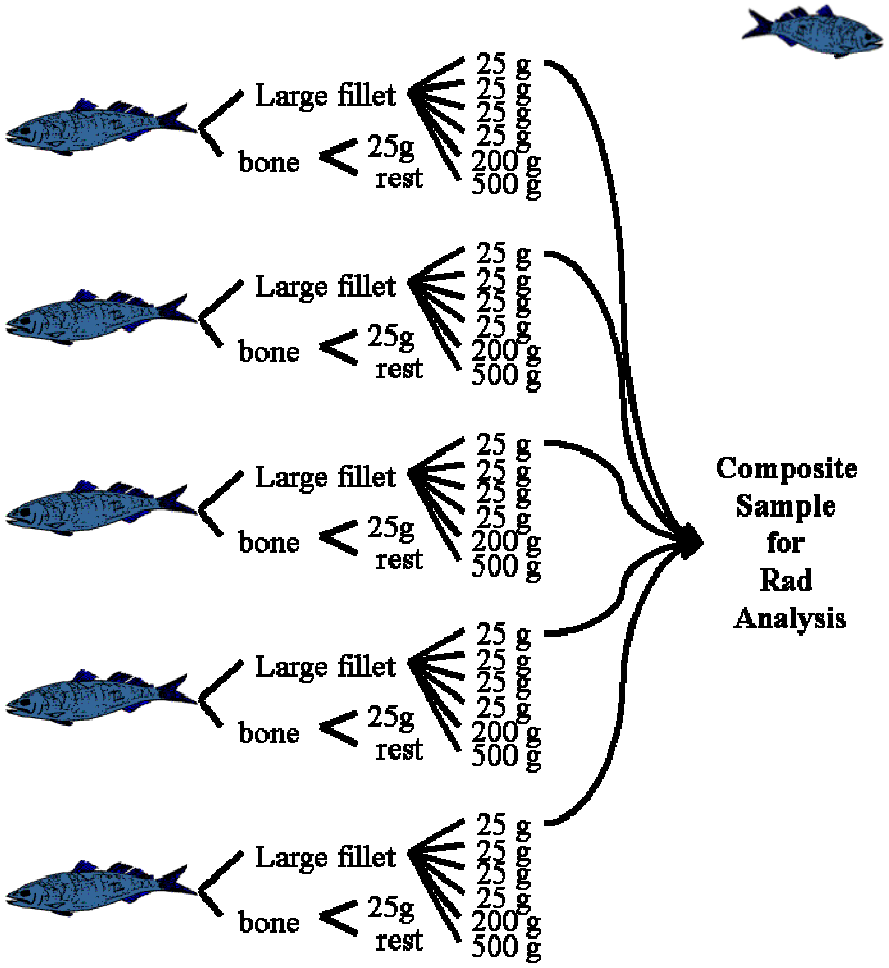
On the main sampling vessel, the different types of samples will be placed in separate freezers so that all the initial samples to be homogenized and composited will be together, and their replicates/duplicates will be in a separate freezer and will be transported in separate coolers.

7.3.1.6. Chain of Custody

All samples will have separate Chain of Custody form attached to them linking them uniquely to the original specimen.

An example of a fish compositing is shown in Fig. 3. The composite for the initial radionuclide analysis will have a separate tracking number.

Example of One Composite: ATKA MACKEREL



a 25g will into a composite for radionuclide analysis
b without head or tail

Fig 3

7.3.2. Invertebrates

7.3.2.1. Avoidance of cross-contamination

Similar procedures to vertebrates.

7.3.2.2. Tissue samples

Soft tissues from all invertebrates will be collected.

7.3.2.3. Samples, replicates, duplicates, archival

We will attempt to obtain the same samples as give in 7.3.1.2. above, although this will depend upon the size of the invertebrates. While we can composite samples from 5 individual avian livers or fish muscle, this will not be possible with

invertebrates. The number of animals in a sample depend upon size, and these decisions will be made on the boat between Jewett and Burger at the time of collection.

7.3.2.4. There will be no single sample packaging except possibly for large crabs and kelp.

7.3.2.5. Sample storage will be the same as above.

7.3.2.6. Chain of Custody will be the same as above.

7.3.3. Labeling

7.3.3.1. Types

All containers will be labeled on the container itself, with labels (if available), and all containers will be associated with a Chain of Custody.

