

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 Sample Collection Date: January 17, 2017

Prepared for: IDE AMERICAS, Inc.

4590 Carlsbad Boulevard Carlsbad, CA 92008

Prepared by: Nautilus Environmental

Submitted: February 7, 2017

Data Quality Assurance:

- Nautilus Environmental is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053). It is also certified by the State of California Department of Health Services Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

California

4340 Vandever Avenue San Diego, California 92120 858.587.7333 fax: 858.587.3961

Results verified by: __

EXECUTIVE SUMMARY

MONTHLY CHRONIC TOXICITY TESTING CARLSBAD DESALINATION PLANT – JANUARY 2017 ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: January 17, 2017

Test Date: January 18, 2017

Sample ID: M-001 Brine Effluent (weekly accelerated)

Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type:			Effluent Limitation Met? (Yes/No)
	NOEC	<u>TU</u> c	
Echinoderm Fertilization	2.5	40	No

INTRODUCTION

A 24-hour composite discharge sample was collected in January 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) to satisfy monthly monitoring requirements according to the permit that was adopted in 2006 (Order No. R9-2006-0065). Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on January 18, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

Client: IDE Americas, Inc.

Sample Collection Date: January 17, 2017

MATERIALS AND METHODS

The sample was collected on January 17, 2017. Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was hand delivered to Nautilus the day of collection. Following arrival at Nautilus, an aliquot of the sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocol described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project: IDE Americas, Inc./ Carlsbad Desalination Plant

Monitoring Period: January 2017 (M-001 monthly monitoring)

Sample ID, Material: M-001, desalination plant brine effluent

Sample Collection Date, Time: 01/17/17, 09:00

Sample Receipt Date, Time: 01/17/17, 11:53

Sampling Method: 24-hour Composite

Table 2. Water Quality Measurements upon Sample Receipt

Sample ID	рН	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as CaCO₃)	Total Chlorine (mg/L)
M-001	7.89	6.2	2.0	63.0	118	0.06

TOXICITY SUMMARY REPORT Test ID: 1701-S139 to S140, and S257

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times: 01/18/17, 15:19 through 15:59

Test Organism: Strongylocentrotus purpuratus (purple sea urchin)

Test Organism Source: Field-collected locally (off Point Loma in San Diego, CA)

Lab Control/Dilution Water: Natural seawater (source: Scripps Institution of Oceanography inlet), 34 ±

2 parts per thousand (ppt); 20-µm filtered

Test Concentrations: 2.5, 5.0, 6.06, 10, and 15 percent unadjusted M-001 sample; lab control.

The same dilution series was also tested with the M-001 after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge (EPS) and CDP discharges). The 10 percent M-001 dilution was also tested with the pH10/0.45 μm filtration toxicity identification evaluation (TIE) treatment.

Client: IDE Americas, Inc.

Sample Collection Date: January 17, 2017

Number of Replicates, Organisms

per Replicate:

5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined

before each test with a preliminary rangefinding test.

Test Chamber Type, Volume per

Replicate:

Glass scintillation vial containing 10 mL of test solution

Protocol Used: EPA/600/R-95/136, 1995 West Coast Marine Chronic

Test Type: Fertilization; 20-min sperm exposure to effluent followed by a 20-min egg

fertilization period

Acceptability Criteria: Mean fertilization ≥70% in the control, and percent minimum significant

difference (PMSD) value <25%

Reference Toxicant Testing: Copper chloride

Statistical Analysis Software: CETIS™, version 1.8.7.20

Statistical analyses were conducted using EPA flowchart specifications as outlined in the test guidance manual (USEPA 1995). Organism performance in each sample dilution series was compared to that observed in the laboratory control exposure. Results were used to calculate the No Observed Effect Concentration (NOEC) and chronic toxic unit (TU_c) values.

In addition to EPA flowchart statistical methods, the results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollution Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA, 2010). Notably, the California State Water Resources Control Board (SWRCB) published a Draft Policy for Toxicity Assessment and Control (SWRCB, 2012), which includes the TST as an alternative method to evaluate toxicity data. This approach applies a modified t-test that takes into account both the statistical power of the test and the magnitude of biological effects in determining the presence of a response. For this sample, the in-stream waste concentration (IWC) is 6.06 percent unadjusted effluent, and results are reported as "Pass" if a sample is considered non-toxic according to the TST calculation, or "Fail" if considered toxic according to the TST. As the TST is not included in the CDP permit at this time, the TST analysis is for comparison purposes only.

RESULTS

There was a statistically significant decrease in fertilization rate in the 5.0, 6.06, 10, and 15 percent concentrations of the unadjusted M-001 sample compared to the lab control, resulting in a NOEC of 2.5 percent effluent and a TU_c equal to 40. This exceeds the maximum daily permit effluent limitation of 16.5 TU_c . The 6.06 percent concentration (IWC) resulted in a 9.6 percent effect compared to the lab control, which was not significantly significant using to the TST calculation.

Client: IDE Americas, Inc.

Sample Collection Date: January 17, 2017

The M-001 sample adjusted to 40 ppt prior to dilution preparation resulted in no statistically significant effects in any of the test concentrations and a TU_c less than 6.67. Statistical results for urchin fertilization toxicity tests are summarized in Table 4, and detailed test results are summarized in Table 5. Raw test data and full statistical analyses can be found in Appendix A. Sample receipt information and a copy of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for M-001 Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC ₅₀ (% sample)	TU _c value (toxic units)	TST Result (Pass/Fail)	Percent Effect
M-001 (unadjusted)	2.5	5.0	>15	40	Pass	9.6
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	2.2

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

 EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms

 $TU_c = Chronic Toxic Unit: 100 \div NOEC$

TST: Pass = sample is non-toxic at the IWC according to the TST calculation; Fail = sample is toxic at the IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only.

Percent effect (PE) from control is calculated as: PE= ((mean response in control-mean response in the IWC)/mean response in control) *100. A negative PE results when organism performance in the sample is greater than that in the control.

Table 5. Detailed Results of Purple Urchin Fertilization Testing for the M-001 Sample

Test Concentration	M-001 l	Jnadjusted Sample	M-001 40 ppt Adjusted ^a			
(% Sample)	Salinity Mean Percent (ppt) Fertilization		Salinity (ppt)	Mean Percent Fertilization		
Lab Control	33.5	87.4	33.4	93.2		
2.5	34.3	84.6	33.7	93.2		
5.0	35.0	77.4*	33.9	90.2		
6.06	35.4	79.0*	34.0	91.2		
10	36.5	67.4*	34.3	89.8		
15	37.9	65.8*	34.6	89.4		

^a For comparison to the M-001 unadjusted sample, the M-001 sample was adjusted with seawater to 40 ppt for this concentration series prior to preparing test concentrations. The 100 percent sample in this series represents 40 ppt adjusted brine effluent.

^{*}An asterisk indicates a statistically significant decrease compared to the lab control using the standard USEPA flowchart statistical method (EPA 1995).

presented in Figure 1, and raw datasheets are in Appendix A.

As part of the ongoing toxicity reduction evaluation and toxicity identification evaluation (TRE/TIE) investigations, the M-001 sample was also tested with the pH 10/filtration TIE treatment. The treatment was performed on the 10 percent sample concentration in order to obtain the highest testable concentration to discern differences between treated and untreated sample while also not having the confounding effect of elevated salinity above the organism tolerance level. Salinity measured in the 10 percent M-001 sample was 37.9 ppt; below the salinity tolerance limit for this organism and test procedure (based on Philips et al. 2012, and internal studies at Nautilus). Results for the sample tested with and without the TIE treatments are

Client: IDE Americas, Inc.

Sample Collection Date: January 17, 2017

The pH 10/filtration treatment improved fertilization over the untreated baseline sample. This response is consistent with previous M-001 samples tested using this treatment. Subsamples of the effluent before and after treatment were submitted to Weck Laboratories for chemical analysis. Those data are pending and will be reported separately.

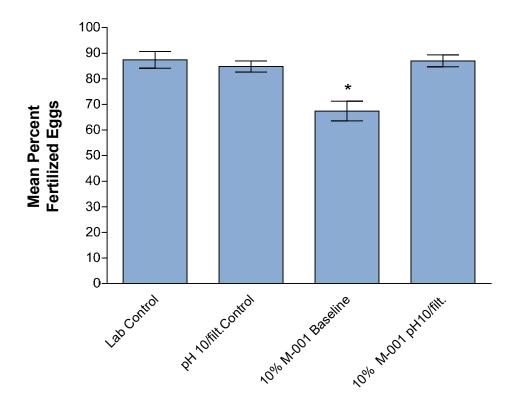


Figure 1. Summary of Urchin Fertilization Results for the M-001 Sample with and without the pH10/filtration TIE treatment (Mean \pm 1SD). *An asterisk indicates a statistically significant decrease compared to the lab control.

QUALITY ASSURANCE

The sample was received on the same day as collection and within the appropriate temperature range. The test was initiated within the allowable holding time of 36 hours. The laboratory controls met the minimum acceptability criteria as set by USEPA. The PMSD values, which are a measure of test variability, were within the acceptable range. Therefore, all test results were deemed valid for reporting purposes.

Client: IDE Americas, Inc.

Sample Collection Date: January 17, 2017

Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to evaluate reliability of the results. Additionally, appropriate alpha levels were used for statistical analyses according to the TST Implementation Document guidelines (USEPA 2010).

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 6 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. Additionally, the median effect concentration (EC_{50} value) was within two standard deviations (SD) of the historical mean, indicating typical test organism sensitivity to copper. A list of qualifier codes used on bench datasheets can be found in Appendix E.

Table 6. Reference Toxicant Test Results

Test Species	Endpoint	EC ₅₀ (μg/L Copper)	Historical Mean EC ₅₀ ±2 SD (μg/L Copper)	CV (%)
Purple Urchin	Fertilization	19.7	39.3 ± 21.4	27.2

 EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms Historical Mean $EC_{50} \pm 2$ SD = Mean of historical test results plus or minus two standard deviations CV = Coefficient of Variation

