Terrestrial Invertebrates Standard Operating Procedures

USGS Western Ecological Research Station SFBE & Nisqually Indian Tribe

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Purpose/Objective:
To assess capacity of the restoration to produce prey resources for juvenile Chinook salmon, we will sample insects that fall into the water from the aerial environment using fall out traps. This sampling is paired with fyke net surveys for juvenile Chinook. These traps measure the direct input of invertebrates from the marsh to the aquatic system by capturing insects that fall or settle onto the surface of the water (Gray et al 2002). Three replicate traps will be deployed monthly between March and August (outmigration season) at each intensive fish sampling site during the low tide and allowed to float with the tide. The traps will be left to sample for 48 hours over an entire tidal cycle.

Figure 1. Fall-out trap, Nisqually, WA.

These protocols provide information on sample collection and sieving. After preservation, samples will be sent to a qualified invertebrate laboratory for sorting, identification, enumeration and weighing for dry biomass. If you decide to sort and identify in-house, refer to the *Invertebrate Lab Manual* (USGS 2010).

Vegetation Composition and Structure (optional)
To explore the relationship between vegetation composition and structure to terrestrial prey availability, we will closely coordinate vegetation sampling with fall-out traps. A vegetation quadrat will be sampled at each fall out trap location for 6 consecutive months (Mar-Aug). Alternatively, vegetation could be sampled in early and late/peak seasons (e.g. Mar & Aug). A 0.5m x 0.5m vegetation quadrat will be used to identify plant species, estimate percent cover for each species, and measure maximum height and stem density of each plant species.

Soil pore-water salinity (optional)
Can be measured in conjunction with salt marsh vegetation quadrats. Salinity data can aid understanding some of the fundamental causes of vegetation change. Soil salinity is measured adjacent to vegetation quadrats. These measurements are taken using substrate extracted water and a refractometer.

Fall-out Trap Surveys:

Fall-out Trap Equipment Needed:

Installation	*Setting up*	*Collecting*
10ft PVC poles or metal conduit (4 per trap)	3-6 Traps/bins (0.25 m^2; 55-cm x 38-cm rectangular plastic basins)	3- 6 containers with lids
1 Trap/bin (0.25 m^2; 55-cm x 38-cm rectangular plastic basins)	String/Fishing line	Rite in the Rain labels
Rubber mallet or post pounder	Soap	70% Ethyl alcohol
GPS	Bucket	#120 sieve (125µmm)
	#120 sieve (125µmm)	Tweezers
	Clock	Spoon
	Datasheet	Clock
		Datasheet
		Knife/Scissors
		Bucket

Methods:

Installation

1. Select trapping locations along channel edge at each fyke netting sampling station.
2. Fall-out traps should be placed upstream of fyke net and far enough away that they are not trampled during fyke netting surveys.
3. Three to six fall-out traps should be installed per fyke net location.
4. For habitat type comparisons, place three replicates in one habitat type (e.g. *Carex lyngbyei*) and another three in a different habitat type (e.g. *Distichlis spicata*).
5. Replicates should be placed at least 1 meter from each other.
6. At each fall-out trap location, pound in PVC or conduit in a diamond-shape pattern (Figure 1) using the rectangular plastic trap as your guide.
7. Record the UTM coordinates of each trap.
8. Drill a small hole into the edge of each plastic trap so that it can be tied to the poles.

Setting up

1. Set traps during low tide.
2. Put traps between the 4 poles.
3. Pour soap in the bottom; not too much as it will clog the sieve when collecting, but just enough to break the water surface tension.
4. Collect a bucket of water from the tidal channel.
5. Pour water through sieve into the trap; only need a few inches. Swirl water to mix with soap.
6. Record time for each trap set up.
7. Tie at least one end of the trap to the poles with the fishing line.