7.3.3.2. Label system

A standard labeling system will be followed for all specimens that includes: species identification -location-collector team-specimen #-specimen type-quantity.

Location = the transect or other, and includes:

I = Island
LS = Long Shot
A = Aleut sampling
TA# - Amchitka trawl #
TR# = trawl reference # (Weston).

Specimen type - M=muscle
L=liver
B=bone

7.3.3.3. Examples

An atka mackerel muscle from longshot transect area by Robert Patrick.

ATKA-LS-RP-1-Mq

Atka mackerel muscle, NOAA trawl (Amchitka trawl)

ATKA-AM-JW-1-Mdq

In both cases, 1a refers to the 1st 25 g sample taken from compositing for initial radionuclide analysis.

TABLE 1. KEY FOR LABELLING SPECIMENS (CRESP-Amchitka Project)

SPECIES-LOCATION-COLLECTOR-SPECIMEN #-TYPE-SUBSAMPLE

Example: ATKA-LS-RP-1Mq ATKA-AT-JW-1Mq

SPECIES:

KELP -Fucus = FUCU Laminaria = LAMI Sea Lettuce=ULVA
Alaria - ALAR Hedophyllum = HEDO

Acorn Barnacle - BARN
Giant Chiton - CHIT
Green Sea Urchin - URCH
Blue Mussel - MUSS
Basket Star - BASK
Rock Jingle - JING
Red King Crab - RECR
Brown King Crab - BRCR
Octopus = OCTP
Common Eider - COEI
Glaucous-winged Gull- GWGU
Pigeon Guillimot - PIGU
Tufted Puffin - TUPU
Bald Eagle - BAEA
Dusky Rockfish - DUSK
Pacific Ocean Perch - PERC
Pacific Salmon - SALM
Dolly Varden - DOLL
Atka Mackerel - ATKA
Rock Greenling - ROCK
Walleye Pollock - WALL
Pacific Halibut - HALI
Turbot - TURB
Sculpin - SCUL
Harbor Seal - SEAL

LOCATION:
LS=Long Shot
ML=Milrow
CA=Cannikin
AM - on Amchitka Island
KI - Kiska
AD = Adak
TA# - NOAA trawl near Amchitka (number as appropriate)
TR# = to be designated by Weston for reference sites

COLLECTOR TEAM
JB = Burger
SJ = Jewett
RP = Aleut
JW = Weston

SPECIMEN # - consecutive within each species

TYPE: M = Muscle L = Liver B = Bone W=Whole E = egg

SUBSAMPLES:
c = 25 g - **Composite** sample to be placed in one bag
5 individual organisms totalling 125g
d = 25 g - **Duplicate** Composite (5 individuals/125g)
r = 25 g - **Replicate** for QC in the initial laboratory.
q = 25 g - **Replicate** sample for QC in reference laboratory.
e = 200 g -in case we need to prepare 1000 gr composite
a = 500 g - **Archival**
g. Larger sample - when limited preparation time on boat

Tidal Relationships of Biota to be collected at Amchitka

Table 1. Major ecoreceptors at risk in the Amchitka Island marine ecosystem.

	Intertidal	Subtidal	Deepwater	Surface
Sessile/benthic	Kelp Sea Lettuce Chiton Blue Mussels	Tunicate, Sea Cucumber, Kelp, Giant Chiton, Blue Mussel, Rock Jingle		
Mobile		Sculpin, Rock Greenling, Octopus, Sea Urchin, Basket Star	King Crab Sculpin Pollock Dusky Rock Fish Ocean Perch Atka Mackerel	Sea Otter Harbor Seal Sea Lion
Migratory	Eagle Oystercatcher Gulls Common Eider		Halibut Pacific Salmon Dolly Varden Pacific Cod	Harbor Seal Sea Lion Eagle Puffins & other seabirds

7.3.3.4. Compositing Labeling

Composited samples will get a separate number for that composited sample, and this number will be recorded in the notebooks as well. START all composites with # 500.

Species-location-team-C- composite #-type (muscle)

An atka mackerel, Long Shot, Patrick composite muscle
ATKA-LS-RP-C501m

** All atka mackerel specimens from Long Shot transects will have consecutive numbers.

8. TRANSPORT ADAK TO RUTGERS AND PORT AUTHORITY (NEWARK)

The current plan is to ship all the primary composites and the individual replicate directly to Nelson Biological Laboratory (Rutgers University). Any specimens requiring lead-bag treatment will also be shipped to Rutgers in a separate cooler.

