GSI Test Facility Validation Report

GSI Facility Blank Testing: Comparison of Control & Treatment Track Effects on Zooplankton with 18 Hour and 5 Day Tank Retention Times

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The objective of these tests was to determine the extent to which there may asymmetrical facility effects on live zooplankton in ballast treatment system testing at the Great Ships Initiative land-based testing facility (RDTE) in Duluth/Superior Harbor.

Methods

Two series of facility blank tests were conducted at the Great Ships Initiative land-based ballast treatment system test site (RDTE) in Duluth-Superior Harbor of Lake Superior in the summer of 2008. The primary objective of the tests was to detect differences in zooplankton densities between the Control (C) and Treatment (T) tracks and between fill and drain sampling locations on and among each given track. Fill water was drawn from the ambient water source (Duluth-Superior Harbor), split, and simultaneously passed through each track and into matched retention tanks. Water was retained in the storage tanks for 18 hours for one set of trials (N=6) and 5 days for a second set of trials (N=4). At the conclusion of the retention period, the matched tanks were drained sequentially, Control tanks first. Samples were collected continuously throughout fill and discharge processes using automated diaphragm valves to control flow. Two replicate samples were collected at each sample location within each test run.

Samples were analyzed within one hour of sample collection. Only zooplankton within taxonomic groups generally greater than 50 um in minimum dimension were included in the analysis. Zooplankton were sorted into live and total status classes.

Mean densities associated with replicate samples at each sample location were averaged, and these averages were compared to averages associated with alternate sample locations and analyzed for significant differences. Appendix A shows the data used in all tests. The statistical procedures were for continuous, normal and paired data because a separate water mass supplied each comparison test, and often there were substantial differences in the kinds and densities of organisms among water masses. Repeated measures ANOVA and the equivalent 2-group paired t-test were performed on the data. Because there were two main factors (control/treatment and fill/drain), the main tests were 2-way repeated measures ANOVA reporting significance of differences associated with control-treatment, fill-drain, and the interaction of the two. Repeated testing often elucidates consistent patterns of results despite sample size differences. When ANOVA

tests showed a significant finding, simple paired t-tests were used to identify the responsible factors.

Results

In all tests, the intake densities of live zooplankton ranged between 10^4 and 10^5 individuals per cubic meter. The data reported here were analyzed for any differential influence of track or sample location on live zooplankton density values. Chart 1 shows the mean densities of live zooplankton at fill and discharge sampling locations by track for each 18 hour trial (N=5). Chart 2 shows the mean densities of live zooplankton at fill and discharge sampling locations by track for each 5 day trial (N=5).

***Chart 1**. Mean densities of live zooplankton at fill and discharge sampling locations by track for each 18 hour trial (N=5).*

