

## **Appendix B. Nautilus Data Report**

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### **Results for the Rainbow Trout Early Life Stages *In-situ* Bioassay – Final Report**

The following pages are Appendix B to the Washington State Department of Ecology report:

*Integrated Ambient Monitoring Follow-up Study in Indian Creek: An Investigation of the Causes of Biological Impairment and a Further Demonstration of the Instream Monitoring Approach*

The report and this appendix are posted at

<https://fortress.wa.gov/ecy/publications/SummaryPages/1403050.html>

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**Washington Department of Ecology - Integrated  
Ambient Monitoring Project**

Rainbow Trout Early Life Stages *In Situ* Bioassay:  
Phase II

**Final Report**

Report date: September 13, 2013

Submitted to:

**WA State Dept. of Ecology**

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## TABLE OF CONTENTS

	Page
TABLE OF CONTENTS .....	2
1.0 INTRODUCTION.....	1
2.0 TEST METHODS.....	1
2.1 Design .....	1
2.2 Field Testing Locations and Site Preparation.....	6
2.3 Field Exposure Apparatus.....	6
2.4 Test Initiation .....	8
2.5 Controls.....	8
2.6 Monitoring of Field Exposures .....	9
2.7 Test Termination.....	9
2.8 Data Analysis .....	10
3.0 QA/QC.....	10
4.0 RESULTS .....	11
4.1 Water Quality Measurements.....	11
4.2 <i>In situ</i> Exposures.....	13
5.0 DISCUSSION .....	16
6.0 REFERENCES .....	18



## LIST OF FIGURES

Figure 1:	Map of locations of sampling creeks, Downstream Indian Creek above, Upstream Indian Creek below.....	4
Figure 2:	Diagram of site locations within Downstream Indian Creek .....	5
Figure 3:	Water temperature data at Upstream (I-1) and Downstream (I-2A, I-2B, I-2C) sites for the duration of the tests.....	12
Figure 4:	Hatching success, post-hatch survival, and both cumulative survival. Bars are standard deviations.....	15
Figure 5:	Length data. Bars are standard deviations. ....	16
Figure 6:	Weight data. Bars are standard deviations.....	16
Figure 7:	Comparison of year to year data from Upstream and Downstream 2C .....	17

## LIST OF TABLES

Table 1:	Summary of the <i>in situ</i> ELS test study design. ....	3
Table 2:	Summary of water quality measurements collected during site visits and control renewals.....	12
Table 3:	Results for hatching success, and survival. The data show the mean and standard deviation. ....	14
Table 4:	Results for growth (mean and standard deviation).....	15

## LIST OF APPENDICES

APPENDIX A – Raw data

APPENDIX B – Statistical Outputs

## 1.0 INTRODUCTION

This report presents the results of the rainbow trout (RBT) early life stages (ELS) *in situ* bioassay, conducted as part of the Washington Department of Ecology's (WDOE) Integrated Ambient Monitoring in Indian Creek: Phase II Study. *In situ* RBT ELS deployments were conducted in 2010 as part of a pilot (Phase I) study to assess the most cost-effective methods for evaluating the biological integrity of urban streams. The 2013 objective is to further investigate the potential causes of mortality seen in the 2010 pilot testing. The *in situ* bioassays and benthic community assessments are intended to provide a direct indication of stream health, especially related to salmonid spawning and rearing. Tissue samples from the trout exposed in the stream were analyzed for metals. Results demonstrated that the RBT *in situ* bioassay can be an effective instream biological monitoring tool for assessing the effects of stormwater discharges on the receiving environment. WDOE investigators collected samples from the stream, stormwater discharges, groundwater seeps, and sediments. All of these samples were analyzed for metals and polycyclic aromatic hydrocarbons (PAHs). In addition, periphyton and benthic macroinvertebrates within the creek were enumerated and analyzed for pollutants. The results of all assessments will be presented in the report to be written by WDOE.

## 2.0 TEST METHODS

Guidance for conducting *in situ* ELS exposures with salmonids is available from a number of sources. The British Columbia Ministry of Environment has a protocol for an *in situ* ELS salmonid test (BC MoE, 2003). Environment Canada has developed both laboratory and *in situ* test procedures for early lifestages of rainbow trout (Environment Canada, 1998). In the U.S., there are two general laboratory protocols for conducting fish early life stage toxicity tests (EPA, 1996; ASTM, 2005). The procedures used in this study reflect refinements made to the general guidance provided in the documents above (Chalmers et al *in press*), and have been used successfully at a number of sites in both Canada and the United States, including the initial pilot study conducted on Indian Creek (Marshall and Era-Miller, 2012).

### 2.1 Design

The study involved monitoring survival, growth and development of eyed-embryos at four stream locations within Indian Creek, Olympia, WA, with three replicates per site. Indian Creek is a small urban stream located in Thurston County, Washington (Figure 1). The Indian Creek

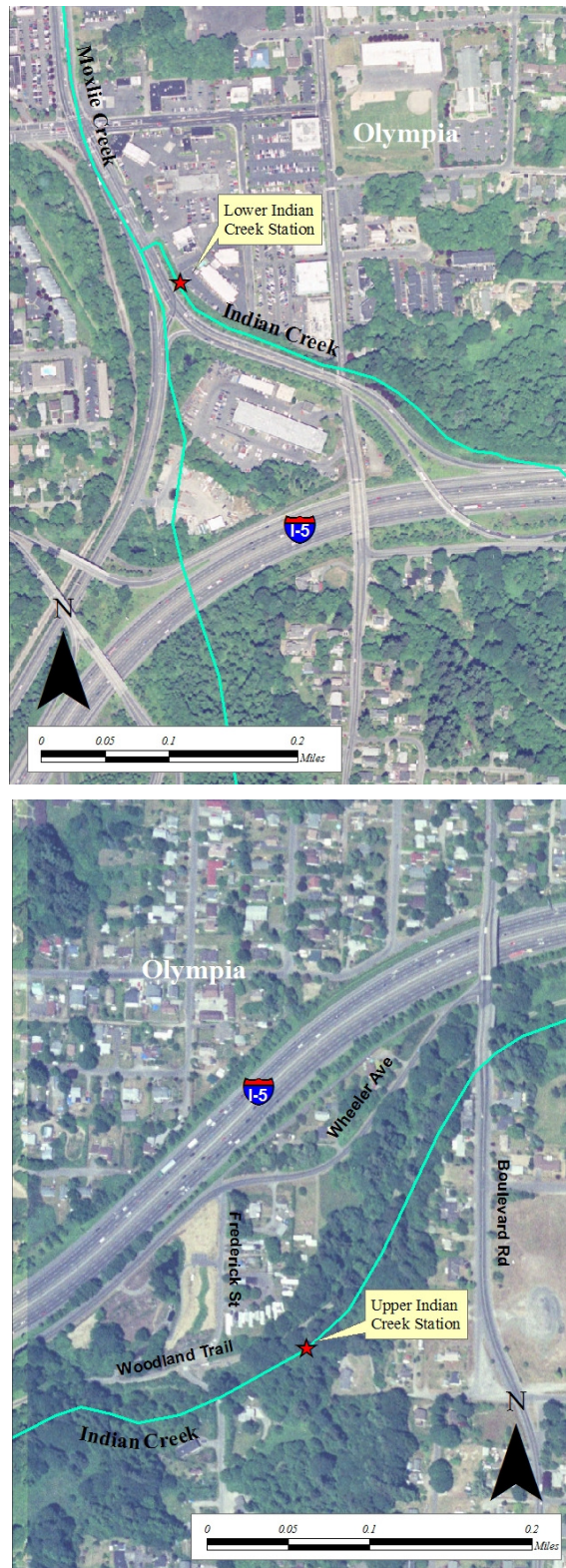
watershed is approximately 1,500 acres, and contains 35% impervious surface (Reynolds and Wood, 2010).

Two of the locations were repeated from the 2010 work, Upstream I-1, and Downstream I-2C. The additional two sites were located within the downstream portion of the Creek (Figure 2). Downstream I-2A was located just below where the Creek daylights, above the suspected stormwater outfall, while Downstream I-2B was below the stormwater outfall, and generally placed where the passive samplers were located in 2010.

The exposure period covered embryo development from the eyed stage, through hatch, the alevin stage (yolk sac present), and the fry stage (also known as swim up fry; the yolk sac absorbed and fish begin feeding). This exposure period included multiple rain/runoff events and corresponding fluctuations in flow, contaminant concentrations, temperature, water chemistry, etc. The test installations were designed to mimic natural spawning conditions in terms of substrate type (i.e., gravel), burial depths (five to seven inches) and habitat types (riffles). Due to the silty substrate found at Downstream I-2A, the replicates at that site were placed above the substrate in gravel contained within PVC crate frames. The eyed eggs were supplied by Trout Lodge (Sumner WA). The organisms were monitored at specific stages of development, and were checked approximately every one to two weeks throughout exposure in order to evaluate hatching, survival, development and growth. Stream temperatures were logged continuously with Tidbit Temperature Loggers supplied by the WDOE (OnSet Computer Corp., Bourne, MA), and weekly temperature measurements were taken to adjust the laboratory controls which were monitored to identify hatch and other developmental events that were used to time field inspections to observe the critical lifestage transitions. The study design is summarized in Table 1.

**Table 1: Summary of the *in situ* ELS test study design.**

Number of stations	No. of replicates	No. of embryos per replicate	No. of embryos required
Indian Creek (Upstream reference I-1)	3	30	90
Indian Creek (Downstream I-2A)	3	30	90
Indian Creek (Downstream I-2B)	3	30	90
Indian Creek (Downstream I-2C)	3	30	90
Laboratory Control	4	30	120



**Figure 1:** Map of Downstream Indian Creek (pictured above), Upstream Indian Creek (pictured below).

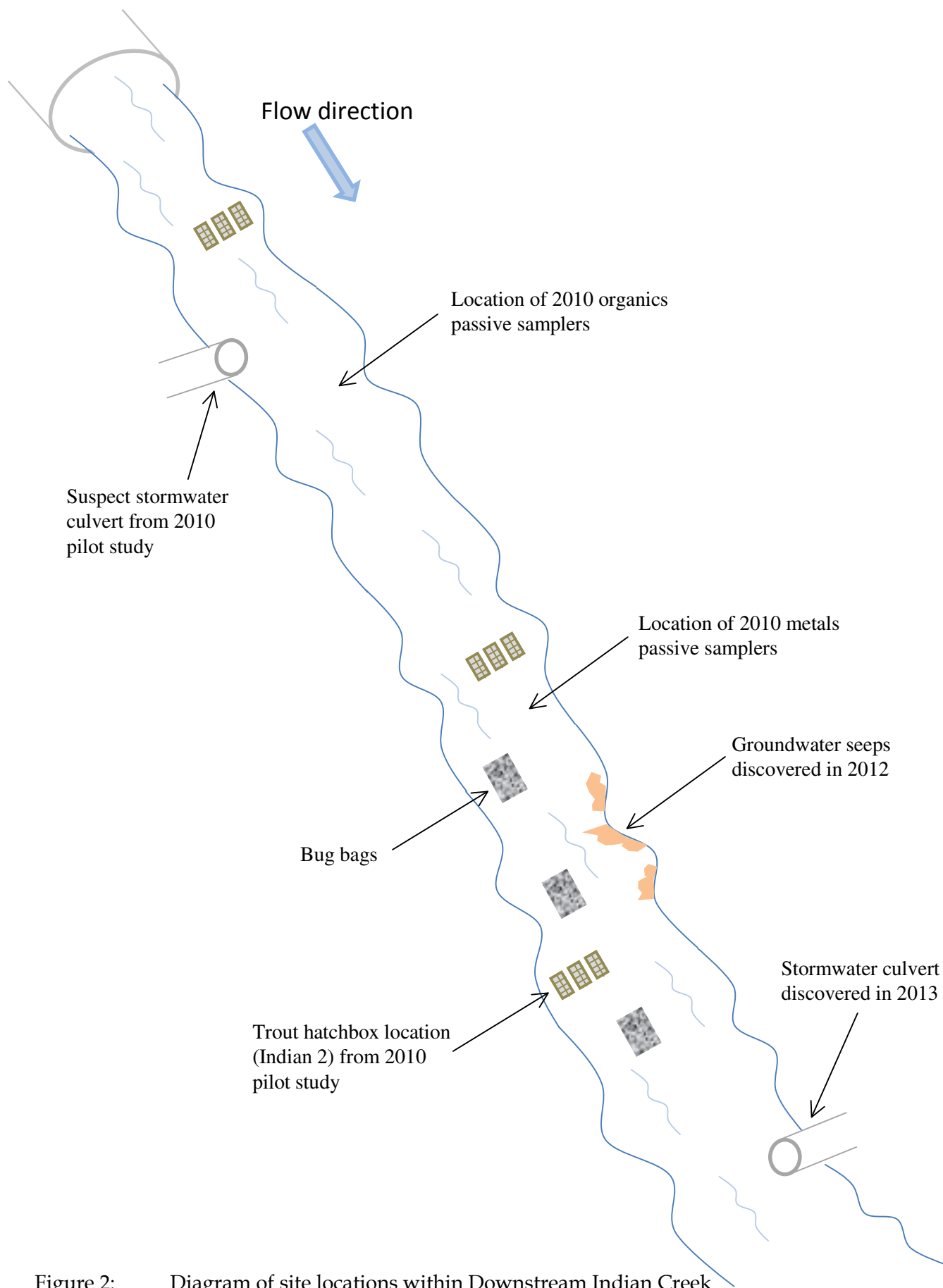


Figure 2: Diagram of site locations within Downstream Indian Creek

## **2.2 Field Testing Locations and Site Preparation**

The exposures were conducted at four Indian Creek sites, 1 Upstream reference site and 3 Downstream exposure sites. Indian Creek was originally chosen for the pilot project because water quality monitoring by the City of Olympia and Thurston County has shown this creek to be at least moderately impacted by stormwater runoff and other sources of pollution. Many of the culverts on Indian Creek are too small or have too much elevation drop to allow for salmon migration, but, despite these barriers, resident trout inhabit various reaches of the stream (City of Olympia, 2010). Monitoring in 2010 showed significantly reduced survival for the trout embryos at the downstream location.