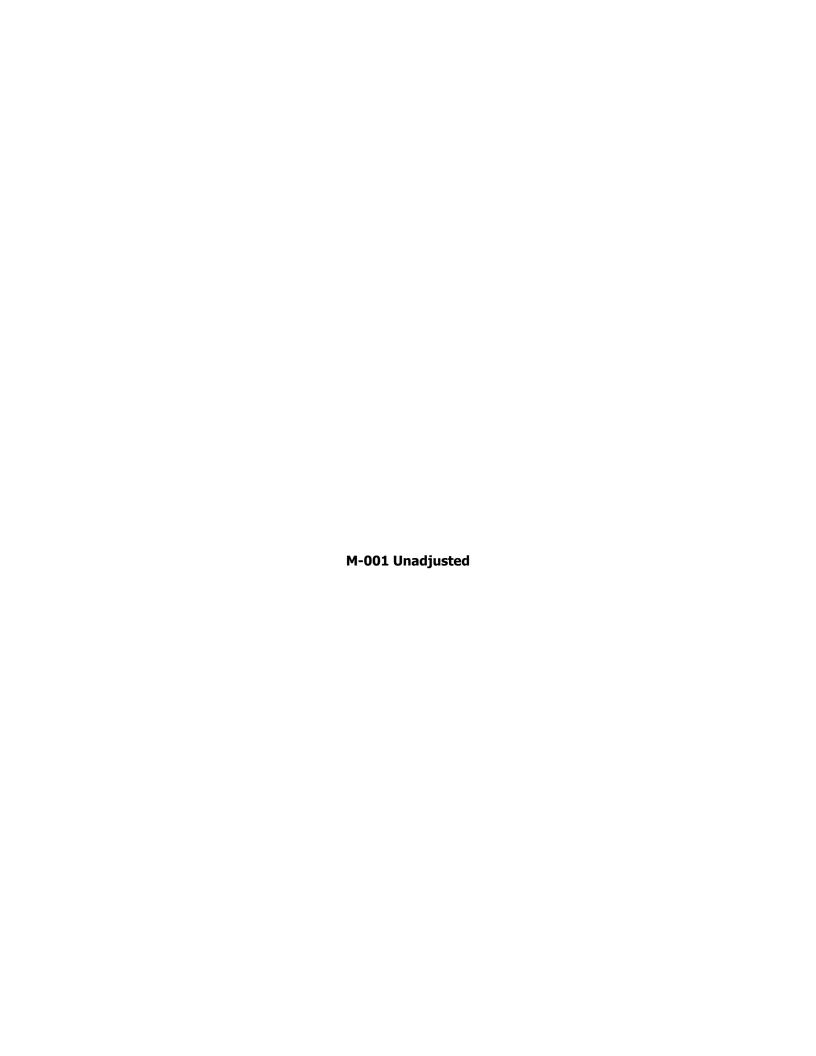
TOXICITY SUMMARY REPORT Client: IDE Americas, Inc.
Test ID: 1701-S139 to S140, and S257 Sample Collection Date: January 17, 2017

REFERENCES

- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.
- Tidepool Scientific Software. 2000-2013. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20
- USEPA. 1991. Methods for Aquatic Toxicity Identification Evaluation Phase I Toxicity Characterization Procedures, 2nd Edition, EPA/600/6-91/003 February 1991.
- USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.
- USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Appendix A

Test Data and Statistical Analyses



CETIS Summary Report

Report Date:

26 Jan-17 12:10 (p 1 of 1)

oe no can	illary repe	,,,,						Test Code:		170	1-S139 20	0-4257-1769
Echinoid Sper	rm Cell Fertiliza	tion Te	st 15C							Nautilus	s Environn	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	03-4943-9750 18 Jan-17 15:1 18 Jan-17 15:5 40m		Test Type: Protocol: Species: Source:	Fertilization EPA/600/R-95 Strongylocentr Pt. Loma		tus		Analyst: Diluent: Brine: Age:		ural Seawate Applicable	эr	
· ·	12-5258-2404 17 Jan-17 09:0 17 Jan-17 11:5 30h (2°C)		Code: Material: Source: Station:	17-0059 Facility Effluer IDE Americas, M-001 (Unadju	, Inc.			Client: Project:	IDE Carl	sbad Desal	Plant	
Comparison S	Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Meth	nod			
05-8210-1401	Fertilization Ra	te	2.5	5	3.536	5.06%	40	Duni	nett N	lultiple Com	parison T e	st
Point Estimate	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Meth	nod			
08-4886-5148	Fertilization Ra	te	EC25 EC50		N/A N/A	N/A N/A	<6.6 <6.6		ar Inte	erpolation (I	CPIN)	
Test Acceptab	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Lim	its	Ove	rlap	Decision		
05-8210-1401	Fertilization Ra	te	Contr	ol Resp	0.874	0.7 - NL		Yes		Passes A	cceptability	Criteria
08-4886-5148	Fertilization Ra	te	Contr	ol Resp	0.874	0.7 - NL		Yes		Passes A	cceptability	/ Criteria
05-8210-1401	Fertilization Ra	te	PMSI)	0.05056	NL - 0.25		No		Passes A	cceptability	^r Criteria
Fertilization R	ate Summary	ere en										
C-%	Control Type	Coun	ıt Mean	95% LCL	. 95% UCL	Min	Мах	Std	Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.874	0.8332	0.9148	0.84	0.91	0.01	47	0.03286	3.76%	0.0%
2.5		5	0.846		0.8961	0.78	0.89	0.01	806	0.04037	4.77%	3.2%
5		5	0.774	0.7305	0.8175	0.73	0.81	0.01		0.03507	4.53%	11.44%
6.06		5	0.79	0.7609	0.8191	0.77	0.82			0.02345	2.97%	9.61%
10		5	0.674		0.7218	0.63	0.72			0.03847	5.71%	22.88%
15		5	0.658	0.6129	0.7031	0.61	0.71	0.01	625	0.03633	5.52%	24.71%
Fertilization R	ate Detail											
C-%	Control Type	Rep '	1 Rep 2	Rep 3	Rep 4	Rep 5						
0	Lab Control	0.88	0.9	0.91	0.84	0.84						
2.5		0.78	0.86	0.85	0.89	0.85						
5		0.81	0.81	0.76	0.76	0.73						
					0.70	0.77						

6.06

10

15

0.77

0.7

0.65

0.81

0.63

0.67

0.82

0.72

0.61

0.78

0.68

0.71

0.77

0.64

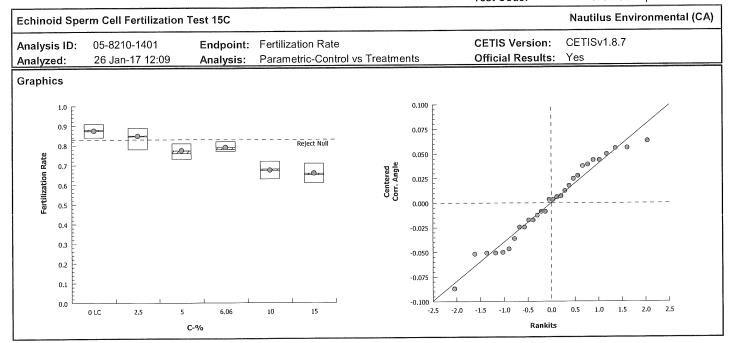
0.65

Report Date: Test Code:

26 Jan-17 12:10 (p 1 of 2) 1701-S139 | 20-4257-1769

	•						Test	Code:	170	-5139 20)-4257-1769
Echinoid Spe	erm Cell Fertiliza	tion Test 1	5C						Nautilus	Environn	nental (CA)
Analysis ID: Analyzed:	05-8210-1401 26 Jan-17 12:0		lpoint: Feri	ilization Rat		ments		S Version: ial Results		8.7	
Data Transfo		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	ΤU
Angular (Corr		NA	C > T	NA	NA		5.06%	2.5	5	3.536	40
Dunnett Mult	tiple Comparisor	n Test									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	2.5		1.477	2.362	0.065 8	0.2316	CDF	Non-Sign	ificant Effect		
	5*		4.893	2.362	0.065 8	0.0001	CDF	Significar	nt Effect		
	6.06*		4.201	2.362	0.065 8	0.0007	CDF	Significar	nt Effect		
	10*		9.013	2.362	0.065 8	< 0.0001	CDF	Significar	nt Effect		
	15*		9.637	2.362	0.065 8	<0.0001	CDF	Significar	nt Effect		
ANOVA Tabl	e										
Source	Sum Squ	ares	Mean Squ	iare	DF	F Stat	P-Value	Decision	ı(a:5%)		
Between	0.282697	8	0.0565395	6	5	30.23	<0.0001	Significa	nt Effect		
Error	0.044890	08	0.0018704	2	24						
Total	0.327587	9			29						
Distribution	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett E	quality of V	ariance	1.632	15.09	0.8974	Equal Var	iances			
Distribution	Shapiro-	Wilk W Nori	mality	0.9647	0.9031	0.4062	Normal D	istribution			
Fertilization	Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.874	0.8332	0.9148	0.88	0.84	0.91	0.0147	3.76%	0.0%
2.5		5	0.846	0.7959	0.8961	0.85	0.78	0.89	0.01806	4.77%	3.2%
5		5	0.774	0.7305	0.8175	0.76	0.73	0.81	0.01568	4.53%	11.44%
6.06		5	0.79	0.7609	0.8191	0.78	0.77	0.82	0.01049	2.97%	9.61%
10		5	0.674	0.6262	0.7218	0.68	0.63	0.72	0.0172	5.71%	22.88%
				0.0400	0.7031	0.65	0.61	0.71	0.01625	5.52%	24.71%
15		5	0.658	0.6129	0.7001	0,00					
	rrected) Transfo			0.6129	0.7001						
	rrected) Transfo			95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Angular (Co		rmed Sumn	nary			Median	Min 1.159	1.266	Std Err 0.02221	4.1%	0.0%
Angular (Co	Control Type	rmed Sumn Count	nary Mean	95% LCL	95% UCL	Median	Min 1.159 1.083	1.266 1.233	Std Err 0.02221 0.02439	4.1% 4.66%	0.0% 3.34%
Angular (Co C-%	Control Type	rmed Sumn Count 5	mary Mean 1.21	95 % LCL 1.148	95% UCL 1.272	Median	Min 1.159 1.083 1.024	1.266 1.233 1.12	Std Err 0.02221 0.02439 0.01882	4.1% 4.66% 3.91%	0.0% 3.34% 11.06%
Angular (Co C-% 0 2.5	Control Type	rmed Sumn Count 5	Mean 1.21 1.17	95% LCL 1.148 1.102	95% UCL 1.272 1.237	Median 1.217 1.173	Min 1.159 1.083	1.266 1.233 1.12 1.133	Std Err 0.02221 0.02439 0.01882 0.01299	4.1% 4.66% 3.91% 2.65%	0.0% 3.34% 11.06% 9.5%
Angular (Co C-% 0 2.5 5	Control Type	rmed Sumn Count 5 5 5	Mean 1.21 1.17 1.076	95% LCL 1.148 1.102 1.024	95% UCL 1.272 1.237 1.129	Median 1.217 1.173 1.059	Min 1.159 1.083 1.024	1.266 1.233 1.12	Std Err 0.02221 0.02439 0.01882	4.1% 4.66% 3.91%	0.0% 3.34% 11.06%

Report Date: Test Code: 26 Jan-17 12:10 (p 2 of 2) 1701-S139 | 20-4257-1769



Report Date:

26 Jan-17 12:10 (p 1 of 1)

Test Code:

1701-S139 | 20-4257-1769

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 08-4886-5148 Analyzed:

26 Jan-17 12:09

Endpoint: Fertilization Rate Analysis:

Linear Interpolation (ICPIN)

CETIS Version: Official Results: Yes

CETISv1.8.7

ı	inear	Interno	lation	Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method

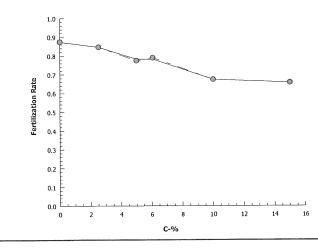
Two-Point Interpolation 263305 1000 Yes Linear Linear

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>15	N/A	N/A	<6.667	NA	NA
EC50	>15	N/A	N/A	<6.667	NA	NA

