Monitoring Protocol for Reporting Impacts of the Salt Tracer Methodology on Stream Biota

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Background

Oregon Department of Forestry (ODF) is conducting a monitoring project – the Riparian Function and Stream Temperature (RipStream) project -- examining effectiveness of riparian management regulations (Forest Practices Act) and strategies (Northwest State Forest Management Plans) in meeting goals for maintaining water quality and protecting the aquatic health and riparian forest structure. One of the key response variables in this study is stream temperature. Another important variable which affects stream temperature and that can act as a co-variant in explaining stream temperature is stream discharge. In 2004, a review team for the RipStream project advised ODF that the flow meters currently being used may be inadequate to measure stream discharge and that a salt tracer conductivity method – specifically the "slug test" tracer method -- was more appropriate. The "slug test" salt conductivity tracer method (henceforth referred to as the salt trace method) involves pouring a solution or "slug" of Sodium Chloride (NaCl) into the stream channel and then calculating the amount of time it takes for the solution (i.e., peak conductivity) to move a specified distance downstream.

In a recent publication of the Watershed Restoration Program of the Government of British Columbia, an excellent review was given on salt tracer methodologies (Moore 2003/2004). Moore (2003/2004) noted that measuring stream discharge using flow meters is often difficult, if not impossible in small streams and is often unreliable in low-flow streams. He further suggests that the salt tracer method is an accurate, cost-effective, and relatively benign method for measuring stream discharge in small, headwater streams. He also notes that although methods using fluorometric dyes are also effective, they require specialized equipment that is expensive and not readily available.

In mid-2004, we entered into discussions with Oregon Department of Fish and Wildlife (ODFW) regarding obtaining a scientific taking permit for the salt tracer method in selected stream reaches of the Coast Range. We solicited comments from district biologists as well as from Oregon Department of Environmental Quality (DEQ) staff on the matter. We also contacted Dr. Bruce Bury and Dr. Dede Olson, herpetologists with Oregon State University, to discuss effects of the salt tracer method on amphibians. Both Dr. Bury and Dr. Olson indicated that they were unaware of any information on effects of the salt tracer method on amphibians. DEQ staff advised ODF that a water quality permit was not necessary, but recommended that ODF monitor and report to DEQ when conductivity levels exceeded a threshold of 400 microSeimens/cm (µS/cm) for an extended period of time. DEQ did not document the rationale for this threshold, but this threshold is conservatively consistent with listed "no observed effect concentration" threshold of 400 mg/l (517 µS/cm) for the frog, *Rana breviceps,* reported in EPA 1988. We forwarded DEQ's suggestion to ODFW for

informational purposes. On August 23, 2004, ODFW issued both a Fish Division, as well as a Wildlife Division, scientific taking permit for our proposed salt tracer method. The Wildlife Division permit required ODF to cease conductivity testing if any amphibian (or other wildlife) mortality occurred. The Fish Division permit required that conductivity testing cease if conductivity exceeded 400 µS/cm at any time during the testing.

On September 1, 2004, we initiated a trial measurement of stream discharge using the salt tracer method. A summary of the methodologies are displayed in Appendix A and conductivity results of this trial are displayed in Appendix B. During the first "slug test" of the trial, our scientific taking permit threshold of 400 µS/cm was exceeded, thus the trial (i.e., necessary repeated applications of the slug test at the site) was terminated before completion. During this initial "slug test", monitoring for response of stream biota was limited to visual observation. The goal of the visual monitoring was to detect any mortality or behavioral changes of amphibians, fish, or macroinvertebrates. We did not detect any fish or amphibians during this first "slug test"; however macroinvertebrates were visually observable during the test. No mortality and no change in activity of macroinvertebrates was noticed.

In December, 2004, we met with staff from ODFW and DEQ to discuss results from this initial application of the salt tracer method. The purpose of the meeting was both to share results and to request a change in the Fish Division's permit condition to cease testing if the conductivity threshold of 400 µS/cm was exceeded. During that meeting, ODFW staff expressed a concern that the high levels of conductivity that we observed during our initial test may pose a risk to stream biota, especially to amphibians and macroinvertebrates. ODFW requested that ODF conduct a literature review to summarize known effects of sodium chloride on stream biota from field studies. In addition, ODFW requested that we develop a more rigorous plan to monitor any impacts of the salt tracer tests on stream biota. This document was prepared to meet in response to these requests.

Literature Review

We conducted a search for published articles regarding effects of sodium chloride (NaCl) on stream-dwelling amphibians and macroinvertebrates. Emphasis was placed publications resulting from field studies with the hope that publications would be discovered on effects of the salt tracer technique on stream fauna. No publications were found regarding potential or real impacts of the salt tracer method on any type of stream biota. The only field studies found were those examining effects of road salt on macroinvertebrates (e.g., Crowther and Hynes 1977, Blasius and Merritt 2002).

Amphibians

No field studies were found on effects of sodium chloride on amphibians. Although we did not focus our literature search on laboratory studies, we did obtain copies of the original scientific papers cited in major summary/review documents on toxicity of amphibians (EPA 1988, Pauli et al. 2000).