Site preparation took place the day before test initiation (April 29, 2013). Using hand tools, a 20-cm depression was excavated in the streambed sufficient to contain all three of the replicates. The excavations were placed so that the replicates were oriented across the stream and parallel to each other. For Downstream 2A, stakes were placed in the substrate for PVC crates to be attached. The stakes and associated crates were placed so that replicate crates rested on the streambed in a row across the stream, perpendicular to the flow.

## **2.3 Field Exposure Apparatus**

The trout embryos were exposed in modified Whitlock-Vibert hatchboxes (Federation of Flyfishers, MO), which are comprised of two rectangular chambers located one above the other; the upper chamber is for embryo development and the lower chamber for rearing of hatched fish. The external sides of the hatchbox are plastic mesh that allows passage of water through the box, but prevents loss of embryos. The face dividing the embryo chamber from the rearing chamber is comprised of slots that are narrow enough to prevent transfer of unhatched embryos, but wide enough to permit migration of hatched fish into the lower rearing chamber. Thus, upon hatch, alevins are able to migrate through the slots from the embryo chamber into the larger rearing chamber. To prevent escape of the hatched fry into the streams upon swim-up, plastic screening material (Darice® size 7 mesh) was attached to the external faces of the rearing chamber and held in place using small plastic zip ties.

At most locations, the hatchboxes were contained within nickel-plated steel wire rotisserie baskets (typically used for barbecuing chicken) that were placed within the streambed. Each basket was half-filled with 1 – 2 inch diameter gravel, the hatchbox placed centrally within the



rocks in the basket, and additional gravel used to fill in the space around the hatchbox. Baskets were held closed with color-coded zip ties. The color-coded zip ties corresponded with the replicate order (1-3).

The baskets were placed into the streambed so that the top of the hatchbox was at approximately the same level as the streambed. Once all of the baskets were in place, surrounding gravels from the excavation, plus additional imported clean gravels, were then used to cover the baskets.

For the replicates at Downstream 2A, the hatchboxes were exposed in plastic crates resting on the stream substrates and were contained within flexible plastic mesh bags surrounded with 1 – 2 inch diameter gravel, with the hatchbox placed centrally within the rocks in the bag (Figure 3). As with the basket approach, the gravel surrounding the hatchboxes was intended to diffuse the current and prevent exposure of the embryos to sunlight. The bags were then placed within the plastic crates that were held in place in the stream by stakes anchored in the substrate.

**Figure 3:** Example of crate set up





## **2.4 Test Initiation**

Eyed-egg stage rainbow trout embryos were obtained from TroutLodge in Sumner, WA, on April 30<sup>th</sup>, 2010. As supplied, the eyed embryos had developed to approximately 245 degree-days<sup>1</sup>, as noted in communication with Troutlodge staff. The embryos were transported to the field locations, where they were randomly counted into replicate units of 30 embryos apiece and transferred into the hatchboxes in the creek that same day. The four remaining replicates were then taken to the Rainier Environmental Laboratory in Fife, WA.

## **2.5 Controls**

Organism controls were used to assess the influence of the following factors on test results:

1. quality (health and viability) of supplied organisms,
2. transport and handling of organisms, and
3. ambient stream temperatures, which influence the time to developmental milestones (e.g., hatch).

The laboratory exposure (four replicates of 30 organisms each) of embryos in moderately hard synthetic water (MHSW) was initiated with the same batch of eyed embryos used to begin the field exposures, and was conducted at Rainier Environmental in Fife, WA. Upon arrival to the laboratory after deployment of all field organisms, the control replicates were maintained in 4-L plastic containers in the laboratory at the average site water temperature,  $\pm 1^{\circ}\text{C}$ , which was adjusted on a weekly basis. Gentle aeration was applied (100-200 bubbles per minute) to the control chambers. The controls were monitored daily for mortalities, and any dead organisms were removed. Water renewals were conducted three times per week (Monday, Wednesday, Friday) using clean MHSW. Dissolved oxygen, pH, conductivity and temperature were measured before and after water renewals. Developmental milestones (hatch and swim-up) were recorded, and used as a prompt for checks on the field organisms on the same or following day.

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<sup>1</sup> Degree-days are used to standardize descriptions of fish development, regardless of rearing temperature. For example, it takes rainbow trout approximately 340 degree-days to reach hatch (Quinn, 2005). Thus, at 8°C, it takes approximately 42 days to reach hatch.

## **2.6 Monitoring of Field Exposures**

Sites were visited approximately every 7-10 days to assess the condition of the test organisms when the controls indicated that specific developmental milestones (i.e., hatch and swim up) had been reached. During these checks, organism survival was recorded, as well as qualitative observations of organism health, site conditions and sedimentation within the boxes. All dead organisms observed were recorded and removed on each visit. These observations were recorded on standard field data sheets included in Appendix A. In addition, temperature, dissolved oxygen, pH and conductivity were measured during the site visits using a MiniSonde®. Stream temperatures were monitored continuously at each site by Tidbit Temperature Loggers supplied by the WDOE. These loggers were attached to one of the individual replicates and downloaded after test termination for water quality measurements.

Monitoring of the installations required removal of the substrate overlying the hatchboxes from either the gravel or the mesh bag in the crate. The embryos or alevins in each hatchbox were then poured into a plastic dishpan containing site water where they were enumerated. They were then returned to the hatchbox, placed back into the basket or crate, and returned to original state. Care was taken to minimize disturbance and damage to the test organisms. In addition, when the boxes were excavated, sedimentation was recorded on a qualitative basis by noting the approximate extent to which the boxes were filled with sediment.

## **2.7 Test Termination**

Upon the 2<sup>nd</sup> field check of organisms (May 17<sup>th</sup>, 2013), only a quarter of the fish were still alive at the farthest downstream site (I-2C). It was determined that having fish available to run tissue metals analyses was important, and that the fish at the downstream site might not survive to test termination. Consequently, it was decided that at least one replicate from each field site be terminated early for collection of tissues for metals analysis. Where possible only one replicate from each site was terminated in the field; however, for 2 of the sites (Downstream 2B, 2C), all replicates were terminated to ensure enough tissue volume for analysis. The remaining field exposures and laboratory controls were terminated when the exposure control organisms (i.e., laboratory controls) reached the swim-up fry stage (i.e., yolk sac absorbed) on May 30<sup>th</sup>, 2013. Surviving organisms were transported back to the Rainier Environmental laboratory and terminated with CO<sub>2</sub> by emersion in Perrier® water. Fish were then wet-weighted (to the nearest 0.0001 gram) on an analytical balance (Mettler AE 240, Mettler Toledo, Columbus, OH) and measured (total length, from tip of snout to end of tail, to the nearest 0.5 cm). Obvious external

malformations were noted. Fish were then saved for tissue metals concentrations. Laboratory exposure control fish were evaluated similarly on the same day.

## **2.8 Data Analysis**

All test data were entered in CETIS (Comprehensive Environmental Toxicity Information System, Tidepool Scientific, McKinleyville, CA), and then analyzed using EPA flow chart methods for all endpoints.

One interim endpoint and four terminal endpoints were evaluated, as described below:

1. Hatching success – an interim measure of the number of eggs hatched the day of the hatch inspection, or determined to have hatched based on the number of alevins present at the subsequent inspection, relative to the total number of eggs originally added;
2. Survival post-hatch - the number of organisms surviving at test termination relative to the number of eggs that hatched (these data help determine whether the majority of mortalities occurred pre- or post-hatch, or were distributed throughout the exposure period);
3. Day 18 survival – the total number of surviving organisms at the May 17<sup>th</sup>, early termination check, relative to the number of embryos in each replicate at the beginning of the exposure;
4. Day 31 survival - the total number of surviving organisms at test termination relative to the number of embryos in each replicate at the beginning of the exposure;
5. Length – Total length, from the tip of the snout to end of the tail, to the nearest 0.5 mm;
6. Weight – to the nearest 0.0001 g.

## **3.0 QA/QC**

The QA/QC program for the field exposure portion involved the following:

- Consistent field staff leader throughout all visits;
- Field documentation to record all primary data; i.e., the names of individuals collecting the samples, the equipment used, sampling location, time of sampling, site conditions (e.g., degree of sedimentation) and other relevant observations, such as weather and any unusual conditions;
- Calibrated field instruments with calibration logs maintained;

- Storing and transporting organisms in sealed containers in a cold, dark environment; cooling sample containers using ice or gel packs;
- Use of transport controls, and rearing these controls in the laboratory to ascertain transport-induced stress;
- Checking field organisms every one to two weeks to monitor development progress, mortality and abnormality in test organisms;

The QA/QC program for the laboratory portion of the study involved:

- Checks and maintenance of control organisms at regular intervals, including recording primary observations and water quality data on standardized data sheets;
- Review of datasheets by project manager;
- Test termination activities (measurements, health assessment) conducted by same staff members to ensure identical assessment of abnormalities across all test sites and controls;
- Use of standard laboratory water for controls.

## **4.0 RESULTS**

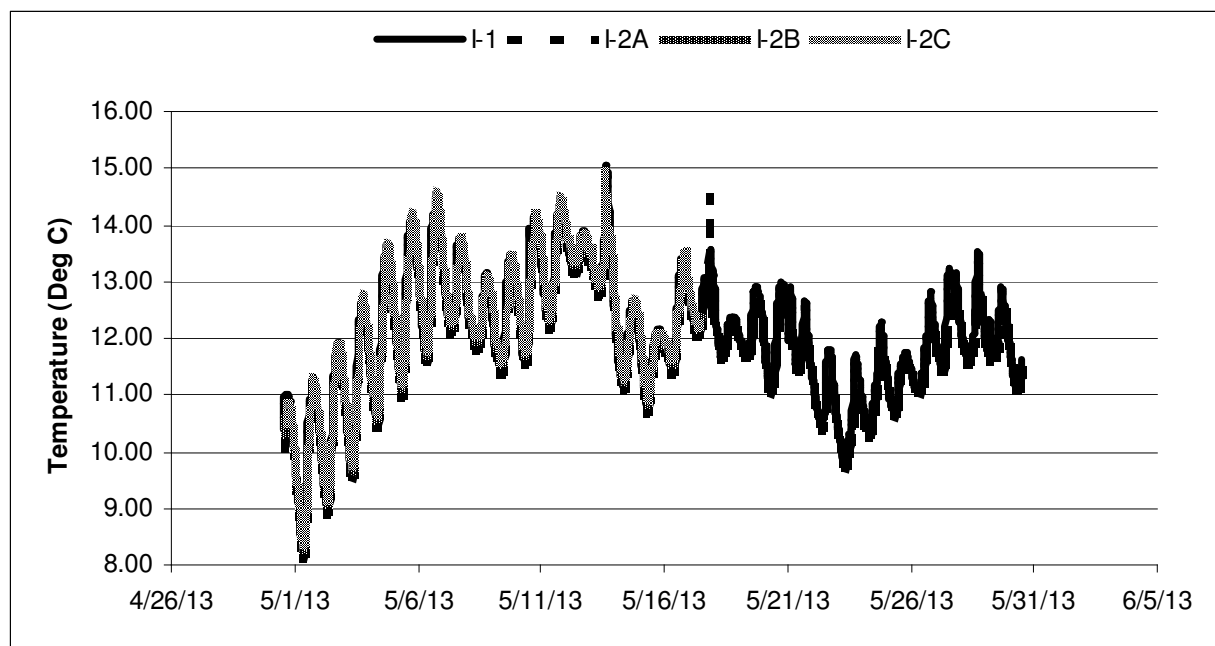
### **4.1 Water Quality Measurements**

Water quality measurements are summarized in Table 2. Temperature data collected at 30-minute intervals from the loggers attached to the hatchboxes are presented in Figure 4.