All duplicate composites and archive specimens will be shipped to the Newark freezer repository. The shipping phase will be handled by D. Volz. Adequate freezer space is available (at Rutgers, or Port Authority facility). There will be a separate Chain of Custody form for coolers during transportation; this Chain of Custody will not be broken until and unless the specimens from that cooler are needed.

9. SPECIMEN HANDLING AT RUTGERS

9.1. Specimen tracking

A specimen tracking system has been set up by V. Vyas, who will manage the electronic data base during and following the voyage (see section 5.3.1.1).

For specimens located at Rutgers, Burger/Gochfeld will track specimens as to location and type so that a given specimen can be found at any time for processing. Similar records will be maintained for the other storage facility (currently Port Authority at Newark).

9.2. Specimen preparation

9.2.1. Specimen selection

Specimens to be prepared each day will be selected by Burger/Gochfeld/Kosson. All steps will be recorded in a laboratory notebook (Burger team), and later entered into the computer data base (V. Vyas).

9.2.2. Specimen handling

Specimens will be defrosted and composites selected. Components will be weighed and trimmed to 20 ± 0.1 g.

9.2.3. Homogenization

Assuming adequate homogenizers have been purchased each sample will be homogenized separately in acid-washed homogenizers.

9.2.4 The five homogenates will be combined and then blended in an homogenizer.

9.2.5. Oven-drying

Assuming adequate ovens have been purchased, the final blend will be oven-dried at 60° and weighed q-3-h until a constant weight is achieved.

9.2.6. Shipment

Oven-dried specimens, with a new Chain of Custody form will be shipped by CRESP to the analytic laboratory.

10. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

This is the overarching feature of the CRESP-Amchitka Project. See also the Quality Assurance Document which is separate from the Implementation Plan. This section includes some duplication of sections 1-9 above, with an emphasis on the Quality Assurance features.

10.1. Objectives

Our objective is to obtain valid and adequate biological specimens representing subsistence foods, commercial fish, and ecosystem trophic levels, for laboratory analysis.

Types of Specimens range from invertebrates to vertebrates which will be collected by a wide variety of methods. Locating the species in the field requires expertise and experience and is the most time consuming part of the sample acquisition. The number of specimens needed was determined in advance to provide adequate power to detect an order of magnitude difference between Amchitka and reference sites. All collections and preparations will be documented and in most cases two people will verify data initially. Adherence to field and laboratory operating procedures will be documented for the data verification process. Quality control in the preparation and compositing phase will be crucial as will quality control in the analytic phase.

This section applies to the subtidal invertebrates (collected by the Jewett team), intertidal organisms (collected by the Jewett team, with help from Burger team if possible), fish and sea mammals (collected by the Patrick and Burger teams), avian and marine mammal specimens (collected by the Burger and Patrick Teams). The following SOP is aimed at achieving Quality Assurance and Quality

Control for the acquisition, preparation, and distribution of biological specimens.

10.1.1. Safety

All field work requires a buddy system for safety as well as for verification of locations, species, and methods.

10.1.2. Voucher specimens.

Two people will sign the initial Chain of Custody form verifying the initial collection data. Voucher specimens and photo-documentation will be obtained. On the NOAA Trawler, Weston will confer with the experienced NOAA fisheries biologists to confirm the identification of each specimen, and will retain whole voucher specimens of each species.

10.1.3. Training

All personnel will receive training for all phases of biological collection, specimen preparation, and specimen handling. Training will be performed on shipboard prior to any collection, by J. Burger, S. Jewett, M. Gochfeld and R. Patrick.

10.2. QA/QC Aspects of biological sampling

There are three features of biological collection are crucial to QA/QC:

1. Correct identification of species
2. Adequate description of collection location physically
3. Adequate description of habitat location.

10.2.1. Correct identification of species

All personnel involved in collection of any biological samples will be under the direction of someone who is expert in that discipline, and has been involved in collections previously:

J. Burger/M. Gochfeld: marine birds, mammals and fish

S. Jewett: invertebrates

J. Weston: marine fish and crabs

10.2.1.1 Voucher specimens

A voucher specimen from each species will be collected whole, frozen entirely, with an appropriate Chain of custody form for later verification if necessary.

10.2.1.2. Photographic voucher

A digital photograph will be taken of each species type for purposes of future verification if necessary.