Current information on effects of sodium chloride on amphibians is lacking. To our knowledge, no research has yet been conducted on effects of sodium chloride in natural settings (e.g., field study). In addition most of our current knowledge of effects of sodium chloride on amphibians appears to come from only three published studies (Mahajan et al. 1979, Parsons et al. 1990, Padhye and Ghate 1992), all of which studied frogs and toads. No studies were found regarding salamanders. Of those three studies, only one examined response of North American species (Parsons et al. 1990) with the other studies examining response of species native to Asia (Padhye and Ghate 1992) and southern Africa (Mahajan et al. 1979). Results from the North American study may not be applicable to the salt tracer method as frogs were directly injected with sodium chloride rather than being exposed via immersion. Only the Asian and southern African studies are listed in the EcoTox Database (EPA 2002). No studies were found regarding effects of sodium chloride on any species of amphibian likely to be found in stream systems of western Oregon.

Major results from the two studies listed in EcoTox (EPA 2002) are summarized in Appendix C. These studies indicate that significant mortality occurs only after long-term exposure (e.g., $\geq$ 48 hours) in solutions containing relatively high concentrations of NaCl (e.g., $\geq$ 5 g/l or 0.3% NaCl [corresponding conductivity of approximately 3500 and 3873 µS/cm, respectively]).

Macroinvertebrates

We found two field studies that examined effects of sodium chloride on macroinvertebrates, both of which examined effects of road deicing salts (Crowther and Hynes 1977, Blasius and Merritt 2002). We provide a brief summary of the field studies on effects of road salt in Appendix D. Appendix E summarizes results on effects of road salts (NaCl) on aquatic macroinvertebrates from both laboratory and field studies (modified from Table 1 presented in Blasius and Merritt 2002).

Existing field studies on effects of NaCl on macroinvertebrates are relatively weak in rigor. The field component of Blasius and Merritt (2002) was observational in nature and examined differences between invertebrate communities below and above sources of salt input (roads) for two streams. Crowther and Hynes (1977) conducted an experimental study in which NaCl was introduced into a single stream which had been split in two using steel, thus creating a "control" and

"treatment" channel. Salt was continuously added to the stream channel. The concentration of the salt solution was not given. In addition, the total duration of time that the solution was added to the stream was not clear, but lasted at least one to two hours. Only one replication was conducted for each of three treatments that varied in concentration of NaCl (two replications of a control treatment were also conducted).

Both Crowther and Hynes (1977) and Blasius and Merritt (2002) conducted laboratory experiments on effects of NaCl on macroinvertebrate drift using artificial stream channels. The artificial stream channels were designed to simulate actual streams and had flowing, recirculated water, a natural-like stream bottom, and similar water chemistry to those observed in the field. Varying concentrations of NaCl were added to the artificial stream channels in replicated experiments lasting 24 hours. Rates of drift were compared between control and treated stream channels. Crowther and Hynes (1977) included three species in their trials: the amphipod *Gammarus pseudolinmaeus* and the caddisflies *Hydropsyche betteni* and *Cheumatopsyche analis*. Blasius and Merritt (2002) included multiple species in their trials, including the amphipod *Gammarus pseudolinmaeus;* the mayflies *Stenonema integrum* and *S. interpunctatum*; the stoneflies *Acroneuria abnormis* and *Agentia capitata*; the caddisflies *Pycnopsyche guttifer*, *P.* l*epida*, and *Brachycentrus numerosus*; and the stonefly *Tipula abdominalis*. Although the exact species may differ, the types of invertebrates listed above are also likely to be found in Pacific Northwest streams (Hafele and Hinton 1996).

Blasius and Merritt (2002) did not observe differences in macroinvertebrate community structure between treated and control reaches for the two streams they studied. Results from both field and laboratory experiments on drift rates of macroinvertebrates appear to indicate that some drift may occur above concentrations of 850 – 5000 mg/l (1214-7143 µS/cm) for some species of macroinvertebrates (Appendix E). Crowther and Hynes (1977) did not observe drift in laboratory experiments for *Gammarus pseudolinmaeus* at concentrations up to 800 mg/l (1143 µS/cm). In contrast, Blasius and Merritt (2002) observed increased drift for *Gammarus pseudolinmaeus* at concentrations ≥ 1000 mg/l (1429 µS/cm), although drift appeared to be most significant at concentrations ≥ 2500 mg/l (3571 µS/cm) in one of two trials. Drift was not observed for *Hydropsyche betteni* or *Cheumatopsyche analis* at concentrations up to 1650 mg/l (2357 µS/cm) by Crowther and Hynes (1977) and no significant drift was observed for any of the other species of macroinvertebrate studied by Blasius and Merritt (2002).

During the field experiment conducted by Crowther and Hynes (1977), they did not observe significant drift during two trials that resulted in peak chloride concentrations up to 500 and 750 mg/l (714 and 1071 µS/cm). Differences in

drift were observed during one trial which resulted in a peak chloride concentration of 2165 mg/l (3093 µS/cm). During that trial, differences in drift between the control and treated channels were observed once the chloride concentration was greater than approximately 850 mg/l (1214 µS/cm). Rates of drift appeared to increase with increasing concentration of chloride, decreased as concentration subsided, and were similar to the control channel once chloride concentrations were again below 800 mg/l (1143 µS/cm). The duration that the stream experienced increased drift (and corresponding high chloride levels) was approximately 6 hours.