**Table 2: Summary of water quality measurements collected during site visits and control renewals.**

Creek		Temperature (°C)	DO (mg/L)	pH (units)	Specific Conductance (uS/cm)
Control	$\bar{X}$ (SD)	11.72 (1.03)	9.47 (0.26)	7.50 (0.09)	273.6 (10.4)
	[Min-Max]	[9.40 - 12.60]	[9.00 - 9.90]	[7.35 - 7.67]	[242.0 - 291.0]
Upstream		11.36 (1.21)	10.80 (0.44)	7.09 (0.14)	126.6 (6.7)
I-1		[10.10 - 12.51]	[10.46 - 11.30]	[6.99 - 7.25]	[121.0 - 134.0]
Downstream		11.32 (1.17)	10.30 (0.45)	7.05 (0.17)	141.0 (8.6)
I-2A		[10.05 - 12.35]	[9.93 - 10.80]	[6.85 - 7.18]	[135.3 - 150.9]
Downstream		11.25 (1.61)	10.50 (0.57)	7.18 (0.05)	144.7 (9.8)
I-2B		[10.11 - 12.38]	[10.09 - 10.90]	[7.14 - 7.21]	[137.7 - 151.6]
Downstream		11.29 (1.41)	11.29 (0.59)	7.21 (0.04)	145.1 (10.2)
I-2C		[10.29 - 12.29]	[10.17 - 11.01]	[7.18 - 7.23]	[137.9 - 152.3]

**Figure 4: Water temperature data at Upstream (I-1) and Downstream (I-2A, I-2B, I-2C) sites for the duration of the tests.**



Temperatures between all the field sites were similar, as were the control temperatures. The field measurements of DO and pH were within the acceptable range (60-100% saturation, 6-9 pH units, respectively) for rainbow trout laboratory bioassays. The pH values were circumneutral, and DO ranged from 9.93 to 11.3 mg/L among all stream measurements. Consequently, although limited to weekly measurements, field DO and pH data suggest good water quality conditions, with no potential for adverse effects on organism survival and development. There was a meter malfunction during one of the checks, and no water quality measurements were taken at that time. The conductivity measurements for all sites showed little variability throughout the test.

## **4.2 In situ Exposures**

The results for hatching success, post-hatch survival, partial cumulative survival, and cumulative survival, are summarized in Table 3, and Table 4 presents the growth endpoints. These same data are presented graphically in Figures 5 through 7 for ease of comparison. As replicates were terminated early, the number of replicates for each endpoint changed, with changes noted for each endpoint.

**Hatching success** – Mean hatching percentage (Day 11) for the controls was 100% (n=4), while 97.8% (n=3) of fish at the Upstream I-1 site hatched. Downstream hatch at I-2A was 88.9% (n=3), I-2B was 81.1% (n=3) and I-2C was 86.7% (n=3). The decrease in hatching success was significantly different from Upstream I-1 only for Downstream I-2B.

**Post-hatch survival** – Control survival of alevins post-hatch was 97.8% (n=3), while Upstream I-1 exhibited 94.9% (n=2) survival post hatch. Fish at the Downstream Indian were limited to I-2A and exhibited 38.2% (n=2) post-hatch survival, which was significantly different from the Upstream I-1 site.

**Day 18 survival** – On Day 18 of the exposure, when sites and replicates were terminated early, control survival was 99.2% (n=4), while Upstream I-1 exhibited survival of 90% (n=3). Conversely, Downstream I-2A exhibited 60% (n=3) survival, I-2B had 4.4% (n=3), and I-2C was at 24.4% (n=3). Only I-2B and I-2C were significantly different from Upstream I-1.

**Day 31 survival** – Control survival from the start of the test to the end was 97.8% (n=3), while Upstream I-1 had survival of 93.3% (n=2) of the fish through the duration of the exposure. Only

35% (n=2) of the embryos initiated at the Downstream I-2A site survived until the end of the test. This result was significantly different from the Upstream I-1 site.

**Growth** – Only fish available at final termination could be assessed for weight and length. Fish grew the largest in the controls, with an average weight of 175.2 mg (n=3). Upstream I-1 fish were slightly smaller at 171.5 mg (n=2), while Downstream I-2A fish averaged 169 mg (n=2). None of these differences were significant. Length was also similar across sites although unable to be tested statistically due to lack of replication in the controls, as only one replicate was assessed for length. It is unlikely differences would be found as the control replicate averaged 2.7 cm, while the two field sites both averaged 2.6 cm.

**Table 3: Results for hatching success, and survival. The data show the mean and standard deviation.**

Site	Hatch (%)	Post Hatch Survival (%)	Day 18 Survival (%)	Day 31 Survival (%)
Control	100 (0)	97.8 (1.9)	99.2 (1.6)	97.8 (1.9)
Upstream I-1	97.8 (1.9)	94.9 (2.3)	90.0 (5.8)	93.3 (0.0)
Downstream I-2A	88.9 (3.8)	<b>38.2 (26.8)</b>	<b>60.0 (20.0)</b>	<b>35.0 (25.9)</b>
Downstream I-2B	81.1 (5.1)	NA	<b>4.4 (7.7)</b>	NA
Downstream I-2C	86.7 (12.0)	NA	<b>24.4 (25.2)</b>	NA

Notes:

1. Values in gray shade are significantly less than control.
2. Bold values are significantly less than Upstream I-1
3. Post hatch survival is based on # of hatched embryos.
4. Partial survival is based on surviving fish at early termination (n=30 per replicate).
5. Cumulative survival is based on number of embryos at test initiation (n=30 per replicate).
6. NA- Replicates terminated early due to elevated mortalities.

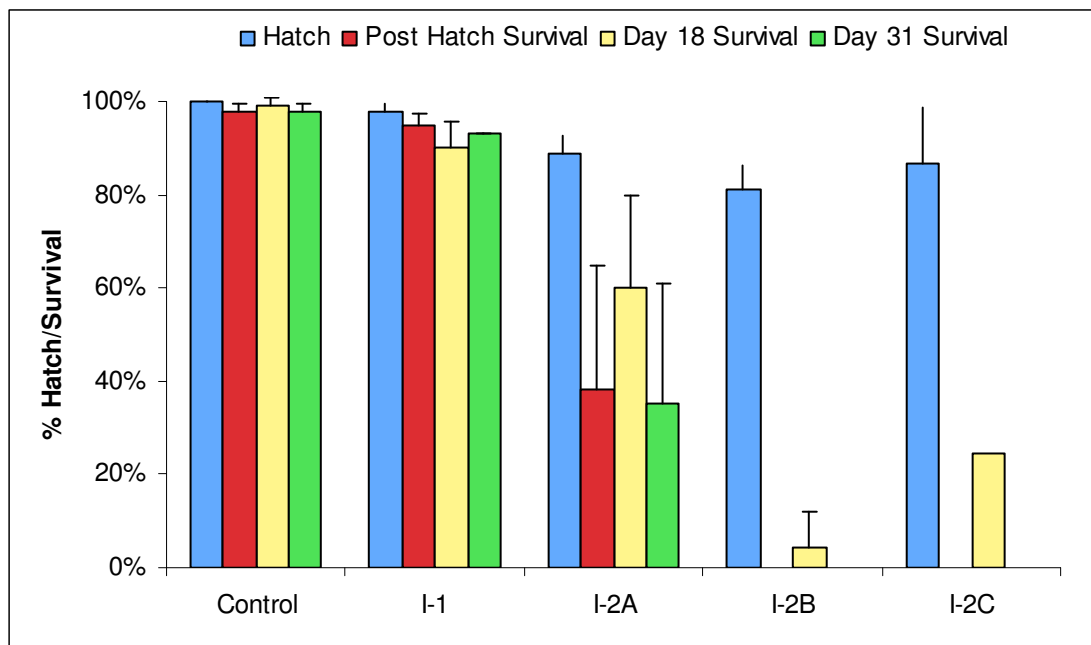
**Table 4: Results for growth (mean and standard deviation).**

Site	Length (cm)	Weight (mg)
Control	2.7	175.2 (7.3)
Upstream I-1	2.6 (0.0)	171.5 (3.5)
Downstream I-2A	2.6 (0.1)	169.0 (8.5)
Downstream I-2B	NA	NA
Downstream I-2C	NA	NA

Notes:

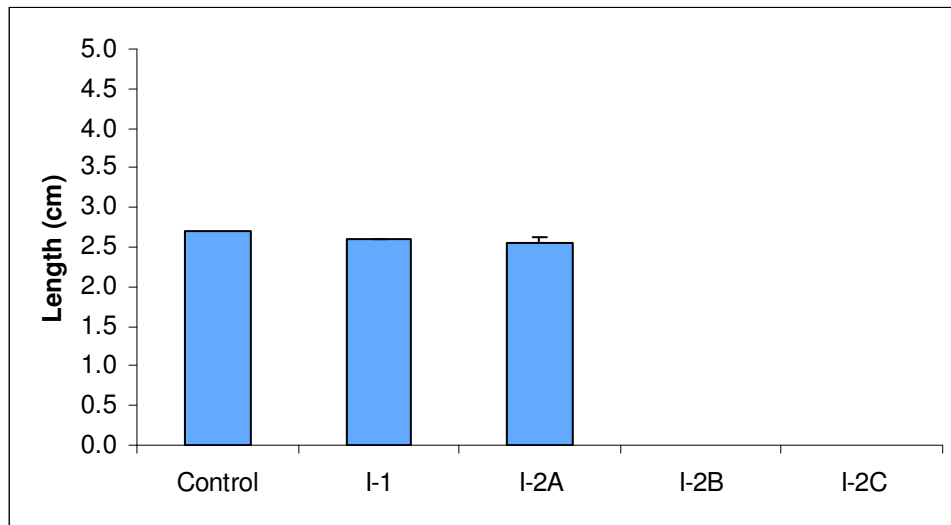
1. NA- Replicates terminated early due to elevated mortalities.