10.2.2. Collection location physically

10.2.2.1. Locations

The locations of all collections will be recorded in the field and in the laboratory notebooks, and checked by a second person. All physical locations will be noted using GPS in digital format.

10.2.2.2. Information recorded will include:
Land or water
Location on the island with GPS coordinates.
Transect number, position, depth
Photo-documentation

10.2.3. Habitat location

10.2.3.1. General habitat will be recorded in the field and in notebooks, using agreed-upon categories to be identified after arrival on Amchitka.

10.2.3.2. Collection of bird specimens will require travel to the northwest end of Amchitka along the Infantry road, in all-weather enclosed and heated vehicles.

10.2.3.3. During this trip we will look for evidence of gull and eider nesting and collect eggs or young from those species.

10.2.3.4. Specific habitats where specimens are obtained will be noted:
Position on land (or in colony)
Location on slope, on structures or vegetation
Tidal designations and depth, aquatic organisms.
Other habitat variables as needed.

10.2.4. Specimen handling

Handling procedures in the field vary for each type of specimen. Where feasible pre-written labels and chain of custody forms will be used for each locality and species, facilitating the recording of data on the label in the field. Terrestrial specimens will be placed in zipped plastic bags of appropriate size, with a field label. Once bagged, eggs will be transported in padded, rigid containers. Other specimens will be double bagged including a chain of custody form.

Specimens obtained by divers will be brought back to their boat for bagging and labeling after each dive. The five diver team will allow four people to be in the water, and one person to handle the specimens as they are brought to the boat.

10.3. QA/QC for Shipboard Specimen handling

10.3.1. Responsibility

All handling of biological samples will be overseen by J. Burger, M. Gochfeld and S. Jewett. This will require at least one of the three dedicated to specimen track and data entry at all times. Thus both the sample preparer and the data tracker will concur on the identification of species and tissue. Training of all specimen preparer will be conducted by J. Burger, M. Gochfeld, and S. Jewett, prior to initiation of operations.

10.3.2. Chain of Custody

Chain of Custody is a critical component of Quality Assurance. Chain of Custody forms (Figure 4) will accompany all specimens. A chain of custody form will be initiated at the time of collection and will follow the specimen to Rutgers where it will remain with the archived specimen. Each subsample will have a new Chain of Custody form initiated, linked to the original form. Persons shipping and receiving specimens will sign the Chain of Custody form as well persons preparing and analyzing the sample. Thus at all times it will be possible to know who has handled a specimen at each step of the process.

10.3.1. Tracking of specimens

All specimens will be associated with a Chain of Custody form which will follow the specimen through every step. Locations of all specimens will be recorded in a separate laboratory notebook with respect to location in freezers or coolers. Separate freezers will be used for different portions of specimens to ensure appropriate duplication. Duplicate specimens will be shipped in separate coolers so that loss of a cooler during transport will not compromise the overall project.

Insofar as possible specimens will be accompanied by one of the project personnel from point to point, from collection to the Rutgers laboratory and freezer facility. Shipment from Rutgers to the analytic laboratories will be by a commercial courier service (UPS, FedExp, or other to be determined). Master records will be kept in laboratory notebooks and on computer file, initialed by the responsible individual. All pages in laboratory notebooks will be initialed by the data entry person and will include the name of the preparer.

10.4. QA/QC for Laboratory sample preparation

10.4.1. Responsibility

During laboratory procedures at Rutgers all specimen preparations will be conducted by two laboratory technicians, each of whom will verify the others' records and procedures. These will be overseen by J. Burger and M. Gochfeld, with additional data management oversight by V. Vyas. At least 2 people will concur on species identification, tissue identification, homogenization and compositing.

10.4.2. Tracking

A key feature is the ability of laboratory personnel to locate specimens in freezers when needed. This is a complex filing task. Freezers will be locked, and a log sheet will be attached to the freezer to document when and who opens it and what specimens

are removed. Copies of completed freezer logs will be retained in the laboratory. All specimen preparations will be conducted by two laboratory technicians, each of whom will verify the others' records and procedures. These will be overseen by J. Burger and M. Gochfeld. All balances have annual factory calibration and certification. Balances are calibrated each day and calibration results are recorded. A standard 20 g weight will be used to calibrate for this project.

10.4.3. Compositing, Homogenization, and Compositing.

There are two compositing phases: on the boat or in the laboratory when a decision is made to put 5 particular, similarly-sized specimens together, and when each sample is homogenized and has to be composited for final drying.