Conclusion

Based on our review of available literature (i.e., laboratory testing, toxicity databases and controlled experiments), we do not feel that there is evidence to indicate that mortality of macroinvertebrates, amphibians or fish is likely to occur due to the salt tracer method. However, due to findings in the literature that suggest possible impact of NaCl in the form of macroinvertebrate drift, we feel monitoring impacts of the salt tracer method on macroinvertebrate drift near the point of slug input is warranted.

Results from the toxicity studies for amphibians reviewed here are not directly applicable to the salt tracer methodology because, unlike the "slug test" being proposed by ODF, they involved long-term exposure to NaCl in closed environments. Nevertheless, these studies do represent an extreme scenario of intense and long-term exposure to NaCl that is useful for gauging possible impacts. Our conclusion is that the results of the literature suggest that the two frog species studied would not be impacted during repeated applications of the "slug test" for measuring stream discharge. This is because 1) the approximate concentrations and corresponding conductivities that caused mortality during toxicity trials exceeded the maximum levels observed during our initial "slug test", and 2) the pulse of increased conductivity observed during each "slug test" was spatially and temporally limited (Appendix B) and would not at all approach the long-term exposure the toxicity tests are based upon. Although we were unable to locate any information on effects of NaCl on any species of amphibian native to the Pacific Northwest, given the short-term and spatially limited extent of increased conductivity associated with "slug tests", we do not feel that amphibians are likely to be negatively impacted by the salt tracer method.

Results from laboratory studies on macroinvertebrates suggest that NaCl may induce drift for some species at concentrations above approximately 1000 mg/l (corresponding conductivity of approximately 1429 µS/cm), however these levels of drift were observed during extended periods of exposure (24 hours of continuous exposure) and results were inconsistent between trials and between

studies. Results from a field study indicated that drift in the field may occur when NaCl concentrations exceed 850 – 1250 mg/l (corresponding conductivity of 1214 - 1786 µS/cm).

Our preliminary test of the salt tracer method resulted in increases in conductivity above 1200 µS/cm for ≤ 5 seconds and only at the point of slug input. Conductivity never exceeded 1200 µS/cm at 10, 40 or 100 feet downstream from the point of slug input (Appendix B). Conductivity exceeded 400 µS/cm (the threshold established in our scientific taking permits) at the point of slug input for approximately 25 seconds for approximately 60 seconds at 10 feet downstream of the point of slug input. Conductivity never exceeded 140 µS/cm at 40 or 100 feet downstream. We feel our maximum conductivity level of 3476 µS/cm (Appendix B) may be high enough to induce some drift for some species of macroinvertebrate. The area of the stream experiencing levels of conductivity above 1200 µS/cm was spatially limited to within 10 feet of the point of insertion of the salt tracer slug. In addition, conductivity was greater than 1200 µS/cm for less than 30 seconds. Thus we feel that the level of drift that might occur would be minimal and that any drifting animals would be likely to settle relatively quickly. We feel that "clearing" of a large area of the stream of food resources (i.e., macroinvertebrates) for fish and amphibians is highly unlikely. Further, we conclude that macroinvertebrate mortality from the salt tracer method is unlikely.

Further Research

Results of the literature review indicate that much is unknown regarding effects of the salt tracer method on stream biota – especially in field settings and for species common to the Pacific Northwest. As a result, we feel that further research is necessary so as to fully understand the possible impacts of the salt tracer method on stream biota. Our literature review suggests the following research questions need to be investigated.

- *Does the salt tracer method cause catastrophic drift of macroinvertebrates? If so, what is the spatial and temporal extent of that drift?* This question would best be answered by conducting a rigorous field experiment, preferably using a Before After Control Impact experimental design to compare drift rates prior to and after insertion of the salt tracer as well as between treated and untreated streams. In order to be rigorous, multiple samples should be conducted both within and across streams.
- *What are the behavioral impacts of the salt tracer method on amphibians and macroinvertebrates?* This question would best be addressed during laboratory trials using artificial stream channels.
- *What are the impacts of the salt tracer method on mortality rates of amphibians and macroinvertebrates?* This question would best be

addressed during laboratory or field trials using caged animals exposed to NaCl during a normal salt tracer test.

- *What are the toxicity thresholds (e.g., LC50 values) for NaCl for Pacific Northwest Amphibians?* This question would require a standard laboratory toxicity study.

Salt Tracer Monitoring Protocol for Reporting Impacts on Stream Biota

Based on results from our literature review, we feel that monitoring impacts on macroinvertebrate drift near the point of slug input is warranted. Thus the protocol laid out below focuses on assessment of drift near the point of salt tracer input. Throughout this protocol the term "stream discharge trial" refers to the work to be conducted for the purpose of measuring stream discharge in the treatment reach of the RipStream study streams. The term "monitoring trial" refers specifically to the salt tracer test that will be conducted to measure impacts of the salt tracer method on invertebrate drift.

Purpose: We will measure and monitor macroinvertebrate drift for the purpose of reporting impacts associated with the use of salt tracers for measuring stream discharge to the permitting agency, ODFW. Our monitoring is not intended to fulfill information gaps on effects of the salt tracer method on stream biota; these gaps are best answered by rigorous, controlled scientific research.

Methods: We will evaluate macroinvertebrate drift in order to determine if the salt tracer methodology causes a significant level of catastrophic drift. In order to be able to separate catastrophic drift from behavioral or constant drift "normally" present in the stream, we will use a before-after, control-impact study design in which levels of drift will be compared prior to and following the insertion of salt tracer in the stream and between control and treated areas of the stream. All salt discharge and monitoring trials will be conducted during periods of low stream flow, primarily in July and August and possibly into September.