**Figure 5: Hatching success, post-hatch survival, partial cumulative survival, and cumulative survival. Bars are standard deviations.**

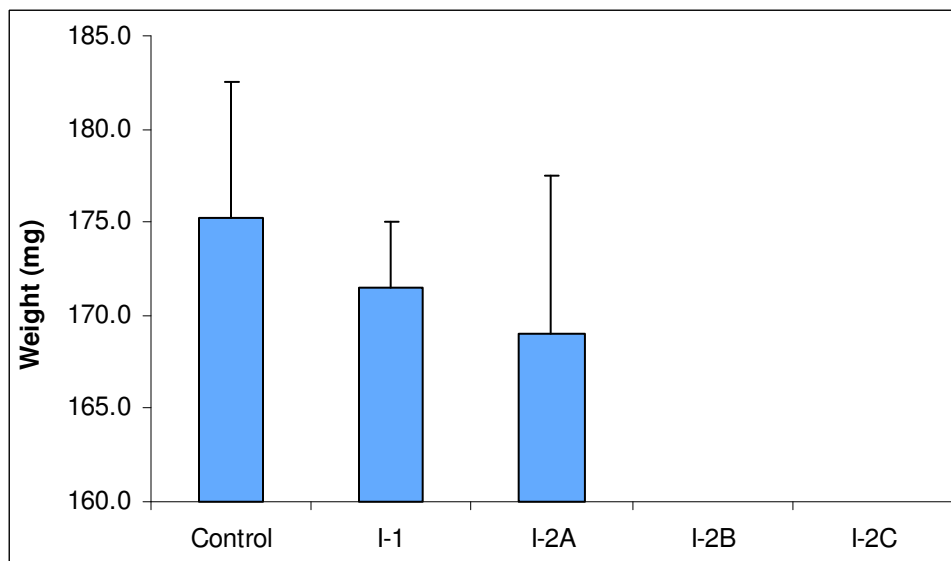




**Figure 6: Length data. Bars are standard deviations.**



**Figure 7: Weight data. Bars are standard deviations.**

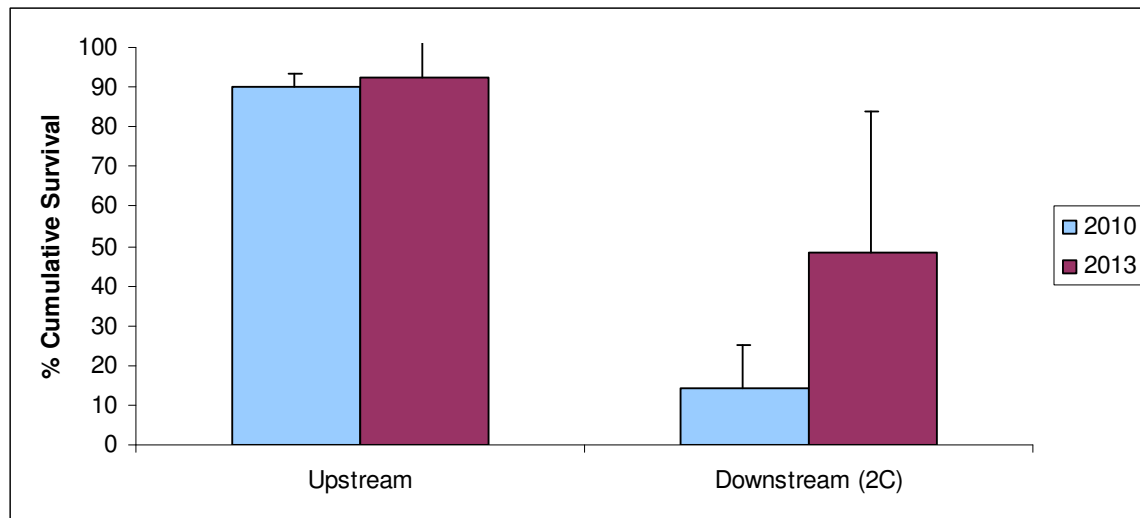


## 5.0 DISCUSSION

The results of the rainbow trout *in situ* early life stage exposure indicated that adverse effects were associated with the Downstream sites, compared with the Upstream site. Day 18 and Day 31 survival were the primary endpoints affected, with reduced survival most clearly associated

with the post-hatch period. The fact that most mortality was not observed until after hatching suggests that hatching and the ensuing early alevin developmental period are critical life stages for salmonids. There were also differences in responses observed between different exposure sites located within the downstream portion of the creek. All of the exposure locations (e.g., I-2A, I-2B and I-2C) are exposed to discharge from a sedimentation basin that receives run-off from US Interstate 5 and discharges into Indian Creek just downstream of the reference site. In addition to discharge from the sedimentation basin, and any other stormwater discharges and groundwater seeps that enter the creek between I-1 and I-2A, Sites I-2B and C are located downstream of a stormwater drain that collects run-off from a local shopping center, office park and hotel complex. I-2A exhibited partial mortality at the early termination check, while the two sites located downstream of the stormwater drain demonstrated almost complete mortality that resulted in their early termination. As part of the larger effort associated with this study, the potential causes of these response may be able to be determined. In addition, these results are similar to those found in the 2010 pilot study; Figure 7 compares cumulative survival at I-2C in 2010 and 2013 (note that the 2013 exposure at I-2C was terminated early in order to obtain sufficient tissue for analytical measurements).

**Figure 8: Comparison of year to year data from Upstream and Downstream 2C**



The consistent positive response of the Upstream I-1 location is a good confirmation that sites in Western Washington urban streams can provide the water quality and habitat conditions necessary to support viable populations of salmonids and continue to do so year after year. However, it is equally apparent that localized sources of contaminants can adversely affect water quality and rearing conditions for salmonids.

## 6.0 REFERENCES

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## **APPENDIX A – Raw data**

## **Field Datasheets**



# Field Raw Datasheet

Initiation

~~SITE I-2A~~

Client: WA. Dept of Ecology

Project ID: Indian Creek 2013

Visit Date: 4-30-13

Personnel:

BEM, PS, CC

Downstream

Location/Time I-2A	DO (mg/L) 10.80 Temp °C 18.05	Conduct (uS) 136.9 pH 6.85	Comments/ Observations	
Eggs Alive/Dead:	Rep 1 30/0	Rep 2 30/0		
	Rep 3 30/0	Rep 4 —		
Alevins Alive/Dead:	Rep 1 —	Rep 2 —		
	Rep 3 —	Rep 4 —		
Weather (circle one)	Sunny	Partly Cloudy	Overcast	Raining
Sedimentation: NA	10%	25%	50%	80%
			Water Level: Base Flow	

Location/Time I-2B	DO (mg/L) 10.90 Temp °C 10.11	Conduct (uS) 137.7 pH 7.14	Comments/ Observations	
Eggs Alive/Dead:	Rep 1 30/0	Rep 2 30/0		
	Rep 3 30/0	Rep 4 —		
Alevins Alive/Dead:	Rep 1 —	Rep 2 —		
	Rep 3 —	Rep 4 —		
Weather (circle one)	Sunny	Partly Cloudy	Overcast	Raining
Sedimentation: NA	10%	25%	50%	80%
			Water Level: Base Flow	

Location/Time I-2C	DO (mg/L) 11.01 Temp °C 10.29	Conduct (uS) 137.9 pH 7.23	Comments/ Observations	
Eggs Alive/Dead:	Rep 1 30/0	Rep 2 30/0		
	Rep 3 30/0	Rep 4 —		
Alevins Alive/Dead:	Rep 1 —	Rep 2 —		
	Rep 3 —	Rep 4 —		
Weather (circle one)	Sunny	Partly Cloudy	Overcast	Raining
Sedimentation:	10%	25%	50%	80%
			Water Level: Base Flow	
			QA: CC	



# Field Raw Datasheet

# Initiation

Client: WA. Dept of Ecology

Project ID: Indian Creek 2013

Visit Date: 4-30-13

Personnel: BEM, S, CC

Location/Time <u>I-1</u> <u>Upstream</u>	DO (mg/L) <u>11.3</u> Temp °C <u>10.1</u>	Conduct (uS) <u>121</u> pH <u>7.04</u>	Comments/ Observations
Eggs Alive/Dead:	Rep 1 <u>30/0</u> Rep 3 <u>30/0</u>	Rep 2 <u>30/0</u> <del>Rep 4</del>	
Alevins Alive/Dead:	Rep 1 <u>-</u> Rep 3 <u>-</u>	Rep 2 <u>-</u> <del>Rep 4</del>	
Weather (circle one)	<u>Sunny</u> Partly Cloudy Overcast Raining		
Sedimentation:	10% <u>25%</u> 50% 80%		
Water Level: <u>Close to base</u>			

Location/Time	DO (mg/L) Temp °C	Conduct (uS) pH	Comments/ Observations
Eggs Alive/Dead:	Rep 1 Rep 3	Rep 2 <del>Rep 4</del>	
Alevins Alive/Dead:	Rep 1 Rep 3	Rep 2 <del>Rep 4</del>	
Weather (circle one)	Sunny Partly Cloudy Overcast Raining		
Sedimentation:	10% 25% 50% 80%		
Water Level:			

Location/Time	DO (mg/L) Temp °C	Conduct (uS) pH	Comments/ Observations
Eggs Alive/Dead:	Rep 1 Rep 3	Rep 2 <del>Rep 4</del>	
Alevins Alive/Dead:	Rep 1 Rep 3	Rep 2 <del>Rep 4</del>	
Weather (circle one)	Sunny Partly Cloudy Overcast Raining		
Sedimentation:	10% 25% 50% 80%		
Water Level:			

QA: ce





# Field Raw Datasheet

Hatch - Day 11

Client: WA. Dept of Ecology

Project ID: Indian Creek 2013

Visit Date: 5-10-13

Personnel: CC + BEM

Downstream

Location/Time <u>I-2A/1340</u>	DO (mg/L) Temp °C /	Conduct (uS) pH /	Comments/ Observations <u>Hydrolab not working at time of trout check.</u> <u>- 2 crayfish</u> <u>- 1 sculpin</u>
Eggs Alive/Dead:	Rep 1 <u>0/0</u>	Rep 2 <u>0/0</u>	
	Rep 3 <u>0/0</u>	Rep 4 <u>—</u>	
Alevins Alive/Dead:	Rep 1 <u>26/4</u>	Rep 2 <u>28/1</u>	
	Rep 3 <u>26/2</u>	Rep 4 <u>—</u>	
Weather (circle one)	<u>Sunny</u> Partly Cloudy	Overcast Raining	
Sedimentation:	10% <u>25%</u>	50% 80%	Water Level: <u>Base flow</u>

Location/Time <u>I-2B/1405</u>	DO (mg/L) Temp °C /	Conduct (uS) pH /	Comments/ Observations
Eggs Alive/Dead:	Rep 1 <u>0/0</u>	Rep 2 <u>0/0</u>	
	Rep 3 <u>0/0</u>	Rep 4 <u>—</u>	
Alevins Alive/Dead:	Rep 1 <u>24/1</u>	Rep 2 <u>26/0</u>	
	Rep 3 <u>23/1</u>	Rep 4 <u>—</u>	
Weather (circle one)	<u>Sunny</u> Partly Cloudy	Overcast Raining	
Sedimentation:	10% <u>25%</u>	50% 80%	Water Level: <u>Base flow</u>

Location/Time <u>I-2C/1430</u>	DO (mg/L) Temp °C /	Conduct (uS) pH /	Comments/ Observations
Eggs Alive/Dead:	Rep 1 <u>0/0</u>	Rep 2 <u>0/0</u>	
	Rep 3 <u>0/1</u>	Rep 4 <u>—</u>	
Alevins Alive/Dead:	Rep 1 <u>22/2</u>	Rep 2 <u>27/2</u>	
	Rep 3 <u>29/0</u>	Rep 4 <u>—</u>	
Weather (circle one)	<u>Sunny</u> Partly Cloudy	Overcast Raining	
Sedimentation:	<u>10%</u> 25%	50% 80%	Water Level: <u>Base flow</u>

QA: CC





## Field Raw Datasheet

Client: WA. Dept of Ecology

Hatch - Day 11

Project ID: Indian Creek 2013

Visit Date: 5-10-13

Personnel: CC + BEM

upstream

Location/Time <u>I-1 1300</u>	DO (mg/L) <u>NA</u>	Conduct (uS) <u>NA</u>	Comments/ Observations <u>Hydrolab not working</u>	
	Temp °C <u>NA</u>	pH <u>NA</u>		
Eggs Alive/Dead:	Rep 1 <u>0%</u>	Rep 2 <u>0%</u>		
	Rep 3 <u>0%</u>	Rep 4 <u>—</u>		
Alevins Alive/Dead:	Rep 1 <u>29/1</u>	Rep 2 <u>30/0</u>		
	Rep 3 <u>29/0</u>	Rep 4 <u>—</u>		
Weather (circle one)	<u>Sunny</u>	Partly Cloudy	Overcast	Raining
Sedimentation:	10%	<u>25%</u>	50%	80%
			Water Level: <u>Base flow</u>	

Location/Time	DO (mg/L) Temp °C	Conduct (uS)	Comments/ Observations	
		pH		
Eggs Alive/Dead:	Rep 1	Rep 2		
	Rep 3	Rep 4		
Alevins Alive/Dead:	Rep 1	Rep 2		
	Rep 3	Rep 4		
Weather (circle one)	<u>Sunny</u>	Partly Cloudy	Overcast	Raining
Sedimentation:	10%	25%	50%	80%
			Water Level:	

Location/Time	DO (mg/L) Temp °C	Conduct (uS)	Comments/ Observations	
		pH		
Eggs Alive/Dead:	Rep 1	Rep 2		
	Rep 3	Rep 4		
Alevins Alive/Dead:	Rep 1	Rep 2		
	Rep 3	Rep 4		
Weather (circle one)	<u>Sunny</u>	Partly Cloudy	Overcast	Raining
Sedimentation:	10%	25%	50%	80%
			Water Level:	

QA: CC



# Field Raw Datasheet

Client: WA. Dept of Ecology

Day 18

Project ID: Indian Creek 2013

Visit Date: 5-17-13

Personnel:

CC + BEM

Location/Time 1200 I2-A	DO (mg/L) 9.93 Temp °C 12.35	Conduct (uS) 150.9 pH 7.18	Comments/ Observations Sacrificed Rep 1 for metals analysis
Eggs Alive/Dead:	Rep 1 0/0 Rep 3 0/0	Rep 2 0/0 Rep 4 —	
Alevins Alive/Dead:	Rep 1 18/8 Rep 3 12/14	Rep 2 24/5 Rep 4 —	
Weather (circle one)	Sunny Partly Cloudy <u>Overcast</u> Raining		
Sedimentation:	10% <u>25%</u> 50% 80%		
Water Level:			

Location/Time 1140 I2-B	DO (mg/L) 10.09 Temp °C 12.38	Conduct (uS) 151.6 pH 7.21	Comments/ Observations * more dead chunks test terminated
Eggs Alive/Dead:	Rep 1 0/0 Rep 3 0/0	Rep 2 0/0 Rep 4 —	
Alevins Alive/Dead:	Rep 1 0/20 Rep 3 0/11 *	Rep 2 4/20 Rep 4 —	
Weather (circle one)	Sunny Partly Cloudy <u>Overcast</u> Raining		
Sedimentation:	10% <u>25%</u> 50% 80%		
Water Level:			

Location/Time 1055 I2-C	DO (mg/L) 10.17 Temp °C 12.29	Conduct (uS) 152.3 pH 7.18	Comments/ Observations test terminated at 1128 All alive fish and combined sacrificed for metals analysis. No lengths & weights Just above base flow
Eggs Alive/Dead:	Rep 1 0/0 Rep 3 0/0	Rep 2 0/0 Rep 4 0/0	
Alevins Alive/Dead:	Rep 1 2/9 Rep 3 3 4/19	Rep 2 16/5 Rep 4 —	
Weather (circle one)	Sunny Partly Cloudy <u>Overcast</u> Raining		
Sedimentation:	10% <u>25%</u> 50% 80%		
Water Level:			
QA: <u>CC</u>			



# Field Raw Datasheet

Day 18

Rainbow LB  
pink  
yellow  
blue  
RB

Client: WA. Dept of Ecology

Project ID: Indian Creek 2013

Visit Date: 5-17-13

Personnel:

CC + BEM

Location/Time I-1 1245	DO (mg/L) 10.46	Conduct (uS) 134.0	Comments/ Observations Ended rep 1 for metals No length or weight just above base flow		
	Temp °C 12.51	pH 7.25			
Eggs Alive/Dead:	Rep 1 0%	Rep 2 0%			
	Rep 3 0%	Rep 4 —			
Alevins Alive/Dead:	Rep 1 25/1	Rep 2 28/1			
	Rep 3 28/0	Rep 4 —			
Weather (circle one)	Sunny	Partly Cloudy		Overcast	Raining
Sedimentation:	10%	< 25%		50%	80%
Water Level:					

Location/Time	DO (mg/L)	Conduct (uS)	Comments/ Observations		
	Temp °C	pH			
Eggs Alive/Dead:	Rep 1	Rep 2			
	Rep 3	Rep 4			
Alevins Alive/Dead:	Rep 1	Rep 2			
	Rep 3	Rep 4			
Weather (circle one)	Sunny	Partly Cloudy		Overcast	Raining
Sedimentation:	10%	25%		50%	80%
Water Level:					

Location/Time	DO (mg/L)	Conduct (uS)	Comments/ Observations		
	Temp °C	pH			
Eggs Alive/Dead:	Rep 1	Rep 2			
	Rep 3	Rep 4			
Alevins Alive/Dead:	Rep 1	Rep 2			
	Rep 3	Rep 4			
Weather (circle one)	Sunny	Partly Cloudy		Overcast	Raining
Sedimentation:	10%	25%		50%	80%
Water Level:					

QA:

CR





# Field Raw Datasheet

Termination of Test  
Day 31

Client: WA. Dept of Ecology

Project ID: Indian Creek 2013

Visit Date: 5-30-13

Personnel: BEM+PS

upstream Location/Time <u>I-1 1100</u>	DO (mg/L) <u>10.64</u> Temp °C <u>11.46</u>	Conduct (uS) <u>124.8</u> pH <u>6.99</u>	Comments/ Observations  Rep 2 had 1 dead alevin (some yolk still apparent) Rep 3 had 2 trout with bent spines, one of which was an alevin. The rest of the fish were at swim-up. Water Level: <u>above base</u>
Eggs Alive/Dead:	Rep 1 <u>—</u> Rep 3 <u>0%</u>	Rep 2 <u>0%</u> Rep 4 <u>—</u>	
Alevins Alive/Dead:	Rep 1 <u>—</u> Rep 3 <u>28/0</u> blue	Rep 2 <u>28/1</u> yellow Rep 4 <u>—</u>	
Weather (circle one)	Sunny Partly Cloudy <u>Overcast</u>	Raining	
Sedimentation:	10% <u>&lt; 25%</u> 50% 80%		

Location/Time <u>I2-A 1220</u>	DO (mg/L) <u>10.17</u> Temp °C <u>11.55</u>	Conduct (uS) <u>135.3</u> pH <u>7.11</u>	Comments/ Observations  Rep 2 had 6 dead alevins Rep 3 had 7 dead alevins one crayfish in the rocks of both reps. Water Level: <u>above base</u>
Eggs Alive/Dead:	Rep 1 <u>—</u> Rep 3 <u>0%</u>	Rep 2 <u>0%</u> Rep 4 <u>—</u>	
Alevins Alive/Dead:	Rep 1 <u>—</u> Rep 3 <u>5/7</u> blue	Rep 2 <u>16/0</u> orange lot debris Rep 4 <u>—</u>	
Weather (circle one)	Sunny Partly Cloudy <u>Raining</u>	Overcast	
Sedimentation:	10% <u>&lt; 25%</u> 50% 80%		

Location/Time	DO (mg/L)	Conduct (uS)	Comments/ Observations
	Temp °C	pH	
Eggs Alive/Dead:	Rep 1	Rep 2	
	Rep 3	Rep 4	
Alevins Alive/Dead:	Rep 1	Rep 2	
	Rep 3	Rep 4	
Weather (circle one)	Sunny Partly Cloudy Overcast Raining		
Sedimentation:	10% 25% 50% 80%		Water Level:

QA: CC

## **Laboratory Datasheets**

Client Name: WDOE Sping 2013Sample ID: Laboratory Controls

# /Container									
Conc.	Cont.	Rep.	Date						
			5/1/13	5/2/13	5/3/13	5/4/13	5/5/13	5/6/13	5/7/13
Control	A	1	30	30	30	30	30	30	30
	B	2	30	30	30	30	30	30	30
	C	3	30	30	30	30	30	30	30
	D	4	30	30	30	30	30	30	30
	Stage E, NH, AH <sup>1</sup>		E	E	E	E	E	E	E
Tech Initials			Et	Et	Et	Et	Et	Et	Et

	Renewal Date					
	5-1	5-3	5-3	5-6	5-6	5-8
	init.- Wed	final- Fri	init.- Fri	final- Mon	init.- Mon	final- Wed
pH	7.35	7.42	7.58	7.51	7.55	7.42
DO (mg/l)	9.1	9.0	9.9	9.2	9.7	9.5
Cond. (µmhos-cm)	278	277	242	255	262	271
Temp (°C)	9.5	<del>6.9</del> 9.4	10.2	10.5	10.3	11.5
Tech Initials		Et	Et	Et	Et	Et

1- Note stage of organism observed, E=egg, NH= Normal hatch, AH= Abnormal hatch, D=Dead; if more than one stage is observed, place / between numbers. Note: Each Rep needs to be denoted the same way

QA Check: CCTarget Temp: 10°

Comments: \_\_\_\_\_

Client Name:

WDOE Sping 2013

Sample ID:

Laboratory Controls

			# /Container						
Conc.	Cont.	Rep.	Date						
			5/8/13	5/9/13	5/10/13	5/11/13	5/12/13	5/13/13	5/14/13
Control	A	1	30/0	32/8	4/36	30	30	30	30
	B	2	27/3	13/17	0/30	30	30	30	30
	C	3	15/15	9/21	1/29	30	30	30	30
	D	4	29/1	19/12	1/29	30	30	30	30
	Stage E, NH, AH <sup>1</sup>		E/NH	E/NH	E/NH	NH	NH	NH	NH
Tech Initials			Et	Et	Et	Et	Et	Et	Et

	Renewal Date					
	5-8	5-10	5-10	5-13	5-13	5-15
	init.- Wed	final- Fri	init.- Fri	final- Mon	init.- Mon	final- Wed
pH	7.51	7.47	7.42	7.48	7.56	7.39
DO (mg/l)	9.3	9.7	9.5	9.4	9.7	9.5
Cond. (µmhos-cm)	268	271	267	273	270	275
Temp (°C)	11.3	11.3	11.2	11.5	11.5	12.5
Tech Initials		Et	Et	Et	Et	Et

1- Note stage of organism observed, E=egg, NH= Normal hatch, AH= Abnormal hatch, D=Dead; if more than one stage is observed, place / between numbers. Note: Each Rep needs to be denoted the same way

QA Check:

CC

Target Temp:

11°

Comments:



Client Name: WDOE Spring 2013Sample ID: Laboratory Controls

			# /Container						
Conc.	Cont.	Rep.	Date						
			5/15/13	5/16/13	5/17/13	5/18/13	5/19/13	5/20/13	5/21/13
Control	A	1	30	30	ENDED	—	—	—	—
	B	2	30	30	0/30	30	30	30	30
	C	3	30	30	0/30	30	30	30	30
	D	4	30	30	1/29	29	29	29	29
	Stage E, NH, AH <sup>1</sup>		NH	NH	D/NH	NH	NH	NH	NH
Tech Initials			Et	Et	Et	Et	Et	Et	Et

	Renewal Date					
	5-15	5-17	5-17	5-20	5-20	5-22
	init.- Wed	final- Fri	init.- Fri	final- Mon	init.- Mon	final- Wed
pH	7.56	7.37	7.48	7.43	7.51	7.41
DO (mg/l)	9.9	9.5	9.2	9.7	9.5	9.8
Cond. (µmhos-cm)	269	275	271	275	273	275
Temp (°C)	12.5	12.4	12.5	12.6	12.6	12.5
Tech Initials	Et	Et	Et	Et	Et	Et

1- Note stage of organism observed, E=egg, NH= Normal hatch, AH= Abnormal hatch, D=Dead; if more than one stage is observed, place / between numbers. Note: Each Rep needs to be denoted the same way

QA Check: CCTarget Temp: 12.5°

Comments: \_\_\_\_\_



Client Name: WDOE Spring 2013Sample ID: Laboratory Controls

# /Container									
Conc.	Cont.	Rep.	Date						
			5/22/13	5/23/13	5/24/13	5/25/13	5/26/13	5/27/13	5/28/13
Control	A	1	—	—	—	—	—	—	—
	B	2	30	30	30	30	30	30	30
	C	3	30	30	30	30	30	30	30
	D	4	29	29	29	29	29	29	29
	Stage E, NH, AH <sup>1</sup>		NH	NH	NH	NH	NH	NH	NH
Tech Initials			et	et	et	et	et	et	et

	Renewal Date					
	5-22	5-24	5-24	5-27	5-27	5-29
	init.- Wed	final- Fri	init.- Fri	final- Mon	init.- Mon	final- Wed
pH	7.67	7.59	7.62	7.55	7.65	7.62
DO (mg/l)	9.1	9.4	9.2	9.5	9.4	9.8
Cond. (µmhos-cm)	285	287	285	291	283	285
Temp (°C)	12.5	12.6	12.4	12.5	12.5	12.4
Tech Initials		et	et	et	et	et

1- Note stage of organism observed, E=egg, NH= Normal hatch, AH= Abnormal hatch, D=Dead; if more than one stage is observed, place / between numbers. Note: Each Rep needs to be denoted the same way

QA Check: CCTarget Temp: 12.5

Comments: \_\_\_\_\_

\_\_\_\_\_

Nautilus Environmental

Pg 5 of 5  
**Raw Data Sheet**  
**Rainbow Trout**  
*(Oncorhynchus mykiss)*  
**Trout Embryo Test**

Client Name: WDOE Spring 2013

Sample ID: Laboratory Controls

# /Container									
Conc.	Cont.	Rep.	Date						
			5/29/13						
Control	A	1	—						
	B	2	30						
	C	3	30						
	D	4	29						
	Stage E, NH, AH <sup>1</sup>		NH						
Tech Initials			EL						

	Renewal Date					
	<u>5-29</u>					
	init.- Wed	final- Fri	init.- Fri	final- Mon	init.-Mon	final-Wed
pH	<u>7.48</u>					
DO (mg/l)	<u>9.2</u>					
Cond. (µmhos-cm)	<u>277</u>					
Temp (°C)	<u>12.4</u>					
Tech Initials	<u>EL</u>					

1- Note stage of organism observed, E=egg, NH= Normal hatch, AH= Abnormal hatch, D=Dead; if more than one stage is observed, place / between numbers. Note: Each Rep needs to be denoted the same way

QA Check: OC

Target Temp: 12.5

Comments: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

## **Test Termination Datasheets**

5-30-13

Indian Creek

Trout In-situ test termination

I-1 Rep 2

	<u>weight(g)</u>	<u>Length (cm)</u>
1.	0.2046	2.7
2.	0.2106	2.7
3.	0.1782	2.7
4.	0.1814	2.6
5.	0.1681	2.7
6.	0.1740	2.7
7.	0.1707	2.6
8.	0.1882	2.7
9.	0.1543	2.6
10.	0.1770	2.6
11.	0.1773	2.7
12.	0.2237	2.8
13.	0.1870	2.7
14.	0.1190	2.4
15.	0.1685	2.6
16.	0.1490	2.5
17.	0.1905	2.7
18.	0.1800	2.7
19.	0.1730	2.7
20.	0.1962	2.6
21.	0.1657	2.6
22.	0.1712	2.7
23.	0.1089	2.4
24.	0.1545	2.7
25.	0.2022	2.7

I-1 Rep 2 cont.