Fertilizat	tion Rate Summary		Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.874	0.84	0.91	0.0147	0.03286	3.76%	0.0%	437	500
2.5		5	0.846	0.78	0.89	0.01806	0.04037	4.77%	3.2%	423	500
5		5	0.774	0.73	0.81	0.01568	0.03507	4.53%	11.44%	387	500
6.06		5	0.79	0.77	0.82	0.01049	0.02345	2.97%	9.61%	395	500
10		5	0.674	0.63	0.72	0.0172	0.03847	5.71%	22.88%	337	500
15		5	0.658	0.61	0.71	0.01625	0.03633	5.52%	24.71%	329	500

Graphics



TST

Report Date: Test Code: 26 Jan-17 12:10 (p 1 of 1) 1701-S139 | 20-4257-1769

Principal Pri				Ī	> $/$			Test	Code:	170	1-\$139 20	0-4257-1769
Paralyzed April Paralyzed April Paralyzed	Echinoid Spe	erm Cell Fertiliza	ition Test 15	5C						Nautilus	Environn	nental (CA)
Pate	Analysis ID:	13-9351-3806	End	point: Fer	tilization Rat	e					.8.7	
Name	Analyzed:	26 Jan-17 12:0)9 A na	l ysis : Par	ametric Bioe	equivalence-	Two Sample	e Offic	ial Results	Yes		
Note	Data Transfo	rm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Control vs C-% Test Stat Critical MSD VF V-Value P-Type Decision(c:5%) Section (c:5%)	Angular (Corr	ected)	NA	C*b < T	NA	NA	0.75	3.43%	10	15	12.25	10
	TST-Welch's	t Test										
Paris	Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(α:5%)		
	Lab Control	2.5*		8.876	1.895	0.056 7	<0.0001	CDF	Non-Signi	ficant Effect		
NOVAY Table		5*		6.712	1.895	0.048 7	0.0001	CDF	Non-Signi	ficant Effect		
Note		6.06*		8.883	1.895	0.040 7	<0.0001		Non-Signi	ficant Effect		
NOVA Table Source Sum Squ² Sq6878 0.0663956 5 0.0018702 24 0.0018702 25 0.		10*		2.258	1.895	0.047 7	0.0293	CDF	Non-Signi	ficant Effect		
Source Sum Squ² → 0.0282697 → 0.0468908 → 0.0468908 → 0.04		15		1.626	1.895	0.045 7	0.0740	CDF	Significan	t Effect		
Between 0.2826978 0.05653956 5 24 24 Total 0.3275879 0.00187042 24 Total 0.3275879 0.00187042 29 Distributional Tests Test Test Stat Critical P-Value Decision(σ:1%) Variances Bartlett ⊆uality of Variance 1.632 15.09 0.8974 Equal Variances Normal Distribution Shapiro-Vulk W Normal V Normal	ANOVA Table	9										
Error 0.048890 ★ 0.018792 24 Total 0.3275879 ★ 29 Distribution Tests Test Stat Critical P-Value Decision(x:1%) Variances Bartlett Equility of Variance 1.632 15.09 0.8974 Equal Variances Distribution Sampior Variances Distribution Sampior Variances Distribution Sampior Variances Distribution Distribution Test Stat Critical Parameters Distribution State In Interview Intervi	Source	Sum Squ	ares	Mean Squ	iare	DF	F Stat	P-Value	Decision(α:5%)		
Distributional Tests	Between	0.282697	8	0.0565395	66	5	30.23	<0.0001	Significan	t Effect		
Distributional Test	Error	0.044890	08	0.0018704	2	24						
Attribute Test Critical P-Value Decisio(α:1%) Variances Distribution Bartlett ⊆uality of Variance Shapiro-Wilk W Norriality 1.632 15.09 0.8974 Equal Variances Normal Distribution Fertilization Rate Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 2.5 5 0.846 0.7959 0.8961 0.85 0.78 0.91 0.0147 3.76% 0.0% 5 0.874 0.73305 0.8961 0.85 0.78 0.99 0.01406 4.77% 3.2% 5 0.774 0.7305 0.8175 0.76 0.73 0.81 0.01568 4.53% 11.44% 6.06 5 0.79 0.7609 0.8191 0.78 0.77 0.82 0.01049 2.97% 9.61% 10 5 0.674 0.6262 0.7218 0.68 0.63 0	Total	0.327587	9			29						
Variances Distribution Bartlett Equality of Variance Shapiro-Wilk W Normality 1.632 0.9031 15.09 0.8974 0.9031 Equal Variances Normal Distribution Fertilization Rate Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 0.874 0.8332 0.9148 0.88 0.84 0.91 0.0147 3.76% 0.0% 2.5 5 0.846 0.7959 0.8961 0.85 0.78 0.89 0.01806 4.77% 3.2% 5 0.774 0.7305 0.8175 0.76 0.73 0.81 0.01668 4.53% 11.44% 6,06 5 0.674 0.6262 0.7218 0.68 0.63 0.72 0.0172 5.71% 22.88% 15 5 0.658 0.6129 0.7031 0.65 0.61 0.71 0.01625 5.52% 24.71% Angula	Distributiona	l Tests										
Distribution Shapiro-Wilk W Normal Distribut	Attribute	Test			Test Stat	Critical	P-Value	Decision((α:1%)			
Fertilization Rate Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 0.874 0.8332 0.9148 0.88 0.84 0.91 0.0147 3.76% 0.0% 2.5 5 0.846 0.7959 0.8961 0.85 0.78 0.89 0.01806 4.77% 3.2% 5 0.774 0.7305 0.8175 0.76 0.73 0.81 0.01568 4.53% 11.44% 6.06 5 0.79 0.7609 0.8191 0.78 0.77 0.82 0.01049 2.97% 9.61% 10 5 0.674 0.6262 0.7218 0.68 0.63 0.72 0.0172 5.71% 22.88% Angular (Corrected) Transformed Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err<	Variances	Bartlett E	quality of Va	ariance	1.632	15.09	0.8974	Equal Var	iances			
C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 0.874 0.8332 0.9148 0.88 0.84 0.91 0.0147 3.76% 0.0% 2.5 5 0.846 0.7959 0.8961 0.85 0.78 0.89 0.01806 4.77% 3.2% 5 0.774 0.7305 0.8175 0.76 0.73 0.81 0.01568 4.53% 11.44% 6.06 - 5 0.79 0.7609 0.8191 0.78 0.77 0.82 0.01049 2.97% 9.61% 10 - 5 0.674 0.6262 0.7218 0.68 0.63 0.72 0.0172 5.71% 22.88% 15 5 0.658 0.6129 0.7031 0.65 0.61 0.71 0.01625 5.71% 22.88% 6.% Control Type Count Mean	Distribution	Shapiro-	Wilk W Norn	nality	0.9647	0.9031	0.4062	Normal Di	istribution			
0 Lab Control 5 0.874 0.8332 0.9148 0.88 0.84 0.91 0.0147 3.76% 0.0% 2.5 5 0.846 0.7959 0.8961 0.85 0.78 0.89 0.01806 4.77% 3.2% 5 0.774 0.7305 0.8175 0.76 0.73 0.81 0.01568 4.53% 11.44% 6.06 5 0.79 0.7609 0.8191 0.78 0.77 0.82 0.01049 2.97% 9.61% 10 5 0.674 0.6262 0.7218 0.68 0.63 0.72 0.0172 5.71% 22.88% 15 0.658 0.6129 0.7031 0.65 0.61 0.71 0.01625 5.52% 24.71% Angular (Corrected) Transformed Summers C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control <td< td=""><td>Fertilization</td><td>Rate Summary</td><td></td><td></td><td></td><td>The state of the s</td><td></td><td></td><td></td><td></td><td></td><td></td></td<>	Fertilization	Rate Summary				The state of the s						
2.5	C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
5 0.774 0.7305 0.8175 0.76 0.73 0.81 0.01568 4.53% 11.44% 6.06 5 0.79 0.7609 0.8191 0.78 0.77 0.82 0.01049 2.97% 9.61% 10 5 0.674 0.6262 0.7218 0.68 0.63 0.72 0.0172 5.71% 22.88% 15 5 0.658 0.6129 0.7031 0.65 0.61 0.71 0.01625 5.52% 24.71% Angular (Corrected) Transformed Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 1.21 1.148 1.272 1.217 1.159 1.266 0.02221 4.1% 0.0% 2.5 5 1.076 1.024 1.129 1.059 1.083 1.233 0.02439 4.66% 3.34% 5	0	Lab Control	5	0.874	0.8332	0.9148	0.88	0.84	0.91	0.0147	3.76%	0.0%
6.06 5 0.79 0.7609 0.8191 0.78 0.77 0.82 0.0149 2.97% 9.61% 10 5 0.674 0.6262 0.7218 0.68 0.63 0.72 0.0172 5.71% 22.88% 15 5 0.658 0.6129 0.7031 0.65 0.61 0.71 0.01625 5.52% 24.71% Angular (Corrected) Transformed Summary Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 1.21 1.148 1.272 1.217 1.159 1.266 0.02221 4.1% 0.0% 2.5 5 1.17 1.102 1.237 1.173 1.083 1.233 0.02439 4.66% 3.34% 5 1.076 1.024 1.129 1.059 1.024 1.12 0.01882 3.91% 11.06% 6.06 5	2.5		5	0.846	0.7959	0.8961	0.85	0.78	0.89	0.01806	4.77%	3.2%
10	5		5	0.774	0.7305	0.8175	0.76	0.73	0.81	0.01568	4.53%	11.44%
15 5 0.658 0.6129 0.7031 0.65 0.61 0.71 0.01625 5.52% 24.71% Angular (Corrected) Transformed Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 1.21 1.148 1.272 1.217 1.159 1.266 0.02221 4.1% 0.0% 2.5 5 1.17 1.102 1.237 1.173 1.083 1.233 0.02439 4.66% 3.34% 5 1.076 1.024 1.129 1.059 1.024 1.12 0.01882 3.91% 11.06% 6.06 5 1.095 1.059 1.131 1.083 1.071 1.133 0.01299 2.65% 9.5% 10 5 0.9636 0.9126 1.015 0.9695 0.9169 1.013 0.01837 4.26% 20.37%	6.06		5	0.79	0.7609	0.8191	0.78	0.77	0.82	0.01049	2.97%	9.61%
Angular (Corrected) Transformed Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 1.21 1.148 1.272 1.217 1.159 1.266 0.02221 4.1% 0.0% 2.5 5 1.17 1.102 1.237 1.173 1.083 1.233 0.02439 4.66% 3.34% 5 1.076 1.024 1.129 1.059 1.059 1.024 1.12 0.01882 3.91% 11.06% 6.06 5 1.095 1.059 1.131 1.083 1.071 1.133 0.01299 2.65% 9.5% 10 5 0.9636 0.9126 1.015 0.9695 0.9169 1.013 0.01837 4.26% 20.37%	10		5	0.674	0.6262	0.7218	0.68	0.63	0.72	0.0172	5.71%	22.88%
C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 1.21 1.148 1.272 1.217 1.159 1.266 0.02221 4.1% 0.0% 2.5 5 1.17 1.102 1.237 1.173 1.083 1.233 0.02439 4.66% 3.34% 5 1.076 1.024 1.129 1.059 1.024 1.12 0.01882 3.91% 11.06% 6.06 5 1.095 1.059 1.131 1.083 1.071 1.133 0.01299 2.65% 9.5% 10 5 0.9636 0.9126 1.015 0.9695 0.9169 1.013 0.01837 4.26% 20.37%	15		5	0.658	0.6129	0.7031	0.65	0.61	0.71	0.01625	5.52%	24.71%
0 Lab Control 5 1.21 1.148 1.272 1.217 1.159 1.266 0.02221 4.1% 0.0% 2.5 5 1.17 1.102 1.237 1.173 1.083 1.233 0.02439 4.66% 3.34% 5 1.076 1.024 1.129 1.059 1.024 1.12 0.01882 3.91% 11.06% 6.06 5 1.095 1.059 1.131 1.083 1.071 1.133 0.01299 2.65% 9.5% 10 5 0.9636 0.9126 1.015 0.9695 0.9169 1.013 0.01837 4.26% 20.37%	Angular (Cor	rected) Transfor	med Summ	ary								
2.5 5 1.17 1.102 1.237 1.173 1.083 1.233 0.02439 4.66% 3.34% 5 5 1.076 1.024 1.129 1.059 1.024 1.12 0.01882 3.91% 11.06% 6.06 5 1.095 1.059 1.131 1.083 1.071 1.133 0.01299 2.65% 9.5% 10 5 0.9636 0.9126 1.015 0.9695 0.9169 1.013 0.01837 4.26% 20.37%	C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
5 1.076 1.024 1.129 1.059 1.024 1.12 0.01882 3.91% 11.06% 6.06 5 1.095 1.059 1.131 1.083 1.071 1.133 0.01299 2.65% 9.5% 10 5 0.9636 0.9126 1.015 0.9695 0.9169 1.013 0.01837 4.26% 20.37% 10 2 0.01837 0.01837 0.01837 0.01837 0.01837 0.01837	0	Lab Control	5	1.21	1.148	1.272	1.217	1.159	1.266	0.02221	4.1%	
5 1.076 1.024 1.129 1.059 1.024 1.12 0.01882 3.91% 11.06% 6.06 5 1.095 1.059 1.131 1.083 1.071 1.133 0.01299 2.65% 9.5% 10 5 0.9636 0.9126 1.015 0.9695 0.9169 1.013 0.01837 4.26% 20.37% 0.0129 0.0129 0.0129 0.0129 0.0129 0.0129 0.0129 0.0129	2.5		5	1.17	1.102	1.237	1.173	1.083	1.233	0.02439	4.66%	3.34%
10 5 0.9636 0.9126 1.015 0.9695 0.9169 1.013 0.01837 4.26% 20.37%	1		5	1.076	1.024	1.129	1.059	1.024	1.12	0.01882	3.91%	11.06%
0.0000 4.000 0.0470 4.000/ 0.4700/	6.06		5	1.095	1.059	1.131	1.083	1.071	1.133	0.01299		
15 5 0.9466 0.8988 0.9943 0.9377 0.8963 1.002 0.0172 4.06% 21.78%	10		5	0.9636	0.9126	1.015	0.9695	0.9169	1.013			
	15		5	0.9466	0.8988	0.9943	0.9377	0.8963	1.002	0.0172	4.06%	21.78%