Collecting

1. Ideally, each trap would be collected 48 hours after it has been set, though collections can occur within 46 – 50 hours of set time.
2. Detach trap by cutting fishing line and lift away from poles.
3. Have partner hold sieve or if confident place sieve on ground and pour the entire contents of the trap through the sieve.
4. Pour a little ethanol solution into a labeled container to minimize soap bubbles. Container should be labeled with site, date, and trap# on the outside and with a Rite in the Rain label on the inside.
5. Transfer contents of sieve into labeled container. Pool a little water in the corner of the sieve and pour invertebrates in, instead of using a tweezer for each individual. Include debris as it may be hiding invertebrates.

6. Add more ethanol to the container making sure that there is more ethanol than water in the container.
7. Place containers in bucket or backpacks. Store samples in a cool, dark location.

Sorting and Identification

After preservation, samples will be sent to a qualified invertebrate laboratory for sorting, identification, enumeration and weighing for dry biomass. If you decide to sort and identify in-house, refer to the *Invertebrate Lab Manual* (USGS 2010) for details on sorting and identification.

When monitoring is focused on invertebrates as prey resources, invertebrate identification to the lowest taxonomic level, although informative, may not be cost effective to answer questions based on prey resources. Rather, the taxonomic categories of interest should consider the known diet of predators of interest and their foraging modes or behavior.

Vegetation Composition and Structure Surveys:

Vegetation Equipment Needed:

Compass
GPS unit
.25 m^2 quadrant
Veg stick (round pole with centimeter increments written on it)
Sharpie
Data sheets
Clipboard
Pencils
Plant list
Ziploc Bags
Digital camera
Binoculars

Figure 2. Vegetation quadrat placed next to fall-out trap, Nisqually, WA.

Methods:

1. Quadrat data is collected adjacent to each fall-out trap.
2. Set the .25 m^2 quadrat against the outside edge of one of the four trap poles (Fig 2). Center the quadrat on this pole and install additional markers (e.g. wood stakes) on the four corners of the quadrat for repeat measures over time.
3. Take a picture of the quadrat from directly overhead. In the photo, include a piece of paper with the name of the study site, fall-out trap, and date so that the photo is easily identifiable (Fig 3).
4. Record the overall % cover vegetated in 1 meter radius from fall-out trap center.
5. The species name, absolute percent cover, rooted stem count, and the maximum height for each species in these quadrants is recorded. Record all species found in the quadrants as well as non-species.

   a) If standing water is present, measure the deepest water depth within the quadrant (record under WD), but do not include standing water in the percent cover.

   b) To estimate percent cover, first record the four letter code for all species and non-species present. Include all plant material intercepting the quadrant area, not just plants rooted in the quadrant. Make ocular

Figure 3. Photo of vegetation quadrat.

estimates of the percent cover for each species etc. When percent cover for all species, etc. are estimated, sum these estimates. If the sum is less than 100%, reconsider estimates. However, due to different canopy layers, total percent cover can exceed 100%.

c) Height is measured from the substrate to the top of the tallest individual of each species in the quadrant. Record to the nearest cm.

d) Stem counts (densities) are done by counting the rooted stems of individual plants inside the quadrant. This measurement is used to report the density of individuals per square meter for each species. If plant foliage is present in the quadrant, but the plant is rooted outside of the quadrant, record 0 as stem count. Stem density is most often used for woody species where the stems are easily discernable. In salt marsh habitats, the stems can be difficult to discern. When counting stems in the salt marsh habitat it is best to try and locate the base of each plant and count each base as one individual stem. When this is not possible without damaging the plants, then estimations in the field based on the individual circumstances must be made. **In these circumstances, make sure to write down your method for counting in the notes.**

e) There are many grass species that may be growing so densely in the quadrant that only a sample of the stems need to be counted and a total count can be estimated, based on the relative size of the sample compared with the total percent cover of that species.

f) Any species that are not identifiable by the observers should be collected as a voucher specimen for further identification purposes and given a number for the survey, until they are properly identified. DO NOT collect plants within the quadrat or along the transect, rather find the same unidentifiable plant nearby to collect. Collect all parts of the plants including roots, place in a baggie and label a temporary code, the date, and the transect it was collected. However, do not collect plants that are growing sparsely in case they have low populations. If you come across this type of plant, use the digital camera to take a close-up picture and record any notes or observations on the plant's growth form, habitat, and characteristics.