	<u>weight(g)</u>	<u>Length (cm)</u>
--	------------------	--------------------

26.	0.1493	2.5
27.	0.1635	2.5
28.	0.1838	2.6

~~29.~~ I-1 Rep 3

1.	0.0863	1.9	alevin stunted
2.	0.1430	2.4	bent spine
3.	0.1812	2.7	bent spine
4.	0.1994	2.8	
5.	0.1450	2.5	
6.	0.1542	2.5	
7.	0.1691	2.6	
8.	0.1718	2.7	
9.	0.1790	2.7	
10.	0.1871	2.7	
11.	0.1384	2.5	
12.	0.1632	2.5	
13.	0.1619	2.7	
14.	0.1642	2.6	
15.	0.1595	2.6	
16.	0.1940	2.5	
17.	0.1349	2.4	
18.	0.1638	2.7	
19.	0.1799	2.6	
20.	0.2205	2.8	
21.	0.1758	2.6	

Fat belly eat something?



5-30-13

I-1 Rep 3 cont.

	<u>weight(g)</u>	<u>Length(cm)</u>
22.	0.1999	2.7
23.	0.1442	2.5
24.	0.1660	2.7
25.	0.1940	2.7
26.	0.2532	2.8
27.	0.1649	2.6
28.	0.1488	2.5

I2A Rep 2

1.	0.1113	2.4
2.	0.1354	2.6
3.	0.1750	2.7
4.	0.1628	2.7
5.	0.1828	2.8
6.	0.1638	2.6
7.	0.1731	2.7
8.	0.1753	2.5
9.	0.1450	2.5
10.	0.1453	2.5
11.	0.1927	2.7
12.	0.1819	2.7
13.	0.1699	2.7
14.	0.1813	2.7
15.	0.1757	2.6
16.	0.1313	2.5

I2A Rep 3

	<u>weight(g)</u>	<u>Length(cm)</u>
1.	0.1692	2.4
2.	0.1829	2.7
3.	0.1755	2.6
4.	0.1898	2.5
5.	0.1565	2.5

Control Rep 2

1.	0.1530	2.7
2.	0.1671	2.7
3.	0.1687	2.7
4.	0.1291	2.4
5.	0.1597	2.7
6.	0.1933	2.7
7.	0.1517	2.7
8.	0.1405	2.6
9.	0.1727	2.7
10.	0.1621	2.7
11.	0.1744	2.6
12.	0.1582	2.6
13.	0.1700	2.7
14.	0.1810	2.7
15.	0.1584	2.6
16.	0.1886	2.8
17.	0.1883	2.8
18.	0.1942	2.7

5-30-13

Control Rep 2 cont.

	<u>weight(g)</u>	<u>Length(cm)</u>
19.	0.1611	2.7
20.	0.2191	2.9
21.	0.1760	2.6
22.	0.1674	2.6
23.	0.1939	2.8
24.	0.1446	2.5
25.	0.1484	2.7
26.	0.1773	2.7
27.	0.1378	2.5
28.	0.1670	2.6
29.	0.1724	2.7

~~30.~~

Control Rep 3 (terminated @ 1730)

Bulk weight(g) = 5.4780

Average weight = 0.1826  $\div$  30

Control Rep 4 (terminated @ 1745)

Bulk weight(g) = 5.0780

Average weight = 0.1751  $\div$  29

Control trout in general are much straighter and narrower. Their bellys aren't bulgy at all like many of the in-situ trout. Are the control trout a bit further along in their development? Control trout also appear cleaner and chromier - more uniform in general.

## **APPENDIX B – Statistical Outputs**

# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 1 of 2)  
Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 17-7634-8629	Endpoint: Final Hatch Rate	CETIS Version: CETISv1.8.0
Analyzed: 29 Jul-13 12:29	Analysis: Parametric-All Pairwise	Official Results: Yes
Batch ID: 21-4402-8388	Test Type: Salmonid ELS (E-A-F)	Analyst:
Start Date: 30 Apr-13	Protocol: EC/EPS 1/RM/28	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 May-13	Species: Oncorhynchus mykiss	Brine:
Duration: 30d 0h	Source: Trout Lodge Fish Farm	Age:

Sample Code	Sample ID	Sample Date	Receive Date	Sample Age	Client Name	Project
Control	05-7015-7859	30 Apr-13	30 Apr-13	NA	WA State Dept of Ecology	
I-1	18-6676-1761	30 Apr-13	30 Apr-13	NA		
2A	11-7967-0876	30 Apr-13	30 Apr-13	NA		
2B	21-2412-8965	30 Apr-13	30 Apr-13	NA		
2c	19-1266-5937	30 Apr-13	30 Apr-13	NA		

Sample Code	Material Type	Sample Source	Station Location	Latitude	Longitude
Control	In Situ Station	Indian Creek - Olympia	Control		
I-1	In Situ Station	Indian Creek - Olympia	Upstream - Frederick St & Woodla		
2A	In Situ Station	Indian Creek - Olympia	Downstream 2A - 59 ft above stor		
2B	In Situ Station	Indian Creek - Olympia	Downstream 2B - 36 ft below stor		
2c	In Situ Station	Indian Creek - Olympia	Downstream 2C - original location		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD
Angular (Corrected)	NA	D<>0	NA	NA	10.4%

### Tukey-Kramer Test

Sample Code	vs	Sample Code	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Control		I-1	1.268	4.576	0.222	5	0.8924		Non-Significant Effect
		2A	5.051	4.576	0.222	5	0.0289		Significant Effect
		2B	7.34	4.576	0.222	5	0.0023		Significant Effect
		2c	5.321	4.576	0.222	5	0.0212		Significant Effect
I-1		2A	3.539	4.576	0.237	4	0.1598		Non-Significant Effect
		2B	5.679	4.576	0.237	4	0.0140		Significant Effect
		2c	3.791	4.576	0.237	4	0.1214		Non-Significant Effect
2A		2B	2.141	4.576	0.237	4	0.5750		Non-Significant Effect
		2c	0.2525	4.576	0.237	4	0.9997		Non-Significant Effect
2B		2c	1.888	4.576	0.237	4	0.6769		Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2943689	0.07359222	4	9.135	0.0017	Significant Effect
Error	0.08861808	0.008056189	11			
Total	0.382987		15			

### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	4.983	7.847	0.0321	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9142	0.8408	0.1359	Normal Distribution

### Final Hatch Rate Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	4	1	1	1	1	1	1	0	0.0%	0.0%
I-1	3	0.9778	0.93	1	0.9667	0.9667	1	0.01111	1.97%	2.22%
2A	3	0.8889	0.7933	0.9845	0.8667	0.8667	0.9333	0.02222	4.33%	11.11%
2B	3	0.8111	0.6846	0.9376	0.8	0.7667	0.8667	0.0294	6.28%	18.89%
2c	3	0.8667	0.5681	1	0.9	0.7333	0.9667	0.06939	13.87%	13.33%



# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 2 of 2)  
Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 17-7634-8629 Endpoint: Final Hatch Rate  
Analyzed: 29 Jul-13 12:29 Analysis: Parametric-All Pairwise

CETIS Version: CETISv1.8.0  
Official Results: Yes

### Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	4	1.479	1.479	1.48	1.479	1.479	1.479	0	0.0%	0.0%
I-1	3	1.418	1.286	1.55	1.387	1.387	1.479	0.03073	3.75%	4.15%
2A	3	1.235	1.073	1.396	1.197	1.197	1.31	0.03754	5.27%	16.55%
2B	3	1.124	0.9579	1.289	1.107	1.067	1.197	0.03851	5.94%	24.05%
2c	3	1.221	0.7716	1.671	1.249	1.028	1.387	0.1046	14.83%	17.43%

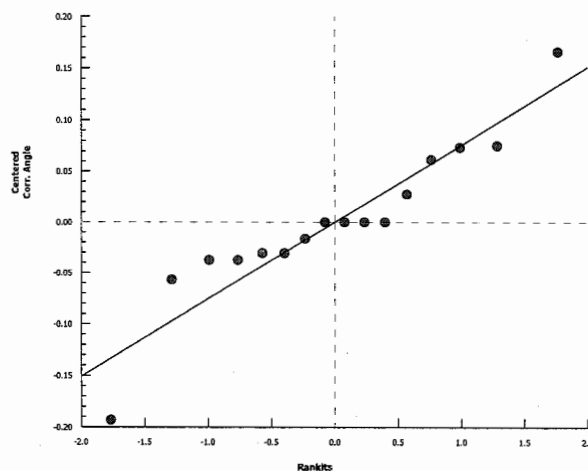
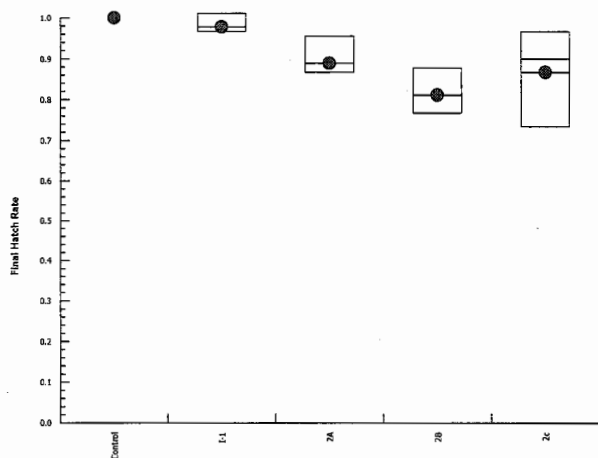
### Final Hatch Rate Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	1	1	1	1
I-1	0.9667	1	0.9667	
2A	0.8667	0.9333	0.8667	
2B	0.8	0.8667	0.7667	
2c	0.7333	0.9	0.9667	

### Angular (Corrected) Transformed Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	1.479	1.479	1.479	1.479
I-1	1.387	1.479	1.387	
2A	1.197	1.31	1.197	
2B	1.107	1.197	1.067	
2c	1.028	1.249	1.387	

### Graphics



# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 1 of 2)

Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 19-0937-4861	Endpoint: 1st Survival Rate	CETIS Version: CETISv1.8.0
Analyzed: 29 Jul-13 12:28	Analysis: Parametric-All Pairwise	Official Results: Yes
Batch ID: 21-4402-8388	Test Type: Salmonid ELS (E-A-F)	Analyst:
Start Date: 30 Apr-13	Protocol: EC/EPS 1/RM/28	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 May-13	Species: Oncorhynchus mykiss	Brine:
Duration: 30d 0h	Source: Trout Lodge Fish Farm	Age:

Sample Code	Sample ID	Sample Date	Receive Date	Sample Age	Client Name	Project
Control	05-7015-7859	30 Apr-13	30 Apr-13	NA	WA State Dept of Ecology	
I-1	18-6676-1761	30 Apr-13	30 Apr-13	NA		
2A	11-7967-0876	30 Apr-13	30 Apr-13	NA		
2B	21-2412-8965	30 Apr-13	30 Apr-13	NA		
2c	19-1266-5937	30 Apr-13	30 Apr-13	NA		