In the homogenization steps all glassware will be acid washed (HNO_3) and rinsed with deionized water between each procedure. Pre-composited samples representing five specimens (approximately 25 g each) will be removed from their plastic bags. Each individual sample will be trimmed and weighed to 20 g to the nearest 0.1 g and will be homogenized separately until a uniform liquid consistency is achieved. The five samples will then be blended together in a freshly cleaned homogenizer for 10 min to assure complete mixing. The samples will be oven-dried at 60°C for 48 hours or until constant weight $\pm$ 1% is achieved. The final weight of the residue will be recorded, and the sample will be placed in a zipper plastic bag and labeled. This bag with a new chain of custody form signed by the laboratory technician, will be placed in an outer zipped plastic bag, for shipping to the analytic laboratory (Vanderbilt, INEEL or other). Bone samples from fish will be cut into 20$\pm$0.1 g segments and bones from five fish will be placed in a zipped plastic bag which will be labeled and placed with chain of custody form into an outer zipped plastic bag. Ashing for bone-seeking radionuclides will be accomplished at INEEL.

10.4.4. Shipment will be by a commercial courier.

FIGURE 4: SAMPLE CHAIN OF CUSTODY

CRESP Amchita Project:Joanna Burger (burger@biology.rutgers.edu)					
FIELD AND COMPOSITE SPECIMEN CHAIN OF CUSTODY (circle one)					
SPECIES			IDENT #		
LOCATION			GPS N		
COLLECTOR			GPS E		
Component##					
Date	**Action**	**Initials**	**Disposition OR Destination**	**Any New numbers for split samples**	**Comp C or D or 200 ---------- COMMENTS**
	Collecte				
	ID-				
	Rad-screen by				NaI GM
	Stored				
	Dissecte				
	Composit				C#
	Stored in box or freezer		Box # Freezer #		
			Freezer #		
	Removed				

CRESP Amchita Project:Joanna Burger (burger@biology.rutgers.edu)
Stephen Jewett (jewett@ims.uaf.edu)

COOLER, BOX OR PACKAGE CHAIN OF CUSTODY (one copy inside & out)

CONTENTS — LOCATION NAME

(see details on attached sheet) — PACKAGE #

COLLECTOR

Date	Action	Initials	Disposition OR Destination		Comments
	Packed by				
	Rad screening (optional)				NaI GM
	Shipped by				
	Received by				
	Stored in box or freezer		Box # Freezer #		
	Removed for final preparation				

11. SAMPLE ANALYSIS SELECTION

This phase will involve which of the samples to analyze, and in what priority order.

11.1. Prioritization: Our underlying assumptions are:

11.1.1. Underlying assumptions

a. 137Cs and 90Sr accumulate to a significant extent in the soft tissue (muscle) that is typically consumed.
b. Other isotopes that are expected to result from the test shots accumulate primarily in skeletal material.
c. U and Pu, which provide information on the potential source of the isotopes, preferentially accumulate in the skeletal material.
d. Therefore, analysis of soft tissue provides the primary insight into human health risks from consumption, while analysis of skeletal material provides primary insights into the potential sources of the radionuclides and foodchain transfer. Analysis of soft tissue is to provide insights into potential human health risk and provide a baseline for comparison with potential future studies. Analysis of soft tissue also will provide insights into food chain transfer in the marine ecosystem. Analysis of skeletal materials is to provide indicators of sources of contaminants, information about foodchain accumulation, and to provide a baseline for comparison with potential future studies.
e. Information of ambient or reference levels for the isotopes of interest in marine species is limited, especially for isotopes other than 137Cs. CRESP continues to review the status of such data.
f. Information on thresholds for individual isotopes that result in ecological risk for the specific marine species of interest is very limited. CRESP continues to review the status of such data.
g. Therefore, analysis of species that are not primary contributors to human consumption is

primarily to obtain a baseline for comparison with potential future studies.

h. Initial screening of marine biota using gross alpha and beta analysis has been determined not to be used because of the tradeoffs between cost, sensitivity, specificity and sample requirements. Instead, isotope specific screening as identified under (3) below will be used.