Because assessment of drift requires placing nets in the stream which may impact rates and/or patterns of stream flow, we cannot conduct our salt tracer test to evaluate discharge rates and simultaneously evaluate drift of macroinvertebrates. However, because macroinvertebrate communities as well as patterns of flow can vary within and between streams, it is important to monitor drift in the same vicinity that the stream discharge trial will be conducted. To resolve these two conflicting concerns, we plan on evaluating macroinvertabrate drift immediately downstream of the RipStream "treatment reach" that will be used to measure stream discharge.

Monitoring of drift will occur primarily 20 to 35 feet below the bottom end of the Rip Stream treatment reach to be used to sample stream discharge. For the monitoring trial, the location of slug input will be 20 feet downstream of the location of the last salt conductivity probe used to sample stream discharge (Figure 1). This will ensure that salt used in our monitoring does not affect results of the stream discharge trial.

Drifting animals will be sampled using standard drift nets (approximately 12" tall, 18" wide, and with 350 – 500 µm mesh) placed 5 and 25 feet below as well as 50 feet above the point of input of the salt tracer slug. The net placed upstream of the salt tracer slug will serve as a control and will be used to determine background levels of drift. Two downstream nets are used so that affects of drift can be evaluated very close to the point of slug input as well as slightly downstream, below where we expect any effects of drift would have dissipated. Nets will be positioned so they are flush with the stream bottom and located to intercept the portion of the stream with the greatest flow. To evaluate stream conductivity that might be associated with invertebrate drift, we will also place a conductivity probe immediately upstream of each net. The nets will be located in areas with similar water velocity and stream depth.

Once nets will be placed, a "dummy" slug consisting of stream water will be poured into the stream. We will then leave the nets in place for one-hour. A duration of one hour was chosen because we feel this is the maximum time at which conductivity levels are expected to be above background levels within 15 feet of the location of slug input. After one hour, the nets will be carefully removed and taken to the stream bank.

Any captured invertebrates will be removed from the net and immediately stored in alcohol. Invertebrates will then be brought back to the laboratory, identified to Order, and counted. In the rare event that we capture any fish or amphibian, they will be identified to Genus or Species and noted. If alive, they will be released; if dead they will be preserved and brought back to the laboratory. Specimens will be donated to the Fisheries and Wildlife Department at Oregon State University.

Nets will then be placed back in the stream, using care to locate them in exactly the same position. A slug containing NaCl will then be poured in exactly the same location as the dummy slug. The nets will again remain in place for one hour. The same methods will be used to remove, identify, and count invertebrates or any captured fish or amphibians.

We recognize the importance of replicating, to the extent possible, the conditions and methods to be used during the stream discharge trial. The monitoring trial will be conducted with care both to 1) replicate the exact methods to be used

during the stream discharge trial (see Appendix A), and 2) to replicate the amount of disturbance (e.g., stepping into the stream bottom) to the stream expected during the actual stream discharge trial. The only extra disturbance to the stream that will occur during the monitoring trial that won't occur during the stream discharge trial will occur during placement of the nets.

We will re-evaluate our procedures following the first two to three streams. If drift rates appear to be too low, we will modify our protocol to extend the duration that nets are in the stream.

Visual Observation

In addition to the monitoring trial, we plan to actively observe the stream during each of the regular stream discharge tests and to qualitatively describe any behavioral changes for any organism that we observe. Because of the cryptic and secretive nature of most stream-dwelling biota, lack of behavioral changes is not sufficient to suggest that behavioral changes did not occur. However, a positive observation of animals that appear to be seeking refuge during the stream discharge test *may* be indicative that a negative behavioral response is occurring. Because we expect that negative effects are likely to occur close to the location where the salt is added to the stream, we will focus our visual observations to the area between 0 and 20 feet from the point of salt insertion. We will note the type of organism (fish, amphibian, macroinvertebrate), the species (if possible), and the nature of the behavior observed (e.g., sudden darting up or downstream, movement towards the edge or bottom of the stream, sudden drift). If any mortality is observed, the animals will be collected, identified, and preserved. The specimens will be donated to the Fisheries and Wildlife Department at Oregon State University. Any mortality will also be reported to ODFW.

***Reporting*:** It will not be possible to confirm any impacts to macroinvertebrates (e.g., increased drift) in the field as this confirmation will be subject to post-field laboratory counting and identification. This laboratory work will be conducted prior to ODF conducting the next stream discharge measurement at a new location. Impacts will be calculated and reported as the observed impact from the monitoring trial multiplied by the number of salt tracer "slug tests" necessary to complete the stream discharge trial. Both the measured macroinvertebrate impacts as well as any visually observed impacts will be reported to the appropriate ODFW District Biologist(s) prior to conducting the next stream discharge trial at a new location.