Report Date:

16 Jan-17 11:39 (p 1 of 1)

Test Code: 1701 -5/39 20-4257-1769/79BF2BF9

Nautilus Environmental (CA)

Echinoid Spe	erm C	ell Fe	rtilizat	tion Test 150	;		Nautilus Environmental (CA)
Start Date: End Date: Sample Date	18 .	Jan-17 Jan-17 Jan-17	,	Species: Strongyloce Protocol: EPA/600/R- Material: Facility Efflu			4AA8E404 17-0059 IDE Americas, Inc. M-001 (Unadjusted)
C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes	
			61	100	82	1/19/17	•
			62	100	71	1	
			63	100	81		
			64	100	91		
			65	601	65		
			66	100	81		
			67	100	81 70		
			68	100	85		
			69	/00	89		
			70	100	86		
			71	100	76		
			72	100	77		
			73	/00	63 65		
			74	/00	65	 	
			75	100	77		
			76	100	73		
			77	100	81		
			78	/60	25		
			79	/ 00	90		
	-		80	/00	68 88		
			81	100	88		
			82	100	72 78	 	
			83	169	78		
		-	84	/00	64		
			86	100	84		
		-	87	100	61		
	-	-	88	/00	67		
	-		89	100	76		
			90	100 3	84 84 20		

3 918 SG 1/19/17 B QNB FB 1/25/17

Report Date:

16 Jan-17 11:39 (p 1 of 1)

Test Code: 1701-513 q 20-4257-1769/79BF2BF9

Echinoid Spe	erm C	ell Fei	rtiliza	tion Test 150	3		Nautilus Environmental (C
Start Date: End Date: Sample Date	nd Date: 18 Jan-17 Protocol: EPA/600/R-95/136 ample Date: 18 Jan-17 Material: Facility Effluent		· ·	Sample Code: 4AA8E404 17-005 (Sample Source: IDE Americas, Inc. Sample Station: M-001 (Unadjusted)			
C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes
0	LC	1	81		www.		
0	LC	2	79				
0	LC	3	64	100	94	EG 1/18/17	
0	LC	4	89				
0	LC	5	85				
2.5		1	83	100	74	AC1/18/17	
2.5		2	70				
2.5		3	68				
2.5		4	69				
2,5		5	78		100000		
5		1	63	100	83	Ac 1/18/17	
5		2	66			7911/	
5		3	88				
5		4	71				
5		5	76				
6.06		1	75				
6.06		2	77	100	86	EL	
6.06		3	61		30		
6.06		4	90				
6.06		5	72				
10		1	67	100	74	EG	
10		2	73	100	73	724	
10		3	82	100	71	ES	
10		4	80	100	79	EG	
10		5	84	100	66	EG	
15		1	74				
15		2	87				
15	1	3	86				

QCEG @ QIBKB1/25/17

62

15

15

Water Quality Measurements

Client: Test Species: S. purpuratus

Sample ID: M-001 (unadjusted) Start Date/Time: 1/18/2017 | 51 9

Sample Log No.: 17- 0069 End Date/Time: 1/18/2017 1559

			Analyst:	RH
		Initial R		
Concentration	DO	рН	Salinity	Temperature (°C)
%	(mg/L)	(units)	(ppt)	
Lab Control	8.5	8.07	33.5	14.9
2.5	8.4	8.07	34.3	15.0
5.0	8.4	8.07	35.0	15.0
6.06	8.4	8.07	35.4	15.0
10	8.4	8.07	36.5	15.0
15	8.4	8.07	37.9	15.2

Comments:		
	m\	as plate
QC Check:	V8112511	Final Review: A D 7 1 7

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Test Species: S. purpuratus Client: IDE/ CDP

Start Date/Time: 1/18/2017 Project ID: M-001 pH 10/filt.

Test No.: 1701-\$1396 **End Date/Time:** 1/18/2017

Sample ID	Total # Counted	# Fertilized	Technician Initials
pH 10 Control A	100	85	SG 1/19/17
pH 10 Control B	100	84	
pH 10 Control C	100	85	
pH 10 Control D	100	88	
pH 10 Control E	/00	<u>88</u> 82	
pH 10 M-001 A	100	88	
pH 10 M-001 B	/00	85	
рН 10 М -001 С	l∞	89 84	
pH 10 M-001 D	100	84	
pH 10 M-001 E	/∞	89	Y

QC Check: 18 13511

Final Review: AC 2/1/17

Water Quality Measurements

Marine Chronic E	oluassay				AAGC: GUAII	ty measurements
Client :	IDE/ CDP		-	Test Species:	S. purpuratus	
Sample ID:	M-001		_	Start Date/Time:	1/18/2017	519
Sample Log No.:	17-0059		_	End Date/Time:	1/18/2017	559
Dilutions made by:	AC, EG		-	Test No:	1701-8	3918Ac2/1/17
				Analyst:	RH	
	Γ		Initial	Readings	L	
	Concentration 10% Treatment	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)	
	pH10/filt. Control	6.2	8.17	33.4	15.4	
	pH 10/filt. 10% M-001	61	8.06	36.1	15.3	
			1			
Comments:						
QC Check:	A 217/17		_	Final Review:	w 21	7/17

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Echinoderm Sperm-Cell Fertilization Worksheet

Client:	IPE					Start Date	e/Time: _1	/18/2017 /	519
Sample ID:	M-001	conadi.	<u> </u>			End Date	e/Time: _1	/18/2017 / 15	559
Test No.:	1701-51	39				Sp	oecies:	S. purpur	atus
	<i>y</i> :	•				Animal S	ource: _	Pt. Loma	
Tech initials:	Ely					Date Col	lected:	12/20/16	
Injection Time:	1440							, 1	
Sperm Absorbance at 40	0 nm:	854	(target range of	f 0.8 - 1.0) for density	of 4x10 ⁶ spe	rm/ml)		
Eggs Counted:	_8 ⁵	Mean:_	<u> 30.6</u> x	50 =	4030	_eggs/ml			
	76	_							
	93	, ,	ounts of 80 eggs ide for a final der	•		edgwick-			
	73	- Nation Si	ide for a fillar der	1151ty 01 40	oo eggs/iii)				
	76								
	4020		- (A): 111		.		(è)		
Initial density:		eggs/ml		ution fact		egg stock		ml	
Final density:	4000	eggs/ml	- 1.0 pa	irt egg sto	ock	seawater	-(A)	ml	

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

parts seawater

				Sperm:	:Egg Ratio			
Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	_50:1_
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1448 1458 1508	Ra	ngefinder Ra 50: 1 100: 1 200: 1 400: 1	7	8 22 14/94 6 19 1	fert.		

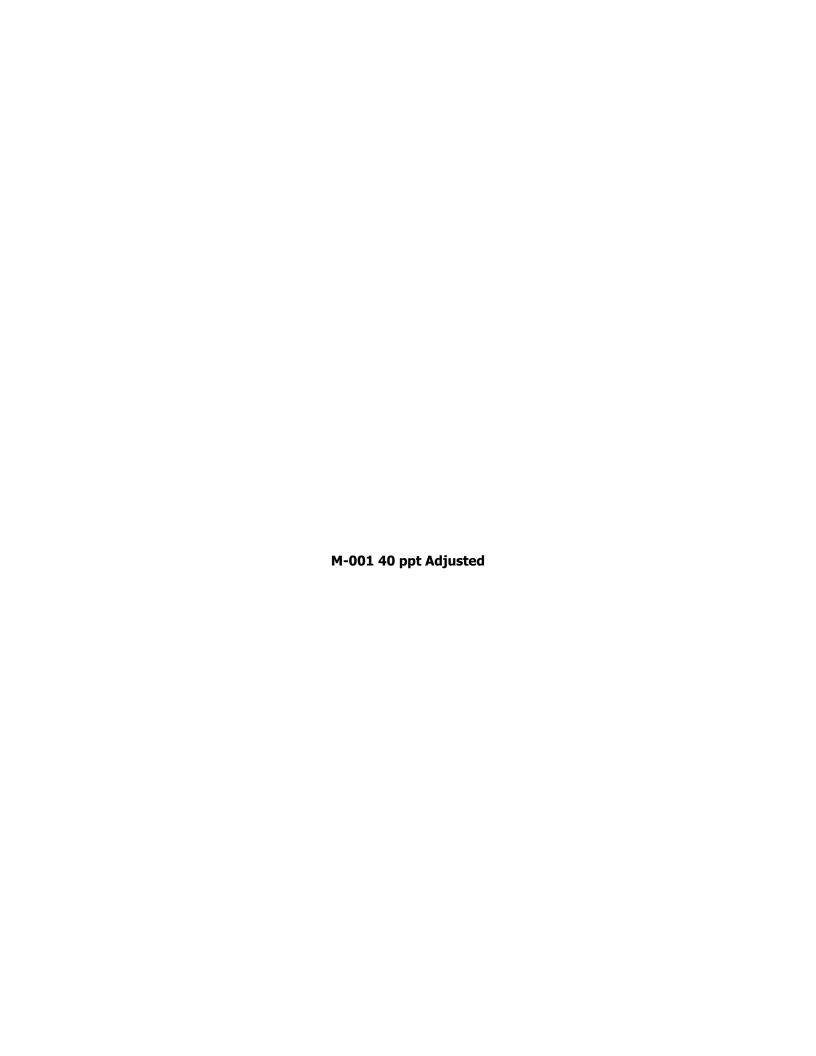
<u>NOTE</u>: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test		Sperm:Egg Ratio Use	ed:	_ \		
Sperm Added (100 μl): Eggs Added (0.5 ml): Test Ended:	Time 1519 1559	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 71 92 0 0	Unfert.		
Comments:	M No Pilution	Needed				
					Δ / 1	