11.1.2. Priority in addressing specific programmatic objectives are as follows:

a. Defining the human health risk posed by the primary consumed components of subsistence and commercially consumed marine biota. Minor diet components are not as important as primary diet components.

b. Identifying the potential sources of radionuclides that are identified to cause significant human health risk.

c. Establishing the baseline of radionuclide concentrations in the primary consumed components of subsistence and commercially consumed marine biota, and in marine biota on different trophic levels.

d. Identifying the potential sources of radionuclides that are above detection limits while establishing the baseline in the primary consumed components of subsistence and commercially consumed marine biota.

e. Analysis of marine biota from different trophic levels that would be indicative of transmission and accumulation of radionuclides through the ecosystem.

f. Analysis of marine samples that are the lowest trophic levels (e.g., kelp and sediments) that potentially may accumulate radionuclides.

11.2. Screening analysis

Screening analysis will be performed on one composite for each species from each sampling location with the intent of identifying the specific species within each trophic level to focus more extensive analyses. Overall, this screening is assumed to be

limited to a maximum of 25 species across all trophic levels. Screening analysis will consist of analysis of muscle for 137Cs and 90Sr and analysis of corresponding skeletal material for the full range of isotopes under consideration in this study. The following potential species and trophic levels have been identified for sample collection (These are all species that form part of the subsistence diet, except for eagle):

Kelp/Ulva
Chiton/Sea Urchin
Blue mussel/Basket Star/Rock Jingle
Octopus
Red king Crab/Brown Crab
Octopus
Ocean Perch
Dolly Varden
Atka Mackerel
Halibut/cod/Pollock
Eagle
Gull/Puffin
Eider/
Guillemot/or other high trophic level seabird
Harbor Seal/Sea Otter

11.3. Sample selection

11.3.1. Initially analyze the one within each group with the sample size that most approaches our target (in most cases, the one with the highest sample size).

11.3.2. Where there are equal sample sizes, select the one that is expected to be the highest accumulator of radionuclides (e.g. top level predators for birds, mammals, fish).

11.3.3. Where there are equal sample sizes, and no obvious difference in their accumulator qualities, select the one that is a traditional subsistence food or commercially viable.

11.4 Multiple replicate selection

For selection of specific species to focus analysis on (e.g., multiple replicates), the following considerations apply:

11.4.1. One species representative of each of the selected trophic levels will be identified for more extensive analysis. More extensive analysis will consist of

11.4.1.1. Up to 10 analysis for each sample location for 137Cs (soft tissue)

11.4.1.2. Up to 4 analysis for each sample location for 90Sr (soft tissue)

11.4.1.3. Up to 4 analysis for each sample location for remaining isotopes of interest (e.g., U and Pu series, 99Tc, other isotopes of interest) in skeletal material

11.4.2. Priority for selection of species for analysis will be based on:

11.4.2.1. Human health risk, if above a nominal minimal threshold of 10E-6 excess cancer risk if consumed at a rate of 300 kg/yr for 75 years.

11.4.2.2. Information about isotope sources based on measurable U and Pu isotope series.

11.4.2.3. Ecological importance

11.4.2.4. The following algorithm would be used for assigning priority within a given trophic level:

$$\textit{Priority number} = \log(10 \times \text{human health risk})/(10E\text{-}6) + (\textit{Pu information} + \textit{U information})$$

Under this approach, the larger the *priority number*, the higher the priority for the more extensive analysis of the species within the designated trophic level. The human health value would equal 1 for a 10E-6 risk level. The *Pu information* would equal 0.5 if characteristic Pu isotope ratios are measurable. The *U information* would equal 0.5 if the characteristic U isotope ratios are measurable. Thus, the value of the source information would be equal to either 0, 0.5 or 1). Tie values would favor human health if the risk value was greater than

10E-6 and would favor source information if the risk value was less than 10E-6.

iv. Measurement of Pu isotope ratios can be used to provide an indicator of whether the source of contamination was from a nuclear test shot. Measurement of U isotope ratios can be used to provide an indicator of whether the source of contamination was from enrichment or reactor accidents or discharges. Absence of information on these ratios does not support that the source of contamination was not from one of these causes.

11.5. In total, approximately the following numbers of analyses can be completed within our current budget:

a. 137Cs – 600 (20% at INEEL, 80% at VU)
b. 90Sr – 200
c. Other isotopes – 200
d. These numbers will be refined after trial runs and validation of all analytical measures. `
e. Reductions in gamma counting times will allow for more 137Cs sa
f. Fewer screening analyses would allow for more replicates focused on specific species.