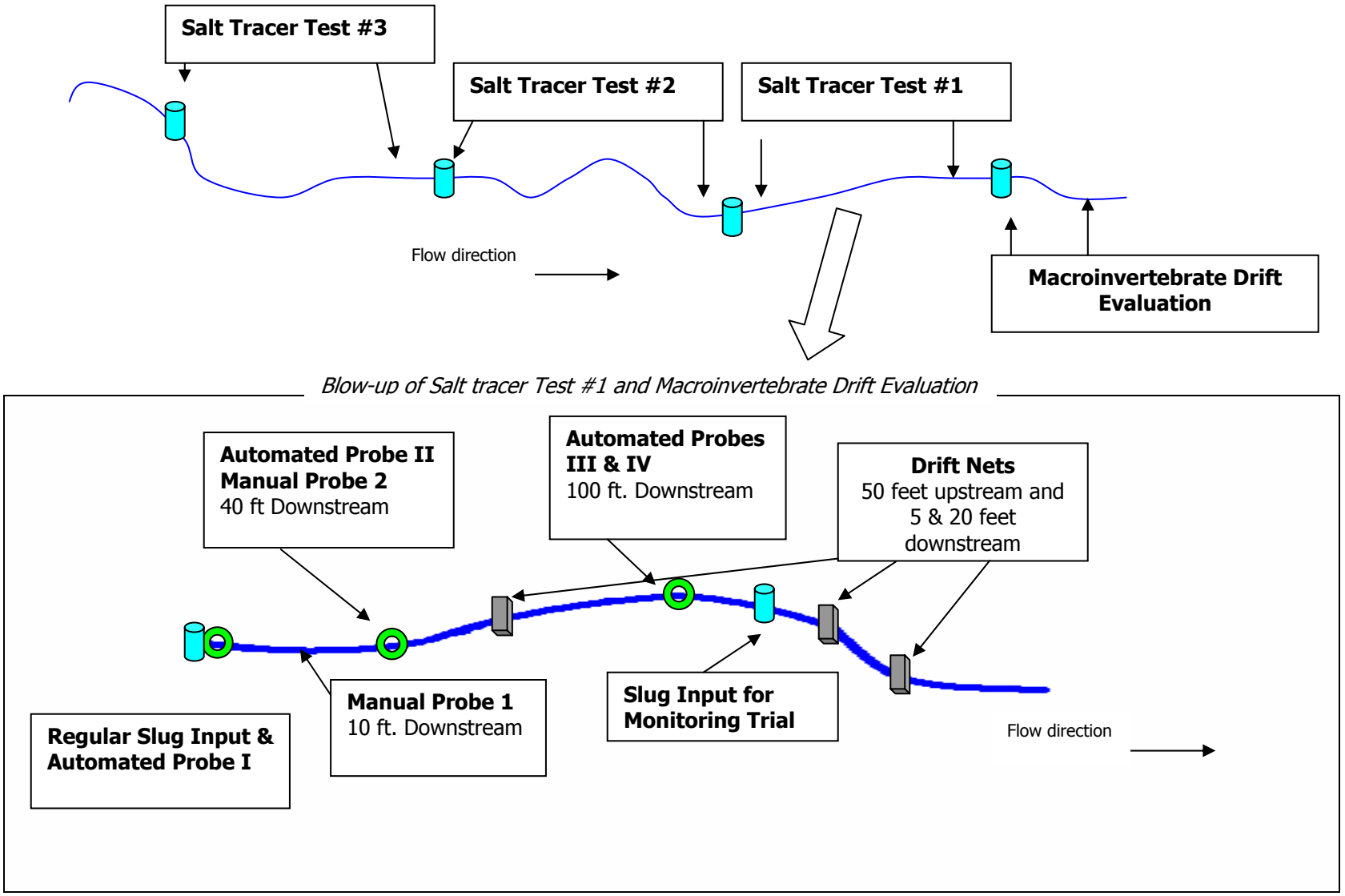

Figure 1: Design used to examine stream discharge using the salt tracer method, including location of probes as well as the relationship of the evaluation of the steam to be evaluated for macroinvertebrate drift to the salt tracer tests.

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Appendix A: Proposed methods for examination of stream discharge using the salt tracer methodology.

For each Salt-Tracer measurement procedure conductivity data-loggers are placed in the stream channel to record conductivity (µS/cm) at one second intervals. The data-loggers are located at the following locations: at the location of slug input location, 40 ft, and 100 feet downstream of location of salt slug input. Two loggers are placed 100 feet downstream to increase our chances of locating a probe where it would best intercept the salt plume as it moved downstream. Data-loggers are placed at the bottom of the stream channel with the sensors positioned to maximize interception of the slug plume. In addition to the automated data-loggers, conductivity is recorded manually (Hanna Instruments model DiST WP 3) every five minutes for a thirty minute duration. Manual readings are taken at the water surface at 10 and 40 feet below the point of slug insertion. Background conductivity (the actual conductivity of the stream prior to input of the slug) is recorded five minutes prior to data-logger installation as well as at time of installation.

The salt-tracer "Slug" solution contains one liter volume of water with 100 grams/liter or 200 grams/liter sodium chloride (table salt) diluted. The slug will be poured in the stream channel within the "mixing" zone.

Continuous observation of stream will be conducted at point of solution application, and throughout the 100 ft. salt-tracer test reach for one hour minimum. Presence of any observable fish, amphibians, or macro invertebrates will be noted. Attempts will be made to continuously observe any readily detectible animals any mortality or behavioral changes (e.g., macroinvertebrate drift) will be noted.

Measurements for the salt tracer experiments will continue for one to two hours.