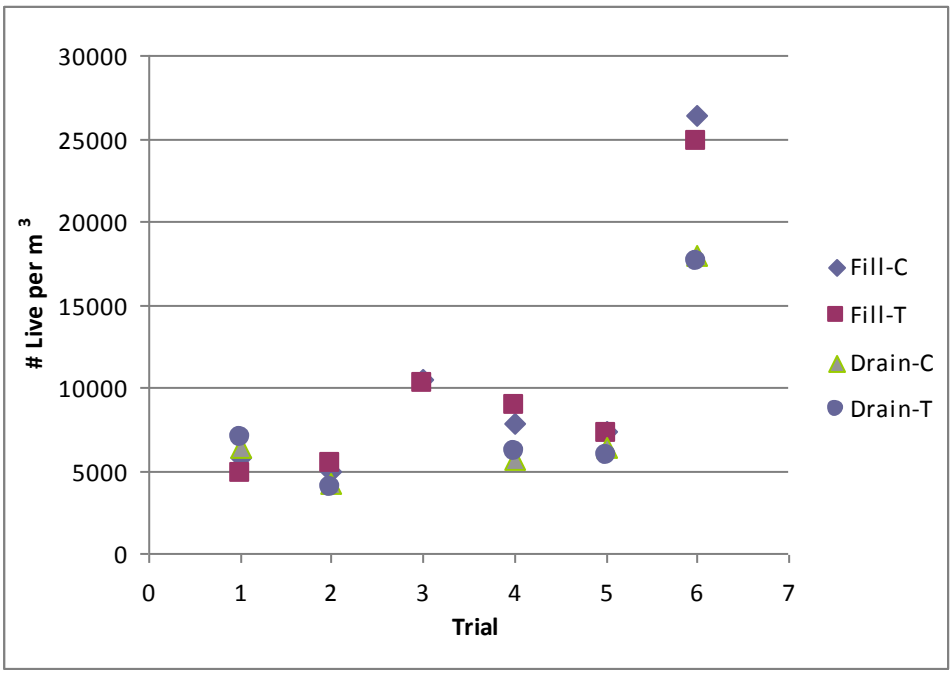
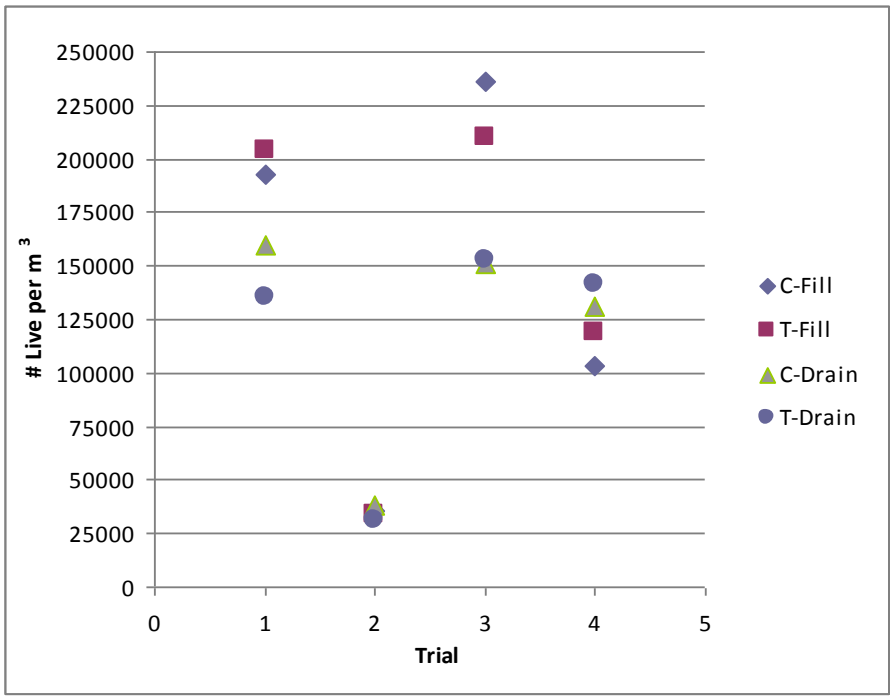


***Chart 2**. Mean densities of live zooplankton at fill and discharge sampling locations by track for each 5 day trial (N=4).*



Control-Track vs. Treatment-Track Intake Streams

Intake zooplankton density values for the matched Control and Treatment tracks from all trials (18 H and 5 D) were combined to evaluate possible differences in C- and T-track intake streams (N=10). Data were analyzed using a paired t-test. Mean densities by track across trials appear in Table 1. The analysis revealed no differences in numbers of live zooplankton in the matched C-track and T-track intake streams.

Table 1. Mean intake densities (#/m^3) by track, and status class, differences in the means and probability of a difference.

Organism class	Fill/Control (mean)	Fill/Treatment (mean)	Difference	P
Live zooplankton	104292	100889	-3403	0.584578
Total zooplankton	207946	198320	-9626	0.27987

Control Track vs. Treatment Track Discharge in an 18 Hour Blank Test

There were N=5 complete 18 hour retention blank trials. Table 2 provides 18 H test results, expressed as mean densities across 18 H facility blank tests. Data are shown in appendix A.

Table 2 shows the results of repeated measures ANOVA tests that compare track, sampling location, and the interaction of the two. All comparisons were clearly non-significant except. In particular, there was no difference in live macro-zooplankton discharge densities in the respective tracks or between intake and discharge from the respective tracks.