QC Check:

48 1/5/17

Final Review: AC 2/1/17



CETIS Summary Report

Report Date:

26 Jan-17 12:06 (p 1 of 1)

Test Code:

1701-S140 | 14-4621-9388

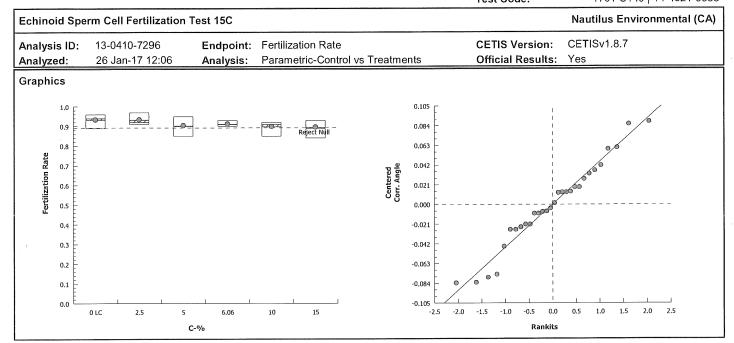
								rest code.			
Echinoid Spe	rm Cell Fertiliza	tion Tes	st 15C						Nautilus ———	s Environn	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	12-7726-4590 18 Jan-17 15:19 18 Jan-17 15:59 40m	9 ! 9 !	Fest Type: Fertilization Protocol: EPA/600/R-95/136 (1995) Species: Strongylocentrotus purpuratus Source: Pt. Loma				tural Seawate Applicable	er			
-			iode: 17-0059 Iaterial: Facility Effluent Iource: IDE Americas, Inc. Itation: M-001 (40 ppt adj)			Client: IDE Project: Car	: Isbad Desal	Plant			
Comparison S	Summary										
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Method			
13-0410-7296	Fertilization Ra	te	15	>15	NA	A 4.28% < 6.66		7 Dunnett N	Multiple Com	parison Te	st
Test Acceptal	oility								popular de la companya del companya de la companya del companya de la companya de		
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Li	mits	Overlap	Decision		
				ol Resp	0.932	0.7 - NL		Yes	Passes A	cceptability	Criteria
	Fertilization Rate		PMSE	•	0.04281	NL - 0.2	5	No	Passes A	cceptability	[,] Criteria
Fertilization R	Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.932	0.8999	0.9641	0.89	0.96	0.01158	0.02588	2.78%	0.0%
2.5		5	0.932	0.9024	0.9616	0.91	0.97		0.02387	2.56%	0.0%
5		5	0.902	0.856	0.948	0.85	0.95	0.01655	0.03701	4.1%	3.22%
6.06		5	0.912	0.8958	0.9282	0.9	0.93	0.005831	0.01304	1.43%	2.15%
10		5	0.898	0.8635	0.9325	0.85	0.92	0.01241	0.02775	3.09%	3.65%
15		5	0.894	0.8505	0.9375	0.84	0.93	0.01568	0.03507	3.92%	4.08%
Fertilization R	Rate Detail										
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.93	0.94	0.96	0.89	0.94					
2.5		0.92	0.94	0.92	0.91	0.97					
5		0.92	0.85	0.89	0.9	0.95					
6.06		0.9	0.92	0.93	0.9	0.91					
				0.04	0.0	0.02					
10		0.85	0.91	0.91	0.9	0.92					

Report Date: Test Code:

26 Jan-17 12:06 (p 1 of 2) 1701-S140 | 14-4621-9388

							rest	Code:	170	1-0140 1-	4-4021-9300
Echinoid Sp	erm Cell Fertiliza	ation Test 1	5C						Nautilus	Environn	nental (CA)
Analysis ID: Analyzed:	13-0410-7296 26 Jan-17 12:0		dpoint: Fer alysis: Par	tilization Rat ametric-Con		tments		S Version:		8.7	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	ΤU
Angular (Corr		NA	C > T	NA	NA		4.28%	15	>15	NA	6.667
Dunnett Mult	tiple Comparisor	n Test									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(a:5%)		
Lab Control	2.5		-0.01504	2.362	0.074 8	0.8377	CDF	Non-Sign	ificant Effect		
	5		1.702	2.362	0.074 8	0.1646	CDF	Non-Sign	ificant Effect		
	6.06		1.276	2.362	0.074 8	0.3042	CDF	Non-Sign	ificant Effect		
	10		1.993	2.362	0.074 8	0.1005	CDF	_	ificant Effect		
	15		2.162	2.362	0.074 8	0.0737	CDF		ificant Effect		
ANOVA Tabl	e										
Source	Sum Squ	ares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	0.023387	34	0.0046774	168	5	1.892	0.1333	Non-Sign	ificant Effect		
Error	0.059344	47	0.0024726	886	24						
Total	0.082731	81			29						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett E	quality of V	'ariance	3.447	15.09	0.6314	Equal Var	iances			
Distribution	Shapiro-	Wilk W Nor	mality	0.9652	0.9031	0.4169	Normal D	istribution			
Fertilization	Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.932	0.8999	0.9641	0.94	0.89	0.96	0.01158	2.78%	0.0%
2.5		5	0.932	0.9024	0.9616	0.92	0.91	0.97	0.01068	2.56%	0.0%
5		5	0.902	0.856	0.948	0.9	0.85	0.95	0.01655	4.1%	3.22%
6.06		5	0.912	0.8958	0.9282	0.91	0.9	0.93	0.005831	1.43%	2.15%
10		5	0.898	0.8635	0.9325	0.91	0.85	0.92	0.01241	3.09%	3.65%
15		5	0.894	0.8505	0.9375	0.89	0.84	0.93	0.01568	3.92%	4.08%
Angular (Co	rrected) Transfor	rmed Sumn	nary								
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.31	1.249	1.372	1.323	1.233	1.369	0.02226	3.8%	0.0%
2.5		5	1.311	1.246	1.376	1.284	1.266	1.397	0.02342	4.0%	-0.04%
5		5	1.257	1.178	1.336	1.249	1.173	1.345	0.02848	5.07%	4.09%
6.06		5	1.27	1.241	1.299	1.266	1.249	1.303	0.01044	1.84%	3.06%
10		5	1.248	1.194	1.302	1.266	1.173	1.284	0.01945	3.49%	4.78%
15		5	1.242	1.173	1.312	1.233	1.159	1.303	0.02501	4.5%	5.19%

Report Date: Test Code: 26 Jan-17 12:06 (p 2 of 2) 1701-S140 | 14-4621-9388



TST

Report Date:

26 Jan-17 12:06 (p 1 of 1)

021107111				757				Code:	170	1701-S140 14-4621-9388		
Echinoid Sp	erm Cell Fertiliza	ation Tes	t 15C						Nautilus	s Environi	mental (CA)	
Analysis ID: Analyzed:	01-9496-1705 26 Jan-17 12:0			ertilization Rat arametric Bioe		-Two Sampl		IS Version		.8.7		
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU	
Angular (Cori	rected)	NA	C*b < T	NA	NA	0.75	3.25%	15	>15	NA	6.667	
TST-Welch's	t Test											
Control	vs C-%		Test Sta	t Critical MSD DF P-Value		P-Type	Decision	n(a:5%)				
Lab Control	2.5*		11.41	1.895	0.054 7	<0.0001	CDF	Non-Sigr	ificant Effect			
	5*		8.303	1.943	0.064 6	<0.0001	CDF	Non-Sigr	nificant Effect			
	6.06*		14.6	1.943	0.038 6	< 0.0001	CDF	Non-Sigr	nificant Effect			
	10*		10.34	1.895	0.049 7	< 0.0001	CDF	Non-Sigr	ificant Effect			
	15*		8.634	1.943	0.058 6	<0.0001	CDF	Non-Sigr	ificant Effect			
ANOVA Tabl	e											
Source	Sum Sqւ	ıares	Mean So	quare	DF	F Stat	P-Value	Decision	ı(<i>a</i> :5%)			
Between	0.023387	34	0.00467	7468	5	1.892	0.1333	Non-Sigr	ificant Effect			
Error	0.059344	47	0.002472	2686	24							
Total	0.082731	81			29	_						
Distribution	al Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)				
Variances	Bartlett E	Equality o	f Variance	3.447	15.09	0.6314	Equal Var	riances				
Distribution	Shapiro-	Wilk W N	lormality	0.9652	0.9031	0.4169	Normal D	istribution				
Fertilization	Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	0.932	0.8999	0.9641	0.94	0.89	0.96	0.01158	2.78%	0.0%	
2.5		5	0.932	0.9024	0.9616	0.92	0.91	0.97	0.01068	2.56%	0.0%	
5		5	0.902	0.856	0.948	0.9	0.85	0.95	0.01655	4.1%	3.22%	
6.06		5	0.912	0.8958	0.9282	0.91	0.9	0.93	0.005831	1.43%	2.15%	
10		5	0.898	0.8635	0.9325	0.91	0.85	0.92	0.01241	3.09%	3.65%	
15		5	0.894	0.8505	0.9375	0.89	0.84	0.93	0.01568	3.92%	4.08%	
Angular (Co	rrected) Transfor	rmed Sur	nmary									
- 0/		C4	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
C-%	Control Type	Count	Wiedii									
0	Control Type Lab Control	5	1.31	1.249	1.372	1.323	1.233	1.369	0.02226	3.8%	0.0%	
					1.372 1.376	1.323 1.284	1.233 1.266	1.369 1.397	0.02226 0.02342	3.8% 4.0%	0.0% -0.04%	
0		5	1.31	1.249								
0 2.5		5 5	1.31 1.311	1.249 1.246	1.376	1.284	1.266	1.397	0.02342	4.0%	-0.04%	
0 2.5 5		5 5 5	1.31 1.311 1.257	1.249 1.246 1.178	1.376 1.336	1.284 1.249	1.266 1.173	1.397 1.345	0.02342 0.02848	4.0% 5.07%	-0.04% 4.09%	

Report Date:

16 Jan-17 11:37 (p 1 of 1)

Test Code: 1701-5140 14-4621-9388/56338E7C

A		
Nautilus	Environmental	(CA)