Appendix B: Methods and results from the Gunn Creek Salt Tracer Experiment

One Salt Tracer stream flow measurement test was performed Wednesday, September 1, 2004, on one, 100ft., reach of Gunn creek, (Oregon Department of Forestry ***RipStream*** study site # 5558), in Sec. 15 of Township 9s, Range 9w, within the proximity of study site station 3W.

The Slug used in the experiment contained one liter water with 100 grams dissolved sodium chloride (100 grams/liter). Conductivity of the "Slug" was approximately 22,000 –24,000 µS /cm. The slug was input into the stream channel at Gunn I (Figure 1) at 14:15:05 PST.

Background conditions for the stream prior to the experiment are as follows:

- "Background" conductivity (Actual stream water conductivity before slug input) = approximately 25-35 µS/cm
- Stream water temperature: 13.54° C
- Stream wetted width: from 5 ft. to 8 ft.

All data-loggers at 6 – 10 inches depth.

Data-loggers were set up to record conductivity in 5 second increments (instrument limit) and placed at substrate level in mixing portion of channel (depth of 6-10 inches).

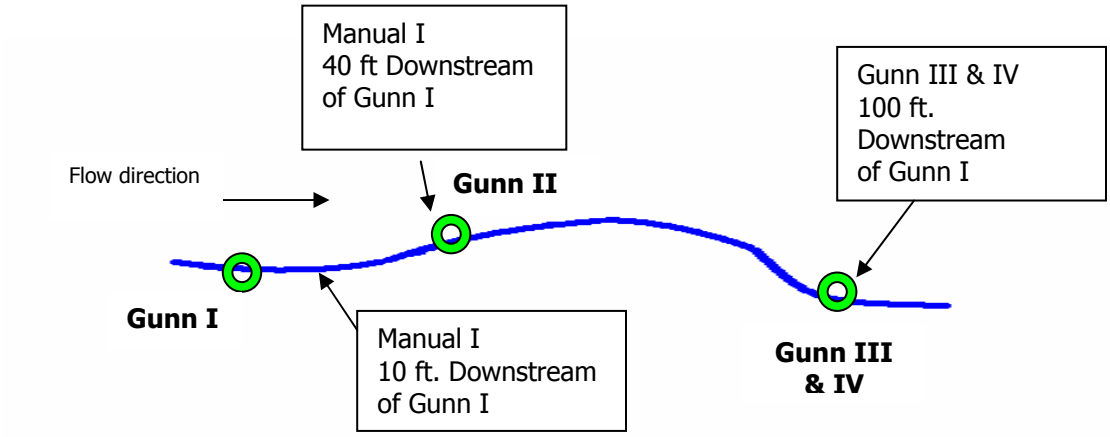

Figure 1: Location of conductivity readers

Results of conductivity measurements:

Conductivity immediately spiked at the point of slug input to 3,476 µS/cm but quickly subsided to levels slightly above background conductivity (approximately 40 µS/cm) within two minutes (Figure 2). At the Gunn II data-logger, conductivity rose to a maximum of 106 µS/cm and conductivity was near background levels within approximately 3 minutes. Conductivity did not rise above background at Gunn III & IV (100 ft below slug input) until approximately 3 minutes after slug input. At that time, conductivity rose slightly with a maximum of 57 µS/cm observed at Gunn III and a maximum of 79 µS/cm observed at Gunn III. Conductivity was near background levels after 2-3 minutes.

Conductivity rose above the allowable threshold (400 µS/cm) established in our scientific taking permits at the point of, and 10 feet below slug input but did not exceed the threshold at 40 or 100 feet (Figure 2). Conductivity was above the allowable threshold at the point of slug input for approximately 25 seconds and at 10 feet below input for approximately 45 seconds.

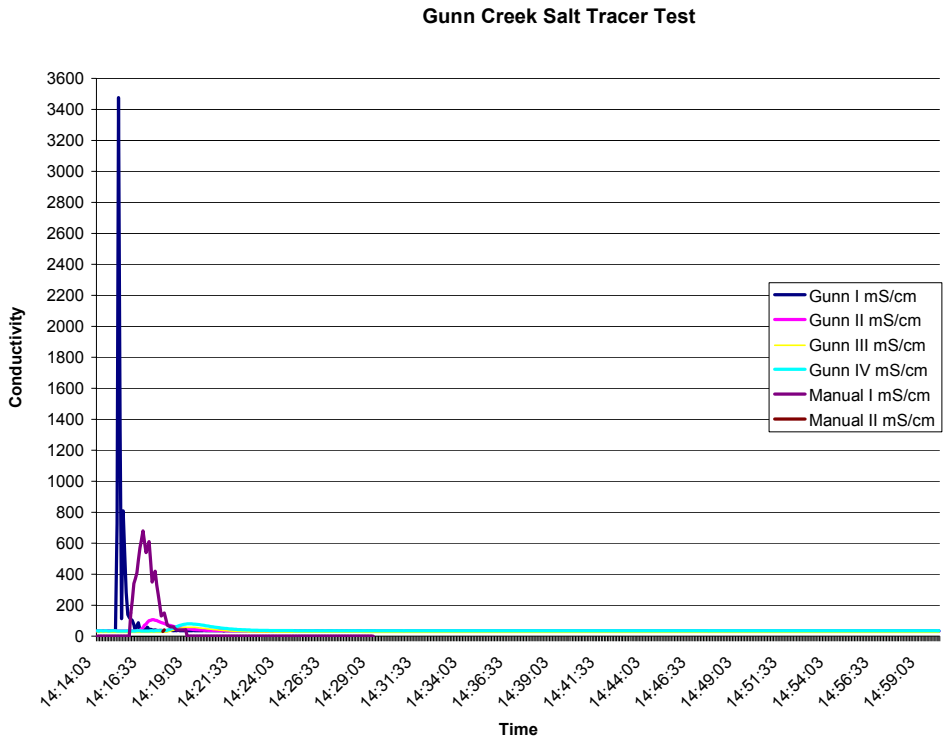

Figure 2: Conductivity (µS/cm) observed over time at the various conductivity readers during the Gunn Creek salt tracer experiment.