Soil Pore-water Salinity Surveys:
Soil Salinity Equipment Needed:

Hand-held refractometer
Trowel of small shovel
Eye dropper
Filter paper (cut-up coffee filters can be used)
Plastic squeeze bottle with freshwater to rinse and calibrate refractometer

Methods:

1. Sampling should coincide with vegetation quadrat sampling.
2. Calibrate (zero) hand-held salinity refractometer with fresh water (tap water is okay) before EACH field day.
3. At a location near the vegetation quadrat a small shovel is used to create a pooling of water to sample from. This is done by pushing the shovel into the ground and wiggling it back a forth a few times.
4. If the water is not pooling around the shovel then a handful of substrate can be extracted and the water squeezed out into the coffee filter. (Multiple handfuls of substrate can be used in order to gather enough water to filter through the filter.)
5. The extracted water should pass through the filter paper and onto the glass plate of the refractometer. If there is not enough water to go through the coffee filter then a few drops

can be tested without filtration, which is better than no reading at all. (The drops can be placed straight onto the refractometer, the reading line will be fuzzy and a best estimate will have to be made)

6. Be sure to record if the water was collected from the pooled or hand squeezed method, if filtration was or was not successful, and record "dry" if no water can be extracted.
7. Read and record the soil water salinity (ppt) on the data sheet.
8. Clean-up. Discard (never re-use) the filter paper. Rinse refractometer with freshwater.

Data Analysis:

Abundances from samples will be standardized to area and reported as average density of invertebrates per square meter. Terrestrial invertebrate data can be used in multiple analyses. Examples include:

1. Analyzing change in insect composition over time in regards to restoration actions.
2. Comparison of restoration insect composition to reference sites (Figure 4).
3. Correlation analysis between insect composition and environmental variables (e.g. vegetation structure, soil pore-water salinity, weather data).
4. Calculation of available prey resources to fish and avian communities.
5. Use in fish diet analyses when fish diet data has been collected (e.g. percent similarity indices between stomach contents and available prey resources; Table 1).