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA					
		18 Jan-17		18 Jan-17 18 Jan-17 : 48 Jan-17		18 Jan-17				centrotus purpuratus R-95/136 (1995) fluent		1 1649D76 17-0-57 IDE Americas, Inc. M-001 (40 ppt adj)
C-%		Rep		# Counted	# Fertilized	1/19/17	Notes					
			31	100	92							
***************************************			32	100	93							
			33	100	95							
			34	100	89							
			35	100	96							
			36	100	84							
			37	100	93							
			38	100	92							
***			39	100	90 90	THE PLANT AND THE PROPERTY COURSE AND ADMINISTRATION OF THE PROPERTY OF THE PR						
			40	100	90							
			41	100	93							
			42	100	91							
			43	100	94							
			44	100	94		,					
			45	100	90							
			46	100	89							
			47	100	91							
			48	100	89							
			49	100	92							
			50	(00	85							
			51	[00]	91		****					
alam Military			52	100	94							
			53	100	91							
			54	(00	92							
			55	100	92							
			56	100	96		normal description of the second definition of the second					
			57	100	89							
A00000			58	100	97							
			59	100	65							

@ Q18 131/25/17

92

Report Date: 16 Jan-17 11:37 (p 1 of 1)

Test Code: 1701 - 5/40 14-4621-9388/56338E7C

Start Date: End Date: Sample Date	18 J	an-17 an-17 an-17		Protoc		centrotus purpuratus R-95/136 (1995) fluent	Sample Code: 11649976 170099 Sample Source: IDE Americas, Inc. Sample Station: M-001 (40 ppt adj)
C-%	Code			# Counted	# Fertilized		Notes
0	LC	1	41	100	96	Eh 1/18/17	
0	LC	2	44				
0	LC	3	35				
0	LC	4	34				
0	LC	5	43		NA ANALYS BY THE		
2.5		1	54				
2.5		2	52				
2.5		3	49	100	90	EG	
2.5		4	42				
2.5		5	58				
5		1	31				
5		2	59				
5		3	57	100	90	Eh	
5		4	40	100	111111111111111111111111111111111111111		
5		5	33				
6.06		1	45		No. of Contract of		
6.06		2	38	100	95	84	
6.06		3	32		1		
6.06		4	56				
6.06		5	53				
10		1	50				
10		2	51				
10		3	47	100	94	EG	
10		4	39				
10		5	60				
15		1	37				
15		2	46				
15		3	36	100	84	Eh	
15		4	55		1		
15		5	48				

Brine Dilution Worksheet

Project: IDE Analyst: EG

Sample ID: M-001 (40 ppt adjusted) Test Date: 1/18/2017

Test No: 170(-Siy) Test Type: Urchin Fertilization

Salinity of Effluent 63.0

Salinity of Seawater 33.5 Date of Brine used: NA

Target Salinity 40.0 Alkalinity of Brine Control: NA mg/L as CaCO3

Effluent Brine Control

Salinity Adjustment Factor: (TS

- SE)/(SB - TS) = 3.54 -6.15

TS = target salinity
SE = salinity of effluent
SB = salinity of brine

Comments: Formula for amount of seawater to dilute sample to 40ppt

100

Use 40 ppt sample as 100% sample for testing.

NA = not applicable; sample not diluted with Nautilus brine.

3.54

QC Check: <u>KB1|25|17</u>

100

Final Review: ACUTITY

353.8

454

Water Quality Measurements

Client:

IDE

Test Species: S. purpuratus

Sample ID:

M-001 (40 ppt adjusted)

Start Date/Time: 1/18/2017 \5\9

Sample Log No.: 17-0050

Dilutions made by:

Test No: 1701-SI40

			Analyst:	RH			
	Initial Readings						
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)			
Lab Control	8.4	8.06	33.4	14.8			
2.5	8.4	8,06	33.7	14.6			
5.0	8.4	8.07	33.9	14.5			
6.06	8.4	8.07	34.0	14.8			
10	8.4	8.07	34.3	14.7			
15	8.4	8.07	34.6	14.6			

_				
\sim	m	ma	nts	
\sim 0	111	1116	1113	

QC Check:

Final Review: AC2/7/17

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Sample ID: Test No.:	102 M-001 (40 pp) adj 1701-5140	Start Date/Time: 1/18/2017 / 1519 End Date/Time: 1/18/2017 / 1559 Species: 5- purpuratus
Tech initials: Injection Time:	<u> </u>	Animal Source: Pt. Lorna Date Collected: 12/20/16
Sperm Absorbance at 4	00 nm:0.851(target ran	ge of 0.8 - 1.0 for density of 4x10 ⁶ sperm/ml)
Eggs Counted:	85 Mean: 80.6	_X 50 = <u>4630</u> eggs/ml
		eggs per vertical pass on Sedgwick- al density of 4000 eggs/ml)
Initial density: Final density:	$\frac{4000 \text{eggs/ml}}{4000 \text{eggs/ml}} = \frac{2}{1.0}$	e_ dilution factor egg stock ml part egg stock seawater ml parts seawater

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

	Sperm:Egg Ratio							
Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75
Sperm Added (100 μl): Eggs Added (0.5 ml): Test Ended:	Time 1448 1458 1508	<u>Ra</u> 	ngefinder Ra 50: 1 100: 1 200: 1 400: 1		-	Unfert. 22 6/6 1		

<u>NOTE</u>: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

<u>Definitive Test</u>		Sperm:Egg Ratio Use	d: <u>160 -</u>	1	
Sperm Added (100 μl): Eggs Added (0.5 ml): Test Ended:	Time 1519 1539 1559	QC1 QC2 Egg Control 1 Egg Control 2	Fert. (4) (9) (0) (0)	Unfert. 9 8 100	
Comments:	@ No Pilotion	needed			
QC Check:	NB 1/25/M			Final Review: _	AC217/17

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Appendix B

Sample Receipt Information

(A)			
0059			
1/17/17 0900			
1/17/17 1153			
2 4L CUBIS			
~5L			
2.0			
Y N	ΥN	Y N	YN
6.2			
7.89			
63.0			
118			
0.06			
MM			
	1/17/17 0900 1/17/17 1153 2 4L CUBIS ~5L 2.0 (Y) N 6.2 7.89	1/17/17 0900 1/17/17 1153 2 4L CUBIS ~5L 2.0 (Y) N Y N 6.2 7.89	1/17/17 0900 1/17/17 1153 2 4L CUDIS ~5L 2.0 (Y) N Y N Y N 6.2 7.89 — 63.0

Test Performed:	Urchin fertilization	Alkalinity: Hardness or Salinity: Hardness or Salinity:
	Additional Control? Y N	=Alkalinity: Hardness or Salinity:
Test Performed:	•	Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:
	Additional Control? Y N	Alkalinity: Hardness or Salinity: = Alkalinity: Hardness or Salinity:
Test Performed:		Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: Alkalinity: Hardness or Salinity:
	Additional Control? Y N	=Alkalinity: Hardness or Salinity:
Notes:	¹ Temperature of sample shoul	d be 0-6°C, if received more than 24 hours past collection time.
Notes:	¹ Temperature of sample shoul	

Sample Check-In Information

Sample Description:	A		
A: Colorless clea	ام 0 ل <u>ل ن _</u> _	855, NO	debris
COC Complete (Y/N)?	?		
A B C			
	N.		
Filtration? Y (N)		
Pore Size:		-	
Organisms	or	Debris	
	_A@		
Salinity Adjustment?		_	
Test:	Source:	_	et ppt:
Test:	Source:	_	et ppt:
Test:	Source:	Targe	et ppt:
pH Adjustment? Y	OC.		
	A	В	С
Initial pH:			
Amount of HCI added:			
Final pH:			
Cl ₂ Adjustment? Y	N)		
	A	В	С
Initial Free Cl ₂ :			
STS added:			
Final Free Cl ₂ :			
Sample Aeration? Y	_		
	A	В	С
Initial D.O.			
Duration & Rate	-		
Final D.O.			
Subsamples for Addi NH3 Othe		ustry Require	ed? Y (N)
Tech Initials A	r		
recii iinuais A	·	- ~	
	QC Ch	eck: <u>KB1</u>	12611
	Final Revi	iew: AC a	217/17

Appendix C

Chain-of-Custody Form



IDE Technologies		CDP laoratory: Entahlpy Laboratory: WECK Laboratory: Nautilus: X AIM: Other:	Turn Around Time Normal:X RUSH (24 hr): 3 Days: 5 Days: 777 Days
Project Name: <u>Monthly NPDES Monitoring</u> Project Manager: <u>Peter Shen</u> Contact Information:	(760) 201-7777	other.	rrr bays
Comments:	ANALY	/SES	NOTES:
24 hour composite sampled via autosampler by a series of consecutive grabs collected at six hour intervals. Sample collected during normal plant operation at 48 MGD to fulfill monthly NPDES requirement. Sample is to be run undiluted as well as	tion		

during normal plant operation at adjusted to 40 ppt. Undiluted san ppt. Start: 1/16/17 @ 09:00, End	nple is to be run	untreated and with p	uirement. Sample is to be pH 10 filtration. Conductivi	run und ty: 84.5	iluted as well as 4 mS/cm, TDS: 60.70	rtilizatic									
		Glass=G Plastic=	:P			ic Fe									
·	Yes=Y No=	N Acid=A Base=B				hron									
Drink	ing Water=DW	Seawater=SW Soil=S		Pre		hin C									
Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	Purple Urchin Chronic Fertilizatic									
Monthly M-001 (16-2880)	1/16-17/17	9:00	24 hr Comp. (Brine)	N	2 x 4L cubi	х									
									-						
Relinquished By:		Date:	Time:		Received By:					Time:		Samı	ole Con	dition Upon Receipt:	
Kennon	~/	1/107/17	1000	(2		111	7/17	7	10.100	之	Iced		Ambient or°C	:
	V	I am lun	11162	1	1	_	-								

Naut. 105 IO: 7060 MM V17/17
17-0059

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date:

26 Jan-17 12:01 (p 1 of 1) 170118sprt | 08-8914-3626

Test Code:	170118sprt	08-8914

										***************************************	· · · · · · · · · · · · · · · · · · ·
Echinoid Sper	m Cell Fertilizati	on Test 15C							Nautilus	Environme	ental (CA)
Batch ID: Start Date: Ending Date: Duration:	11-9574-7644 18 Jan-17 15:19 18 Jan-17 15:59 40m	Test Type: Protocol: Species: Source:		EPA/600/R-95/136 (1995) Strongylocentrotus purpuratus					ıral Seawate Applicable	r	
Sample ID: Sample Date: Receive Date: Sample Age:		Code: Material: Source: Station:	Reference Toxi	170118sprt Copper chloride Reference Toxicant Copper Chloride				Inter	nal		
Comparison S	Summary										
Analysis ID	Endpoint	NOE	L LOEL	TOEL	PMSD	TU	Met				+
12-8205-2347	Fertilization Rate	<10	10	NA	7.61%		Stee	l Man	y-One Rank	Sum Test	
Point Estimate	e Summary										
Analysis ID	Endpoint	Leve	l μg/L	95% LCL	95% UCL	TU	Met	hod			
00-6318-6085	Fertilization Rate	e EC50		18.07	21.37		Trim	med S	Spearman-K	ärber	
Test Acceptab	nility										
Analysis ID	Endpoint	Attri	oute	Test Stat	TAC Limi	ts	Ove	rlap	Decision		
00-6318-6085	Fertilization Rate		rol Resp	0.918	0.7 - NL		Yes		Passes Ac	ceptability	Criteria
	Fertilization Rate		rol Resp	0.918	0.7 - NL		Yes		Passes Ac	ceptability	Criteria
ł	Fertilization Rate		•	0.07613	NL - 0.25		No		Passes Ac	ceptability	Criteria
Fertilization R	ate Summary									9,90	
C-µg/L	Control Type	Count Mea	n 95% LCL	95% UCL	Min	Ma	x Std	Err	Std Dev	CV%	%Effect
0	Lab Control	5 0.91	8 0.8738	0.9622	0.87	0.9	7 0.01	594	0.03564	3.88%	0.0%
10		5 0.63	2 0.5975	0.6665	0.59	0.6	6 0.01	241	0.02775	4.39%	31.15%
20		5 0.49	6 0.3972	0.5948	0.39	0.6	0.03	558	0.07956	16.04%	45.97%
40		5 0.12	4 0.01273	0.2353	0.03	0.2	6 0.04	007	0.08961	72.27%	86.49%
80		5 0.00	2 0	0.007553	0	0.0	1 0.00)2	0.004472	223.6%	99.78%
160		5 0	0	0	0	0	0		0		100.0%
Fertilization R	tate Detail										
C-µg/L	Control Type	Rep 1 Rep	2 Rep 3	Rep 4	Rep 5						
0	Lab Control	0.87 0.92	0.97	0.92	0.91						
10		0.64 0.66	0.62	0.59	0.65						
20		0.39 0.6	0.45	0.52	0.52						
40		0.07 0.1	0.16	0.03	0.26						
80		0 0.01	0	0	0						
160		0 0	0	0	0						

Analyst: VB QA: VFP 1/21/17

CETIS™ v1.8.7.20

000-089-187-3

Report Date: Test Code: 26 Jan-17 12:01 (p 1 of 2) 170118sprt | 08-8914-3626

							Test				
Echinoid Spe	erm Cell Fertiliza	tion Test 1	5C						Nautilus	Environme	ental (CA)
Analysis ID:	12-8205-2347			ilization Rate		F 1		S Version: ial Results:	CETISv1. Yes	8.7	
Analyzed:	26 Jan-17 12:0	1 Ana	ı lysis: Non	parametric-(Control VS	reatments	Onic	iai Kesuits.			
Data Transfo	rm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	ected)	NA	C > T	NA	NA		7.61%	<10	10	NA	
Steel Many-C	ne Rank Sum Te	est									
Control	vs C-μg/L		Test Stat	Critical	Ties D	P-Value	P-Type	Decision(α:5%)		
Lab Control	10*		15	17	0 8	0.0158	Asymp	Significant	Effect		
	20*		15	17	0 8	0.0158	Asymp	Significant	Effect		
	40*		15	17	0 8	0.0158	Asymp	Significant	Effect		
	80*		15	17	0 8	0.0158	Asymp	Significant	Effect		
ANOVA Table	e										
Source	Sum Squ	ares	Mean Squ	are	DF	F Stat	P-Value	Decision(a:5%)		
Between	4.6696		1.1674		4	184	<0.0001	Significan	t Effect		
Error	0.1269165	5	0.0063458	25	20						
Total	4.796517				24						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett E	quality of V	/ariance	13.53	13.28	0.0090	Unequal \	/ariances			
Distribution	Shapiro-\	Wilk W Nor	mality	0.9511	0.8877	0.2655	Normal D	istribution			
Fertilization	Rate Summary						,				
	Rate Summary Control Type	Count	Mean	95% LCL	95% UCI	. Median	Min	Max	Std Err	CV%	%Effect
C-µg/L	Control Type		Mean 0.918	95% LCL 0.8738	95% UCI 0.9622	. Median	Min 0.87	Max 0.97	Std Err 0.01594	3.88%	0.0%
C-μg/L	•	5									0.0% 31.15%
C-μg/L 0 10	Control Type	5 5	0.918	0.8738	0.9622	0.92	0.87	0.97	0.01594 0.01241 0.03558	3.88%	0.0% 31.15% 45.97%
C-μg/L 0 10 20	Control Type	5 5 5	0.918 0.632 0.496	0.8738 0.5975	0.9622 0.6665	0.92 0.64	0.87 0.59	0.97 0.66	0.01594 0.01241	3.88% 4.39%	0.0% 31.15% 45.97% 86.49%
C-μg/L 0 10 20 40	Control Type	5 5 5 5	0.918 0.632 0.496 0.124	0.8738 0.5975 0.3972	0.9622 0.6665 0.5948	0.92 0.64 0.52 0.1	0.87 0.59 0.39	0.97 0.66 0.6	0.01594 0.01241 0.03558	3.88% 4.39% 16.04%	0.0% 31.15% 45.97% 86.49% 99.78%
C-μg/L 0 10 20	Control Type	5 5 5	0.918 0.632 0.496	0.8738 0.5975 0.3972 0.01273	0.9622 0.6665 0.5948 0.2353	0.92 0.64 0.52 0.1	0.87 0.59 0.39 0.03	0.97 0.66 0.6 0.26	0.01594 0.01241 0.03558 0.04007	3.88% 4.39% 16.04% 72.27%	0.0% 31.15% 45.97% 86.49%
C-μg/L 0 10 20 40 80 160	Control Type	5 5 5 5 5 5	0.918 0.632 0.496 0.124 0.002	0.8738 0.5975 0.3972 0.01273	0.9622 0.6665 0.5948 0.2353 0.007553	0.92 0.64 0.52 0.1	0.87 0.59 0.39 0.03	0.97 0.66 0.6 0.26 0.01	0.01594 0.01241 0.03558 0.04007 0.002	3.88% 4.39% 16.04% 72.27%	0.0% 31.15% 45.97% 86.49% 99.78%
С-µg/L 0 10 20 40 80 160	Control Type Lab Control	5 5 5 5 5 5	0.918 0.632 0.496 0.124 0.002	0.8738 0.5975 0.3972 0.01273	0.9622 0.6665 0.5948 0.2353 0.007553	0.92 0.64 0.52 0.1 0	0.87 0.59 0.39 0.03	0.97 0.66 0.6 0.26 0.01 0	0.01594 0.01241 0.03558 0.04007 0.002 0	3.88% 4.39% 16.04% 72.27% 223.6%	0.0% 31.15% 45.97% 86.49% 99.78% 100.0%
C-μg/L 0 10 20 40 80 160 Angular (Co	Control Type Lab Control rrected) Transfor	5 5 5 5 5 5 5	0.918 0.632 0.496 0.124 0.002 0	0.8738 0.5975 0.3972 0.01273 0	0.9622 0.6665 0.5948 0.2353 0.007553	0.92 0.64 0.52 0.1 0 0 Median	0.87 0.59 0.39 0.03 0	0.97 0.66 0.6 0.26 0.01	0.01594 0.01241 0.03558 0.04007 0.002 0 Std Err 0.03141	3.88% 4.39% 16.04% 72.27% 223.6% CV% 5.46%	0.0% 31.15% 45.97% 86.49% 99.78% 100.0% %Effect 0.0%
C-μg/L 0 10 20 40 80 160 Angular (Co C-μg/L 0	Control Type Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 5 mrmed Sumr	0.918 0.632 0.496 0.124 0.002 0 mary	0.8738 0.5975 0.3972 0.01273 0 0	0.9622 0.6665 0.5948 0.2353 0.007553 0	0.92 0.64 0.52 0.1 0	0.87 0.59 0.39 0.03 0	0.97 0.66 0.6 0.26 0.01 0	0.01594 0.01241 0.03558 0.04007 0.002 0 Std Err 0.03141 0.01282	3.88% 4.39% 16.04% 72.27% 223.6% CV% 5.46% 3.12%	0.0% 31.15% 45.97% 86.49% 99.78% 100.0% %Effect 0.0% 28.56%
C-µg/L 0 10 20 40 80 160 Angular (Co C-µg/L 0 10	Control Type Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 rmed Sumr Count	0.918 0.632 0.496 0.124 0.002 0 mary Mean 1.287	0.8738 0.5975 0.3972 0.01273 0 0 95% LCL	0.9622 0.6665 0.5948 0.2353 0.007553 0	0.92 0.64 0.52 0.1 0 0 Median	0.87 0.59 0.39 0.03 0 0	0.97 0.66 0.6 0.26 0.01 0	0.01594 0.01241 0.03558 0.04007 0.002 0 Std Err 0.03141 0.01282 0.03582	3.88% 4.39% 16.04% 72.27% 223.6% CV% 5.46% 3.12% 10.25%	0.0% 31.15% 45.97% 86.49% 99.78% 100.0% %Effect 0.0% 28.56% 39.27%
C-µg/L 0 10 20 40 80 160 Angular (Co C-µg/L 0 10 20	Control Type Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 rmed Sumr Count 5 5	0.918 0.632 0.496 0.124 0.002 0 mary Mean 1.287 0.9192	0.8738 0.5975 0.3972 0.01273 0 0 95% LCL 1.199 0.8836	0.9622 0.6665 0.5948 0.2353 0.007553 0 95% UC 1.374 0.9548	0.92 0.64 0.52 0.1 0 0 - Median 1.284 0.9273	0.87 0.59 0.39 0.03 0 0 Min 1.202 0.8759	0.97 0.66 0.6 0.26 0.01 0 Max 1.397 0.9483 0.8861 0.5351	0.01594 0.01241 0.03558 0.04007 0.002 0 Std Err 0.03141 0.01282 0.03582 0.06174	3.88% 4.39% 16.04% 72.27% 223.6% CV% 5.46% 3.12% 10.25% 40.36%	0.0% 31.15% 45.97% 86.49% 99.78% 100.0% %Effect 0.0% 28.56% 39.27% 73.41%
C-µg/L 0 10 20 40 80 160 Angular (Co C-µg/L 0 10	Control Type Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 mmed Sumr Count 5 5	0.918 0.632 0.496 0.124 0.002 0 mary Mean 1.287 0.9192 0.7813	0.8738 0.5975 0.3972 0.01273 0 0 95% LCL 1.199 0.8836 0.6819	0.9622 0.6665 0.5948 0.2353 0.007553 0 95% UC 1.374 0.9548 0.8808	0.92 0.64 0.52 0.1 0 0 - Median 1.284 0.9273 0.8054	0.87 0.59 0.39 0.03 0 0 Min 1.202 0.8759 0.6745	0.97 0.66 0.6 0.26 0.01 0 Max 1.397 0.9483 0.8861	0.01594 0.01241 0.03558 0.04007 0.002 0 Std Err 0.03141 0.01282 0.03582	3.88% 4.39% 16.04% 72.27% 223.6% CV% 5.46% 3.12% 10.25%	0.0% 31.15% 45.97% 86.49% 99.78% 100.0% %Effect 0.0% 28.56% 39.27%