Monitoring:

Water clarity and flow volume conditions were ideal for observing any movement of biota which may have been affected by the plume of slug material. Aquatic insects could be observed moving about the substrate material before and after slug input with no discernable change in behavior.

Field personnel surveyed the stream, throughout the test reach and downstream, for biota damage or mortality prior to slug input, as well as during the testing (one hour) with no impact noted.

Appendix C: Review of studies on toxic effects of salt (NaCl) on amphibians from laboratory studies.

Table C1: Cumulative mortality of Rana breviceps tadpoles exposed to various concentrations of NaCl for various periods of time (from Mahajan et al. 1979).

Concentration g/l	Conductivity[1] µS/cm	% cumulative mortality at different times (hrs)						
		8	24	48	72	76	120	144
0	0	0	0	0	0	0	0	0
1	700	0	0	0	0	0	0	0
3	2100	0	0	0	7	13	47	66
5	3500	0	0	0	20	53	80	93
6	4200	0	0	7	40	67	93	100
7	4900	0	0	13	53	87	100	n/a
10	7000	0	0	67	93	100	n/a	n/a

Table C2: Mean LC50 values (95% Confidence Interval) for various life stages of *Microhyla ornata.*

Life Stage	Concentration (% NaCl)		Electrical Conductivity (µS/cm)[1]	
	Mean	95% CI	Mean	95% CI
Late Gastrula Embryo	0.2711%	0.2493 – 0.2947	3873	3561 - 4210
8-day old tadpole	0.5027%	0.4623 - 0.5466	7181	6604 - 7809
Hind-limb stage tadpole	0.6929%	0.6014 – 0.7985	9899	8591 - 11407

[1] Conversion calculated by ODF staff using the equation EC=TDS/0.7 (obtained from Wyoming Department of Agriculture, http://wyagric.state.wy.us/aslab/wmterms.htm)

Appendix D: Summary of field studies on effects of road salt.

Blasius and Merritt 2002

Location: Michigan

Study Design:

- Field sampling of two streams for macro-invertebrate species composition as well as for leaf litter decomposition. Each site had a reference reach located above and a "treatment" reach located below a road exposed to sodium chloride used for deicing.
- Laboratory drift study examining drift of macro-invertebrates in artificial stream channels. Exposure to 1000 (1429 µS/cm), 2500 (3571 µS/cm), 5000 (7143 µS/cm), and 10000 mg/l (14286) NaCl over a 24 hour period (two trials per concentration using 5-24 individual invertebrates).
- Laboratory acute toxicity study (96 hour test) using concentrations of 0, 1000, 5000, and 10000 mg/l NaCl. 1-4 individuals per taxon were examined for each concentration.

Key Results:

- Field Study
    - Amount of salt added to roads unknown. Chloride concentrations were only slightly higher in treatment than in reference reach (16.12 vs 15.23 and 9.19 vs. 8.49 mg/l)
    - Salt runoff did not change trophic nature of the invertebrate community (no difference in functional feeding group composition between reference and treatment reaches—$P<0.05$).
    - Differences in leaf litter decomposition were observed, however they were due to increased sedimentation and not from road salt toxicity.
- Laboratory Drift Studies
    - No mortality observed for any NaCl concentration
    - Drift response observed for the amphipod, *Gammarus pseudolimnaeus*, but results inconsistent between trials. During the first trial, drift was low (10%) in the control tests, 23% at 1000 mg/l, 55% at 2500 and 5000 mg/l and 68% at 10000 mg/l. During the second trial, drift was 0 and 45% in the control tests and between 80 and 100% during all treatment tests. Authors describe their results in the abstract by noting that a drift response occurred above 5000 mg/l.
    - Drift was highly variable and not clearly related to dose for all other taxa tested.

    - Test organisms (taxa not specified) exhibited a behavior response by reacting less vigorously to prodding at the 2500, 5000, and 10000 mg/l doses.
- Laboratory Acute Toxicity Study
    - No significant mortality for any treatment for two perlid stonefly and one crane fly species (LC50>10000 mg/l NaCl).
    - The amphipod G. *pseudolimnaeus exhibited a dose response. 96 hour LC50 values was 7700 (95% cl = 6563 – 9034).*
    - *The caddisfly Pycnopsyche spp. Also exhibited a dose response. 96 hour LC50 values was 3526 (95% cl =2364 – 5258).*

<u>Crowther and Hynes 1977</u>

Location: Ontario, Canada.

Study Design:

- Laboratory study examining drift using artificial stream channels in the laboratory. Exposure of chloride at concentrations of 800 mg/l (1143 µS/cm) for *Gammarus* and 1650 (2357 µS/cm) for *Trichoptera*. Two control and three treatment channels were used for 24-hour long trials.
- Also conducted field trials by dividing 15 m of stream with steel, one half which was treated with salt and the other half which acted as a control. Two control and three treatments replicates of the experiment were conducted on different dates in the winter. Salt was added continuously to the stream (not all at once).