Sample Code	Material Type	Sample Source	Station Location	Latitude	Longitude
Control	In Situ Station	Indian Creek - Olympia	Control		
I-1	In Situ Station	Indian Creek - Olympia	Upstream - Frederick St & Woodla		
2A	In Situ Station	Indian Creek - Olympia	Downstream 2A - 59 ft above stor		
2B	In Situ Station	Indian Creek - Olympia	Downstream 2B - 36 ft below stor		
2c	In Situ Station	Indian Creek - Olympia	Downstream 2C - original location		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD
Angular (Corrected)	NA	D<>0	NA	NA	10.4%

### Tukey-Kramer Test

Sample Code	vs	Sample Code	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Control		I-1	1.268	4.576	0.222	5	0.8924		Non-Significant Effect
		2A	5.051	4.576	0.222	5	0.0289		Significant Effect
		2B	7.34	4.576	0.222	5	0.0023		Significant Effect
		2c	5.321	4.576	0.222	5	0.0212		Significant Effect
I-1		2A	3.539	4.576	0.237	4	0.1598		Non-Significant Effect
		2B	5.679	4.576	0.237	4	0.0140		Significant Effect
		2c	3.791	4.576	0.237	4	0.1214		Non-Significant Effect
2A		2B	2.141	4.576	0.237	4	0.5750		Non-Significant Effect
		2c	0.2525	4.576	0.237	4	0.9997		Non-Significant Effect
2B		2c	1.888	4.576	0.237	4	0.6769		Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2943689	0.07359222	4	9.135	0.0017	Significant Effect
Error	0.08861808	0.008056189	11			
Total	0.382987		15			

### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	4.983	7.847	0.0321	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9142	0.8408	0.1359	Normal Distribution

### 1st Survival Rate Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	4	1	1	1	1	1	1	0	0.0%	0.0%
I-1	3	0.9778	0.93	1	0.9667	0.9667	1	0.01111	1.97%	2.22%
2A	3	0.8889	0.7933	0.9845	0.8667	0.8667	0.9333	0.02222	4.33%	11.11%
2B	3	0.8111	0.6846	0.9376	0.8	0.7667	0.8667	0.0294	6.28%	18.89%
2c	3	0.8667	0.5681	1	0.9	0.7333	0.9667	0.06939	13.87%	13.33%

# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 2 of 2)  
Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 19-0937-4861 Endpoint: 1st Survival Rate  
Analyzed: 29 Jul-13 12:28 Analysis: Parametric-All Pairwise

CETIS Version: CETISv1.8.0  
Official Results: Yes

### Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	4	1.479	1.479	1.48	1.479	1.479	1.479	0	0.0%	0.0%
I-1	3	1.418	1.286	1.55	1.387	1.387	1.479	0.03073	3.75%	4.15%
2A	3	1.235	1.073	1.396	1.197	1.197	1.31	0.03754	5.27%	16.55%
2B	3	1.124	0.9579	1.289	1.107	1.067	1.197	0.03851	5.94%	24.05%
2c	3	1.221	0.7716	1.671	1.249	1.028	1.387	0.1046	14.83%	17.43%

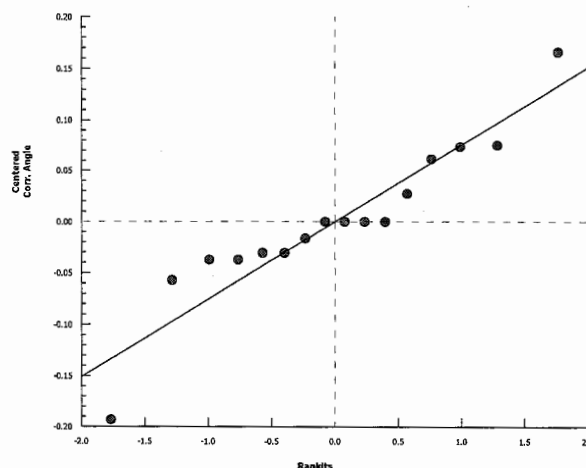
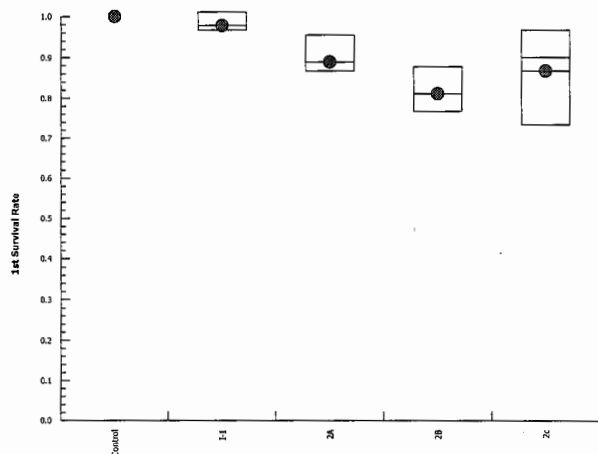
### 1st Survival Rate Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	1	1	1	1
I-1	0.9667	1	0.9667	
2A	0.8667	0.9333	0.8667	
2B	0.8	0.8667	0.7667	
2c	0.7333	0.9	0.9667	

### Angular (Corrected) Transformed Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	1.479	1.479	1.479	1.479
I-1	1.387	1.479	1.387	
2A	1.197	1.31	1.197	
2B	1.107	1.197	1.067	
2c	1.028	1.249	1.387	

### Graphics



## CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 1 of 2)

Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 09-1743-6083	Endpoint: 2nd Survival Rate	CETIS Version: CETISv1.8.0
Analyzed: 29 Jul-13 12:29	Analysis: Parametric-All Pairwise	Official Results: Yes
Batch ID: 21-4402-8388	Test Type: Salmonid ELS (E-A-F)	Analyst:
Start Date: 30 Apr-13	Protocol: EC/EPS 1/RM/28	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 May-13	Species: Oncorhynchus mykiss	Brine:
Duration: 30d 0h	Source: Trout Lodge Fish Farm	Age:

Sample Code	Sample ID	Sample Date	Receive Date	Sample Age	Client Name	Project
Control	05-7015-7859	30 Apr-13	30 Apr-13	NA	WA State Dept of Ecology	
I-1	18-6676-1761	30 Apr-13	30 Apr-13	NA		
2A	11-7967-0876	30 Apr-13	30 Apr-13	NA		
2B	21-2412-8965	30 Apr-13	30 Apr-13	NA		
2c	19-1266-5937	30 Apr-13	30 Apr-13	NA		

Sample Code	Material Type	Sample Source	Station Location	Latitude	Longitude
Control	In Situ Station	Indian Creek - Olympia	Control		
I-1	In Situ Station	Indian Creek - Olympia	Upstream - Frederick St & Woodla		
2A	In Situ Station	Indian Creek - Olympia	Downstream 2A - 59 ft above stor		
2B	In Situ Station	Indian Creek - Olympia	Downstream 2B - 36 ft below stor		
2c	In Situ Station	Indian Creek - Olympia	Downstream 2C - original location		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD
Angular (Corrected)	NA	D<>0	NA	NA	29.3%

## Tukey-Kramer Test

Sample Code	vs	Sample Code	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Control		I-1	2.106	4.576	0.434	5	0.5892		Non-Significant Effect
		2A	5.94	4.576	0.434	5	0.0104		Significant Effect
		2B	13.39	4.576	0.434	5	0.0002		Significant Effect
		2c	10.24	4.576	0.434	5	0.0003		Significant Effect
I-1		2A	3.587	4.576	0.464	4	0.1517		Non-Significant Effect
		2B	10.56	4.576	0.464	4	0.0002		Significant Effect
		2c	7.609	4.576	0.464	4	0.0018		Significant Effect
2A		2B	6.97	4.576	0.464	4	0.0034		Significant Effect
		2c	4.023	4.576	0.464	4	0.0938		Non-Significant Effect
2B		2c	2.948	4.576	0.464	4	0.2919		Non-Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.667754	0.9169385	4	29.7	<0.0001	Significant Effect
Error	0.3396198	0.03087453	11			
Total	4.007374		15			

## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.831	13.28	0.1451	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9445	0.8408	0.4075	Normal Distribution

## 2nd Survival Rate Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	4	0.9917	0.9651	1	1	0.9667	1	0.008333	1.68%	0.0%
I-1	3	0.9	0.7566	1	0.9333	0.8333	0.9333	0.03333	6.42%	9.24%
2A	3	0.6	0.1032	1	0.6	0.4	0.8	0.1155	33.33%	39.5%
2B	3	0.04444	0	0.2357	0	0	0.1333	0.04444	173.2%	95.52%
2c	3	0.2444	0	0.8714	0.1333	0.06667	0.5333	0.1457	103.3%	75.35%

# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 2 of 2)  
Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 09-1743-6083  
Analyzed: 29 Jul-13 12:29

Endpoint: 2nd Survival Rate  
Analysis: Parametric-All Pairwise

CETIS Version: CETISv1.8.0  
Official Results: Yes

### Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	4	1.456	1.383	1.53	1.479	1.387	1.479	0.02305	3.17%	0.0%
I-1	3	1.257	1.028	1.485	1.31	1.15	1.31	0.05313	7.32%	13.72%
2A	3	0.8926	0.3678	1.418	0.8861	0.6847	1.107	0.122	23.67%	38.71%
2B	3	0.1855	-0.2195	0.5905	0.09141	0.09141	0.3738	0.09413	87.87%	87.26%
2c	3	0.4846	-0.2479	1.217	0.3738	0.2612	0.8188	0.1702	60.85%	66.73%

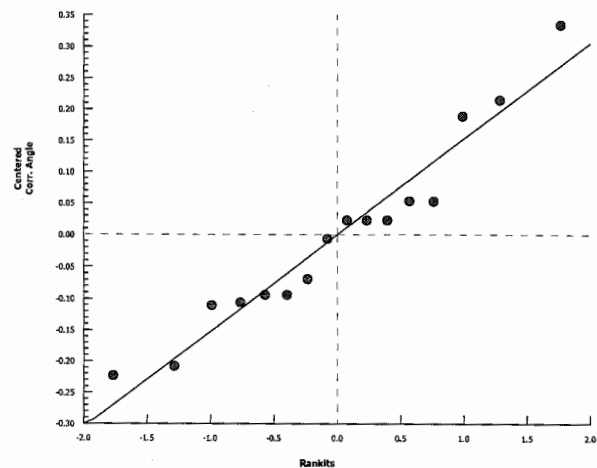
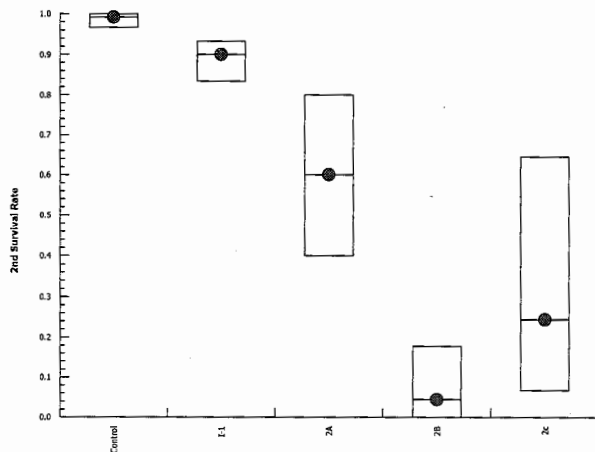
### 2nd Survival Rate Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	1	1	1	0.9667
I-1	0.8333	0.9333	0.9333	
2A	0.6	0.8	0.4	
2B	0	0.1333	0	
2c	0.06667	0.5333	0.1333	

### Angular (Corrected) Transformed Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	1.479	1.479	1.479	1.387
I-1	1.15	1.31	1.31	
2A	0.8861	1.107	0.6847	
2B	0.09141	0.3738	0.09141	
2c	0.2612	0.8188	0.3738	

### Graphics



## CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 1 of 2)

Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 00-3888-4641	Endpoint: Final Survival Rate	CETIS Version: CETISv1.8.0
Analyzed: 29 Jul-13 12:30	Analysis: Parametric-All Pairwise	Official Results: Yes
Batch ID: 21-4402-8388	Test Type: Salmonid ELS (E-A-F)	Analyst:
Start Date: 30 Apr-13	Protocol: EC/EPS 1/RM/28	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 May-13	Species: Oncorhynchus mykiss	Brine:
Duration: 30d 0h	Source: Trout Lodge Fish Farm	Age:

Sample Code	Sample ID	Sample Date	Receive Date	Sample Age	Client Name	Project
Control	05-7015-7859	30 Apr-13	30 Apr-13	NA	WA State Dept of Ecology	
I-1	18-6676-1761	30 Apr-13	30 Apr-13	NA		
2A	11-7967-0876	30 Apr-13	30 Apr-13	NA		

Sample Code	Material Type	Sample Source	Station Location	Latitude	Longitude
Control	In Situ Station	Indian Creek - Olympia	Control		
I-1	In Situ Station	Indian Creek - Olympia	Upstream - Frederick St & Woodla		
2A	In Situ Station	Indian Creek - Olympia	Downstream 2A - 59 ft above stor		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD
Angular (Corrected)	NA	D<>0	NA	NA	37.4%

## Tukey-Kramer Test

Sample Code	vs	Sample Code	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Control		I-1	1.151	5.045	0.475	3	0.7155		Non-Significant Effect
		2A	8.486	5.045	0.475	3	0.0087		Significant Effect
I-1		2A	6.696	5.045	0.52	2	0.0199		Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.8283132	0.4141566	2	19.5	0.0087	Significant Effect
Error	0.0849563	0.02123907	4			
Total	0.9132695		6			

## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	15.34	30.82	0.0266	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9255	0.5629	0.5134	Normal Distribution

## Final Survival Rate Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	3	0.9778	0.93	1	0.9667	0.9667	1	0.01111	1.97%	0.0%
I-1	2	0.9333	0.9314	0.9353	0.9333	0.9333	0.9333	0	0.0%	4.55%
2A	2	0.35	0	1	0.35	0.1667	0.5333	0.1833	74.08%	64.2%

## Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	3	1.418	1.286	1.55	1.387	1.387	1.479	0.03073	3.75%	0.0%
I-1	2	1.31	1.309	1.311	1.31	1.31	1.31	0	0.0%	7.64%
2A	2	0.6196	-1.91	3.15	0.6196	0.4205	0.8188	0.1991	45.44%	56.3%

## Final Survival Rate Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	0.9667	1	0.9667	
I-1	0.9333	0.9333		
2A	0.5333	0.1667		

# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 2 of 2)  
Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

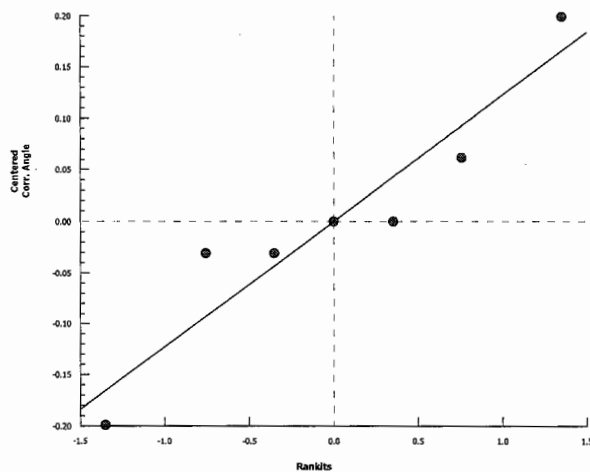
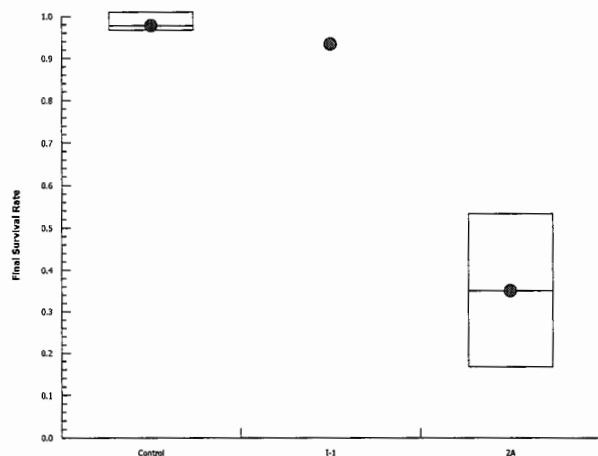
Analysis ID: 00-3888-4641      Endpoint: Final Survival Rate  
Analyzed: 29 Jul-13 12:30      Analysis: Parametric-All Pairwise

CETIS Version: CETISv1.8.0  
Official Results: Yes

### Angular (Corrected) Transformed Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	1.387	1.479	1.387	
I-1	1.31	1.31		
2A	0.8188	0.4205		

### Graphics



# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 1 of 2)  
Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 08-6848-2463	Endpoint: Post-Hatch Survival	CETIS Version: CETISv1.8.0
Analyzed: 29 Jul-13 12:31	Analysis: Parametric-All Pairwise	Official Results: Yes
Batch ID: 21-4402-8388	Test Type: Salmonid ELS (E-A-F)	Analyst:
Start Date: 30 Apr-13	Protocol: EC/EPS 1/RM/28	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 May-13	Species: Oncorhynchus mykiss	Brine:
Duration: 30d 0h	Source: Trout Lodge Fish Farm	Age:

Sample Code	Sample ID	Sample Date	Receive Date	Sample Age	Client Name	Project
Control	05-7015-7859	30 Apr-13	30 Apr-13	NA	WA State Dept of Ecology	
I-1	18-6676-1761	30 Apr-13	30 Apr-13	NA		
2A	11-7967-0876	30 Apr-13	30 Apr-13	NA		

Sample Code	Material Type	Sample Source	Station Location	Latitude	Longitude
Control	In Situ Station	Indian Creek - Olympia	Control		
I-1	In Situ Station	Indian Creek - Olympia	Upstream - Frederick St & Woodla		
2A	In Situ Station	Indian Creek - Olympia	Downstream 2A - 59 ft above stor		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD
Angular (Corrected)	NA	D<>0	NA	NA	38.9%

## Tukey-Kramer Test

Sample Code	vs	Sample Code	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Control		I-1	0.7356	5.045	0.488	3	0.8662		Non-Significant Effect
		2A	7.888	5.045	0.488	3	0.0112		Significant Effect
I-1		2A	6.529	5.045	0.534	2	0.0216		Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.7756494	0.3878247	2	17.3	0.0107	Significant Effect
Error	0.08968111	0.02242028	4			
Total	0.8653305		6			

## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.594	9.21	0.1658	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.942	0.5629	0.6571	Normal Distribution

## Post-Hatch Survival Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	3	0.9778	0.93	1	0.9667	0.9667	1	0.01111	1.97%	0.0%
I-1	2	0.9494	0.745	1	0.9494	0.9333	0.9655	0.01609	2.4%	2.9%
2A	2	0.3819	0	1	0.3819	0.1923	0.5714	0.1896	70.2%	60.95%

## Angular (Corrected) Transformed Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	3	1.418	1.286	1.55	1.387	1.387	1.479	0.03073	3.75%	0.0%
I-1	2	1.347	0.8743	1.819	1.347	1.31	1.384	0.03719	3.91%	5.01%
2A	2	0.6555	-1.905	3.217	0.6555	0.454	0.8571	0.2016	43.48%	53.77%

## Post-Hatch Survival Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	0.9667	1	0.9667	
I-1	0.9333	0.9655		
2A	0.5714	0.1923		



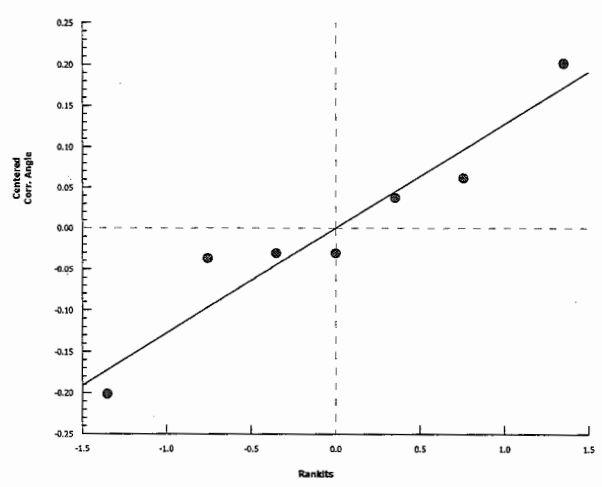
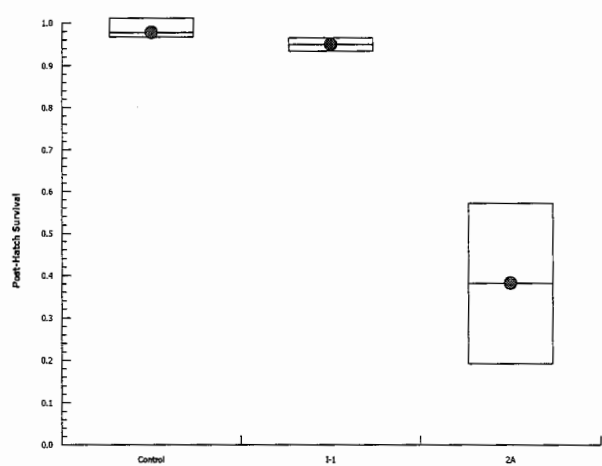
# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 2 of 2)  
Test Code: Indian13 | 05-3811-2389

Salmonid Early Lifestage (E-A-F) Test				Nautilus Environmental	
Analysis ID:	08-6848-2463	Endpoint:	Post-Hatch Survival	CETIS Version:	CETISv1.8.0
Analyzed:	29 Jul-13 12:31	Analysis:	Parametric-All Pairwise	Official Results:	Yes

Angular (Corrected) Transformed Detail				
Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	1.387	1.479	1.387	
I-1	1.31	1.384		
2A	0.8571	0.454		

## Graphics



# CETIS Analytical Report

Report Date: 29 Jul-13 14:34 (p 1 of 2)

Test Code: Indian13 | 05-3811-2389

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 00-4392-4531	Endpoint: Weight	CETIS Version: CETISv1.8.0
Analyzed: 29 Jul-13 12:31	Analysis: Parametric-All Pairwise	Official Results: Yes
Batch ID: 21-4402-8388	Test Type: Salmonid ELS (E-A-F)	Analyst:
Start Date: 30 Apr-13	Protocol: EC/EPS 1/RM/28	Diluent: Mod-Hard Synthetic Water
Ending Date: 30 May-13	Species: Oncorhynchus mykiss	Brine:
Duration: 30d 0h	Source: Trout Lodge Fish Farm	Age:

Sample Code	Sample ID	Sample Date	Receive Date	Sample Age	Client Name	Project
Control	05-7015-7859	30 Apr-13	30 Apr-13	NA	WA State Dept of Ecology	
I-1	18-6676-1761	30 Apr-13	30 Apr-13	NA		
2A	11-7967-0876	30 Apr-13	30 Apr-13	NA		

Sample Code	Material Type	Sample Source	Station Location	Latitude	Longitude
Control	In Situ Station	Indian Creek - Olympia	Control		
I-1	In Situ Station	Indian Creek - Olympia	Upstream - Frederick St & Woodla		
2A	In Situ Station	Indian Creek - Olympia	Downstream 2A - 59 ft above stor		

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD
Untransformed	NA	D<>0	NA	NA	14.1%

### Tukey-Kramer Test

Sample Code	vs	Sample Code	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Control		I-1	0.837	5.045	22.51	3	0.8319		Non-Significant Effect
		2A	1.397	5.045	22.51	3	0.6216		Non-Significant Effect
I-1		2A	0.5115	5.045	24.66	2	0.9317		Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	48.84162	24.42081	2	0.5111	0.6343	Non-Significant Effect
Error	191.1059	47.77646	4			
Total	239.9475		6			

### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.5176	9.21	0.7720	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9451	0.5629	0.6850	Normal Distribution

### Weight Summary

Sample Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Control	3	175.2	157.1	193.4	175.1	168	182.6	4.215	4.17%	0.0%
I-1	2	171.5	139.7	203.3	171.5	169	174	2.5	2.06%	2.13%
2A	2	169	92.76	245.2	169	163	175	6	5.02%	3.56%

### Weight Detail

Sample Code	Rep 1	Rep 2	Rep 3	Rep 4
Control	168	182.6	175.1	
I-1	174	169		
2A	163	175		

## Salmonid Early Lifestage (E-A-F) Test

Nautilus Environmental

Analysis ID: 00-4392-4531

Endpoint: Weight

CETIS Version: CETISv1.8.0

Analyzed: 29 Jul-13 12:31

Analysis: Parametric-All Pairwise

Official Results: Yes

## Graphics