Table 2. Probabilities (p) from 18-hour retention facility blank tests for two-way repeated measures ANOVA tests regarding differences among control (C) and treatment (T) track samples at the fill and drain locations for zooplankton densities (#/m^3) with interaction significance. *Data are shown in Appendix A.*

Organism class	Control vs Treatment	Fill vs Drain	C/T & F/D interaction	Findings
Live zoo-plankton	0.9462	0.8485	0.2859	No evidence that there were any differences.
Total zoo-plankton	0.6364	0.7657	0.2350	No evidence that there were any differences.

Control Track vs. Treatment Track Discharge in 5 Day Blank Tests

Chart 2 reports the average densities by track (Control and Treatment), sampling location (fill and drain) of live zooplankton for the 5-day blank tests (N=4). There were generally higher organism densities in intake water than for the 18-hr tests, with mean densities consistently in the 10^5 order of magnitude.

Table 3 shows the results of repeated measures ANOVA tests that compare track, sampling location, and the interaction of the two for the 5-Day blank tests. There were no significant differences by track and sample location.

Table 3. Probabilities (p) from 5-day facility blank tests for two-way repeated measures ANOVA tests regarding differences among control (C) and treatment (T) track samples at the fill and drain locations for zooplankton densities (#/m^3) with interaction significance. Table entries are the same as described for Table 2.

Organism class	Control vs Treatment	Fill vs Drain	C/T & F/D interaction	Findings
Live zoo-plankton	0.7189	0.6543	0.7207	No evidence that there were any differences.
Total zoo-plankton	0.8791	0.8979	0.2874	No evidence that there were any differences.

Summary of Findings

1. Intake and discharge densities of live zooplankton ranged between 10^4 and 10^5 organisms per cubic meter of water.

2. There was no significant attrition (decline in live densities) associated with either track.

3. There were no significant differences in live organism densities between samples retrieved from matched Treatment and Control *intakes* at the GSI land-based test facility.

4. There were no significant differences in organism densities between samples retrieved from the facility's C-track and T-tracks discharge after either a 5 day or more abbreviated 18 hour holding time.

Discussion

Overall, no facility biases were detected relative to live densities of organisms, the most critical cohort for treatment testing. Further, no facility biases were detected in zooplankton densities irrespective of status (live or dead).

Appendix A

Data: Zooplankton density (#/m3); Average of sub-samples.
18 Hour retention tests

Group	Test ID	Location	Control	Treatment
Zoopl Live	18 H1	Fill	56257	83334
	18 H2	Fill	22445	22149
	18 H3	Fill	97475	93592
	18 H4	Fill	67704	52324
	18 H5	Fill	29935	24014
	18 H6	Fill	202212	166637
	18 H1	Drain	61465	57359
	18 H2	Drain	16935	23703
	18 H3	Drain		
	18 H4	Drain	36802	54809
	18 H5	Drain	38010	31372
	18 H6	Drain	176983	193021
Zoopl TOAL	18 H1	Fill	138079	179328
	18 H2	Fill	45569	39578
	18 H3	Fill	212835	195858
	18 H4	Fill	129822	93025
	18 H5	Fill	55401	52143
	18 H6	Fill	392291	387668
	18 H1	Drain	148430	153455
	18 H2	Drain	32103	44128
	18 H3	Drain		
	18 H4	Drain	84314	120705
	18 H5	Drain	66054	60851
	18 H6	Drain	324426	337790

Data: Zooplankton density (#/m3); Average of sub-samples
5 Day retention tests

Group	Test ID	Location	Control	Treatment
Zooplank Live	5D1	Fill	192534	203817
	5D2	Fill	35181	33828
	5D3	Fill	236000	209859
	5D4	Fill	103180	119333
	5D1	Drain	159361	135445
	5D2	Drain	38213	31633
	5D3	Drain	150708	152500
	5D4	Drain	131163	141834
Zooplank ALL	5D1	Fill	385408	374856
	5D2	Fill	78508	73979
	5D3	Fill	462286	401792
	5D4	Fill	179261	184968
	5D1	Drain	301380	266276
	5D2	Drain	72368	70468
	5D3	Drain	387906	409160
	5D4	Drain	224703	293596