Key Results:

- Laboratory Drift Studies
    - No observable drift was observed for G. pseudolimnaeus when exposed to chloride pulses up to 800 mg/l (1143 µS/cm) or for the Trichoptera at concentrations of 1650 mg/l (2357 µS/cm). Drift rate similar between control and treatments.
    - No mortality observed.
- Field Study
    - No difference in drift at chloride levels (peaks) of 500 (714 µS/cm) and 750 mg/l (1071 µS/cm).
    - Differences in drift were observed during one trial which resulted in a peak chloride concentration of 2165 mg/l (3093 µS/cm). Differences in drift between control and treated channels occurred once the chloride concentration was roughly 850 (1214 µS/cm) to 1200 mg/l (1714 µS/cm) and subsided once the chloride concentration was again below 800 mg/l (1143 µS/cm).
    - Duration that chloride concentration was above 800 mg/l (1143 µS/cm) during this test was approximately 6 hours.

Appendix E: Review of studies on effects of salt (NaCl) on aquatic macroinvertebrates. Adapted from Table 1 of Blasius and Merritt (2002); results from laboratory studies unless otherwise noted.

Unit of measure for NaCl	mg/l (TDS)[1]	uS/cm (EC)[2]	Reference
100% mortality of *Chironomus attenatus* (Diptera: Chironomidae)@ 12h exposure	9995	14279	Thornton and Sauer (1972)
100% mortality of *Nais variabilis* (Oligochaeta: Naididae) @ 48h exposure	3735	5336	Hamilton et al. (1975)
100% mortality of *Crieotopus trifaseia* (Diptera: Chironomidae) @ 48h exposure	8865	12664	Hamilton et al. (1975)
100% mortality for 48-h exposure for *Hydroptila angusta* (Trichoptera: Hydroptilidae)@ 48h exposure	10136	14480	Hamilton et al. (1975)
No effect on drift or mortality for *Gammarus pseudolinmaeus* (Amphipoda: Gammaridae)	800	1143	Crowther and Hynes (1977)
No effect on drift or mortality for *Hydropsyche betteni and Cheumatopsyche analis* (Trichoptera: Hydropsychidae)	1650	2357	Crowther and Hynes (1977)
Some drift activity of macroinvertebrates observed in field experiments (>1000 mg/l)	1000	1429	Crowther and Hynes (1977)
No effect on composition or diversity if Ephemeroptera, Plecoptera, Trichoptera and Coleoptera (field)	67	96	Molles (1980)
Mortality of *Hydropsyche betteni, H. bronta, and H. slossonae* (Trichoptera: Hydropsychedae) unaffected after 10 days.	800	1143	Kersey (1981)
80% mortality for 6-day exposure for *H. betteni*.	6000	8571	Kersey (1981)
Similar growth rates among different treatments for *Hexagenia limbata* (Ephemeroptera: Ephemeridae)	0	0	Chadwick (1997)
Similar growth rates among different treatments for *Hexagenia limbata* (Ephemeroptera: Ephemeridae)	2000	2857	Chadwick (1997)
Similar growth rates among different treatments for *Hexagenia limbata* (Ephemeroptera: Ephemeridae)	4000	5714	Chadwick (1997)
Similar growth rates among different treatments for *Hexagenia limbata* (Ephemeroptera: Ephemeridae)	8000	11429	Chadwick (1997)
LC50 at 28° C for H. limbata after 96 h	2400	3429	Chadwick (1997)
LC50 at 28° C for H. limbata after 96 h	6300	9000	Chadwick (1997)
LC50 for Tricorythus sp. (Ephemeridae: Tricorythidae) after 96 hrs.	2200-	1540-	Goetsch and Palmer

Unit of measure for NaCl	mg/l (TDS)[1]	uS/cm (EC)[2]	Reference
	4500	3150	(1997)
Approximate LC50 for *Hydropsyche betteni* (Trichoptera: Hydropsychedae); no dose-response tested.	13308	19011	Kundman (1998)
No significant drift observed for *Hydropsyche betteni* in lab (2000-8000 mg/l)	8000	11429	Kundman (1998)
50% mortality for 96-h exposure for *Lepidostoma sp*. (Trichooptera: Lepidostomatidae)	6000	8571	Williams et al. (2000)
70% mortality for 96-h exposure for *Nemoura trispinosa* (Plecoptera: Nemouridae)	6000	8571	Williams et al. (2000)
100% mortality for 96-h exposure for *Gammarus pseudolimnaeus*	6000	8571	Williams et al. (2000)
Increases in drift by *Gammarus pseudolimnaeus* for 24 hour exposure	5000	7143	Blasius and Merritt 2002
LC50 for *Gammarus pseudolimnaeus* after 24 hours.	7700	11000	Blasius and Merritt 2002
LC50 for *limnephlid caddisflies (2 species)* after 24 hours.	3526	5037	Blasius and Merritt 2002

[1] Value presented in Blasius and Merritt (2002)
[2] Conversion calculated by ODF staff using the equation EC=TDS/0.7 (obtained from Wyoming Department of Agriculture, http://wyagric.state.wy.us/aslab/wmterms.htm