Analyst: VB QA: WTP 126/17

0.2

0.0

0 LC

20

C-µg/L

80

160

Report Date: Test Code: 26 Jan-17 12:01 (p 2 of 2) 170118sprt | 08-8914-3626

2.0

Nautilus Environmental (CA) **Echinoid Sperm Cell Fertilization Test 15C CETIS Version:** CETISv1.8.7 Endpoint: Fertilization Rate Analysis ID: 12-8205-2347 Nonparametric-Control vs Treatments Official Results: Yes Analysis: Analyzed: 26 Jan-17 12:01 Graphics 0,20 1.0 0.9 0.15 8.0 Fertilization Rate Centered Corr. Angle 0.7 9999 0.6 0.00 0.5 0.4 -0.05 0.3

-0.20

-2.5

-2.0 -1.5

-0.5 0.0

Rankits

Report Date:

26 Jan-17 12:01 (p 1 of 1) 170118sprt | 08-8914-3626

Test Code:

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: Analyzed:

00-6318-6085 26 Jan-17 12:01 Endpoint: Fertilization Rate Trimmed Spearman-Kärber Analysis:

CETIS Version: Official Results:

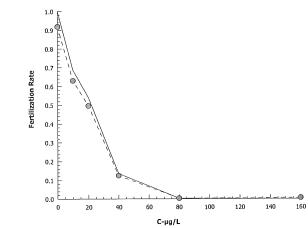
Yes

CETISv1.8.7

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.082	31.15%	1.293	0.01822	19.65	18.07	21.37

Fertilizati	on Rate Summary			Calculated Variate(A/B)							
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.918	0.87	0.97	0.01594	0.03564	3.88%	0.0%	459	500
10		5	0.632	0.59	0.66	0.01241	0.02775	4.39%	31.15%	315	500
20		5	0.496	0.39	0.6	0.03558	0.07956	16.04%	45.97%	248	500
40		5	0.124	0.03	0.26	0.04007	0.08961	72.27%	86.49%	62	500
80		5	0.002	0	0.01	0.002	0.004472	223.6%	99.78%	1	500
160		5	0	0	0	0	0		100.0%	0	500

Graphics



Report Date:

26 Jan-17 12:02 (1 of 1)

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization

Organism: Strongylocentrotus purpuratus (Purpl

Material:

Copper chloride

Protocol:

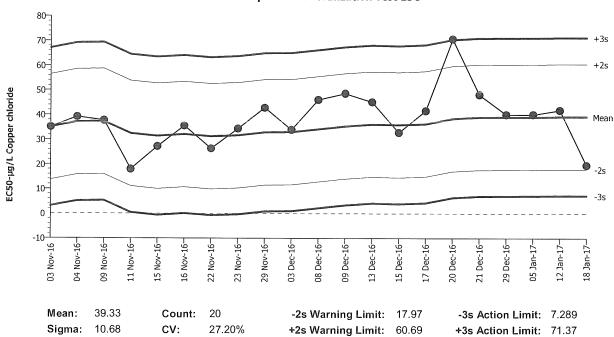
EPA/600/R-95/136 (1995)

Endpoint: Fertilization Rate

Source:

Reference Toxicant-REF

Echinoid Sperm Cell Fertilization Test 15C



Qual	ity C	ontrol	Data
------	-------	--------	------

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Nov	3	15:45	35.12	-4.215	-0.3946			20-3136-0658	11-0001-2879
2			4	15:36	39.19	-0.1379	-0.01291			20-6041-1964	14-0189-3509
3			9	12:42	37.73	-1.603	-0.1501			14-1208-8602	07-4301-7295
4			11	16:16	17.98	-21.35	-1.999			05-3831-5746	09-0240-4709
5			15	14:41	27.24	-12.09	-1.132			05-8975-3204	09-5350-2298
6			16	15:57	35.58	-3.747	-0.3509			11-0010-5225	08-2681-2077
7			22	17:20	26.36	-12.97	-1.215			15-6301-8591	13-1300-2147
8			23	16:18	34.42	-4.913	-0.46			14-8399-9000	17-7821-1750
9			29	16:03	42.87	3.542	0.3316			21-1597-0375	02-3336-7521
10		Dec	3	15:05	33.99	-5.34	-0.5			02-8144-9736	08-6113-6246
11			8	15:04	46.14	6.811	0.6377			11-3755-6520	02-1834-2654
12			9	16:26	48.68	9.348	0.8753			11-3406-8076	17-4460-9811
13			13	15:11	45.26	5.929	0.5552			10-6683-7365	04-6270-7422
14			15	17:33	32.87	-6.458	-0.6047			01-7454-5472	05-8893-7899
15			17	15:22	41.72	2.388	0.2236			08-9842-8510	01-1488-1013
16			20	15:17	70.85	31.52	2.951	(+)		16-6092-1425	02-2928-0983
17			21	12:12	48.26	8.934	0.8365			14-5051-2365	16-1479-8388
18			29	16:22	40.16	0.8328	0.07798			17-0784-9661	08-0208-3856
19	2017	Jan	5	14:34	40.21	0.8818	0.08257			04-1406-8806	15-3393-3643
20			12	17:54	41.95	2.619	0.2452			14-8351-4083	12-3796-8723
21			18	15:19	19.65	-19.68	-1.842			08-8914-3626	00-6318-6085

CETIS Test Data Worksheet

Report Date:

16 Jan-17 11:35 (p 1 of 1)

08-8914-3626/170118sprt Test Code:

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date:	18 Jan-17	Species:	Strongylocentrotus purpuratus	Sample Code:	170118sprt
End Date:	18 Jan-17	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant
Sample Date:	18 Jan-17	Material:	Copper chloride	Sample Station:	Copper Chloride

mpie Dati					ai. Copper cir		 e Station: Copper	
C-μg/L	Code	Rep	Pos	# Counted	# Fertilized	1/19/17	Notes	
			1	(00)	Ô		 	
			2	100	64 3			
			3	100	3			
			4	100	16			
			5	100	60			
			6	100	45		 	
			7	100	45 39		 	
			8	100	66			
			9	100	7			
			10	160	87			
			11	(00	0			
			12	100	26			
			13	100	0			
			14	100	10			/ 10 %
			15	100	62		 	
			16	(00)	0			
			17	100	52			
			18	100	52			
			19	100	0			
			20	100	6			
			21	(00)	65			MANAGEMENT .
			22	(00)				
			23	100	97			
			24	100	92			
			25	100	- (
			26	100	92			
			27	(00	0			
			28	100	91		 	
			29	100	59		 	
			30	100	0			

CETIS Test Data Worksheet

Echinoid Sperm Cell Fertilization Test 15C

Report Date: Test Code:

16 Jan-17 11:35 (p 1 of 1) 08-8914-3626/170118sprt

Nautilus	Environmental (CA)

Start Date:	18 Jan-17	Species:	Strongylocentrotus purpuratus	Sample Code:	170118sprt
End Date:	18 Jan-17	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant
Sample Date:	18 Jan-17	Material:	Copper chloride	Sample Station:	Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	10	100	93	EG 1/18/17
0	LC	2	24			
0	LC	3	23			
0	LC	4	26			
0	LC	5	28			
10		1	2			
10		2	8	100	72	
10		3	15			
10		4	29		1 2000000000000000000000000000000000000	
10		5	21			
20		1	7			
20		2	5	100	43	Ely
20		3	6			
20		4	17			
20		5	18			
40		1	9	100	11	Eh.
40		2	14	100 XB		
40		3	4			
40		4	3			
40		5	12			
80		1	11			
80		2	25	100	0	Eh
80		3	19			
80		4	16			
80		5	27			
160		1	20			
160		2	13			
160		3	1	(00	0	EG
160		4	30			,
160		5	22			

QC: EL

Water Quality Measurements

liei	-+		
 	111	-	

Internal

Test Species: S. purpuratus

Sample ID:

CuCl₂

Start Date/Time: 1/18/2017

1519

Test No:

170118sprt

End Date/Time: 1/18/2017

1559

Dilutions made by:

High conc. made (μg/L): 160 8.7 Vol. Cu stock added (mL):

500 Final Volume (mL):

9,800

Cu stock concentration (µg/L):

Analyst:

RH

	Initial Readings						
Concentration	DO	На	Salinity	Temperature			
(μg/L)	(mg/L)	(units)	(ppt)	(°C)			
Lab Control	8.6	8.02	33.4	14.9			
10	8.5	8,00	33.4	14.6			
20	8.4	8.01	33.4	14.6			
40	g. 3	\$.03	33.5	14.9			
80	8.3	8.04	33.2	14.7			
160	8.3	8.04	3 3.0	14.8			

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QC Check:

Final Review: VFP 1/24/17

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Definitive Test

QC Check:

Echinoderm Sperm-Cell Fertilization Worksheet

Start Date/Time: 1/18/2017 / Client: End Date/Time: 1/18/2017 / Sample ID: Species: 7011850r-Test No.: Animal Source: Date Collected: 12/20/16 Tech initials: 4440 Injection Time: 0.854

(target range of 0.8 - 1.0 for density of 4x10⁶ sperm/ml) Sperm Absorbance at 400 nm: $80.6 \times 50 = 4030$ Eggs Counted: (target counts of 80 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 4000 eggs/ml)

4020 dilution factor egg stock Initial density: eggs/ml part egg stock 4000 Final density: eggs/ml parts seawater

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Sperm: Egg Ratio 100:1 50:1 400:1 200:1 800:1 2000:1 1600:1 1200:1 Rangefinder Test: 2.5 1.25 5.0 30 20 10 ml Sperm Stock 50 40 47.5 48.75 20 30 40 45 0.0 10 ml Seawater

Unfert. Rangefinder Ratio: Fert. Time 50:1 78 Sperm Added (100 µl): 100:1 458 Eggs Added (0.5 ml): 2000 508 Test Ended: 400 - V 100

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g.,

organism health, stage of reproductive season, site conditions).

Sperm:Egg Ratio Used:

Time Unfert. QC1 Sperm Added (100 µl): QC2 Eggs Added (0.5 ml): Egg Control 1 100 Test Ended: Egg Control 2

Pilotion Comments:

Final Review: Ktp 1/26/17

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Appendix E

Qualifier Codes



Glossary of Qualifier Codes:

- Q1 Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 Temperatures out of recommended range; no action taken, test terminated same day
- Q3 Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L
- Q4 Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 Test initiated with aeration due to an anticipated drop in D.O.
- Q6 Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 Salinity out of recommended range
- Q8 Spilled test chamber/ Unable to recover test organism(s)
- Q9 Inadequate sample volume remaining, 50% renewal performed
- Q10 Inadequate sample volume remaining, no renewal performed
- Q11 Sample out of holding time; refer to QA section of report
- Q12 Replicate(s) not initiated; excluded from data analysis
- Q13 Survival counts not recorded due to poor visibility or heavy debris
- Q14 D.O. percent saturation was checked and was ≤ 110%
- Q15 Did not meet minimum test acceptability criteria. Refer to QA section of report.
- Q16 Percent minimum significant difference (PMSD) was <u>below</u> the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.
- Q17 Percent minimum significant difference (PMSD) was <u>above</u> the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.
- Q18 Incorrect Entry
- Q19 Illegible Entry
- Q20 Miscalculation
- Q21 Other (provide reason in comments section)
- Q22 Greater than 10% mortality observed upon receipt and/or in holding prior to test initiation.
 Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.
- Q23 Test organisms received at a <u>temperature</u> greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.
- Q24 Test organisms received at <u>salinity</u> greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Updated: 6/30/15