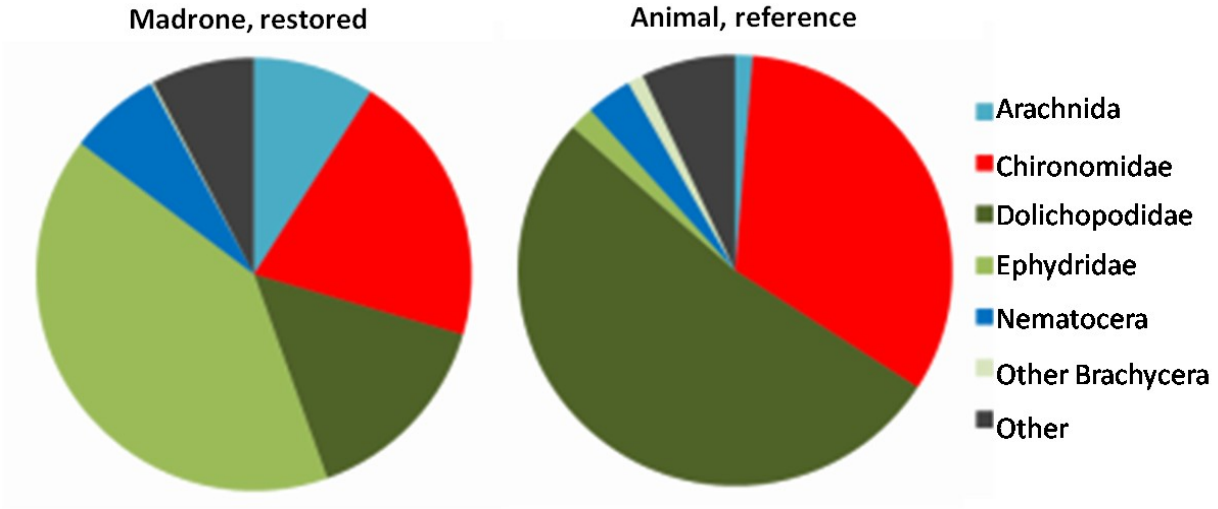

Figure 4. Total catch composition in 2010 at a restored (Madrone) and control (Animal) tidal slough, Nisqually. Fall-out sites were trapped once monthly from March to July.

Table 1. Percent similarity index (PSI) comparisons between Chinook diets and fallout trap invertebrate catches at restoration (Phase I, Phase II) and reference (Phase I Control, sites in 2007, Nisqually estuary).

2007 Fallout Samples

2007 Diets	Phase I Control May	Phase I Restoration May	Phase II Road May	Phase II Fyke May	Phase II Control May	Phase II Road June	Phase II Fyke June	Phase II Control June
Control U2	9	6	4	4	6	4	4	6
Control H2	46	16	18	18	38	18	14	12
Phase I H2	24	**74**	42	42	28	**75**	33	**74**
Phase II U2	26	11	13	13	26	13	9	7
Phase II H2	45	15	17	17	37	16	12	10

	Phase I Control March	Phase I Restoration March	Phase II Road March	Phase II Fyke March	Phase II Road April	Phase II Fyke April	Phase II Control April
Phase II U1	5	5	5	5	5	5	5

References:

Fall-out trap Procedures modified from: Simenstad, C.A., A.J. Wick, J.R. Cordell, R.M. Thom, and G.D. Williams, 2001. Decadal development of a created slough in the Chehalis River estuary: year 2000 results. Report to U.S. Army Corps of Engineers, Seattle District.

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Gray, A., C.A. Simenstad, D.L. Bottom, and T.J. Cornwell. 2002. Contrasting functional performance of juvenile salmon habitat in recovering wetlands of the Salmon River Estuary, Oregon, U.S.A. Restoration Ecology 10:514-526.

US Geological Survey. 2010. Invertebrate lab manual. Unpublished benthic invertebrate sieving and sorting protocols. USGS, Western Ecological Research Center, San Francisco Bay Estuary Field Station, Vallejo, CA.

Fall-Out Sampling Form

Fall-Out Insect Traps

Site Name_________ Observers_________

Site#	Trap#	Date Set	Time Set	Date Coll	Time Coll	Notes

Site Name_________ Observers_________

Site#	Trap#	Date Set	Time Set	Date Coll	Time Coll	Notes

Site Name_________ Observers_________

Site#	Trap#	Date Set	Time Set	Date Coll	Time Coll	Notes

Fall-Out Vegetation Quadrants

Trap #	WD:	Pic #					
Comments							
Species	%	Ht.(m)	Dens	Species	%	Ht.	Dens
% Veg*:				Soil Salinity (ppt):			

Trap #	WD:	Pic #					
Comments							
Species	%	Ht.(m)	Dens	Species	%	Ht.	Dens
% Veg*:				Soil Salinity (ppt):			

Trap #	WD:	Pic #					
Comments							
Species	%	Ht.(m)	Dens	Species	%	Ht.	Dens
% Veg *:				Soil Salinity (ppt):			

Trap #	WD	Pic #					
Comments							
Species	%	Ht.(m)	Dens	Species	%	Ht.	Dens
% Veg *				Soil Salinity (ppt):			

Trap #	WD	Pic #					
Comments							
Species	%	Ht.(m)	Dens	Species	%	Ht.	Dens
% Veg*:				Soil Salinity (ppt):			

Trap #	WD	Pic #					
Comments							
Species	%	Ht.(m)	Dens	Species	%	Ht.	Dens
% Veg *:				Soil Salinity (ppt):			

***Overall % cover vegetated in 1 meter radius from FO trap center**