

# Chronic Toxicity Test Results for the Carlsbad Desalination Plant

Sample ID: M-001 (Daily/Weekly) Sample Collection Date: September 5, 2017

Prepared for: IDE AMERICAS, Inc.

4590 Carlsbad Boulevard Carlsbad, CA 92008

Prepared by: Nautilus Environmental

Submitted: September 22, 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.
- o All test results have met internal Quality Assurance Program requirements.

California

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Results verified by: \_\_\_\_\_\_\_ Advienne libor\_\_\_\_\_

# **EXECUTIVE SUMMARY**

# CHRONIC TOXICITY TESTING CARLSBAD DESALINATION PLANT — SEPTEMBER 2017 ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: September 5, 2017

<u>Test Date:</u> September 5, 2017

Sample ID: M-001 (pre-treatment off-spec period)

Effluent Limitation: 16.5 TU<sub>c</sub>

# Results Summary:

	Effluent Te	est Results	Effluent Limitation
Bioassay Type: Urchin Fertilization	NOEC	TU₀	Met? (Yes/No)
OTCHILI FELLINZATION	6.06	16.5	Yes

Client: IDE Americas, Inc. Test ID: 1709-S036 Sample ID: M-001

Sample Date: September 5, 2017

### INTRODUCTION

A discharge sample was collected in September 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for daily and weekly chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a period of off-spec plant operation. Chronic toxicity testing for the effluent sample was conducted during this time 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 September 5, 2017 using the purple urchin (Strongylocentrotus purpuratus) chronic fertilization test.

### **MATERIALS AND METHODS**

Sample collection and delivery were performed by IDE Americas, Inc. (IDE) personnel. Following arrival at Nautilus, an aliquot of the water sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. The sample was stored at 4° C in the dark until used for testing. 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 protocols described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./Carlsbad Desalination Plant
Sample ID:	M-001 (weekly, and daily pre-treatment off-spec period)
Monitoring Period:	September 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	9/5/17, 08:00
Sample Receipt Date, Time:	9/5/17, 12:16

Table 2. Water Quality Measurements for the M-001 Sample upon Receipt

Sample Collection	рН	DO	Temp	Salinity	Alkalinity	Total Chlorine
Date		(mg/L)	(°C)	(ppt)	(mg/L as CaCO <sub>3</sub> )	(mg/L)
9/5/17	7.90	8.0	3.0	33.7	122	<0.02

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Client: IDE Americas, Inc. Test ID: 1709-S036 Sample ID: M-001

Sample Date: September 5, 2017

### Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times: 9/5/17, 17:07 through 17:47

Test Organism: Strongylocentrotus purpuratus (purple sea urchin) Test Organism Source: Field-collected 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 M-001 sample; lab control

Number of Replicates, Organisms

per Replicate:

before each test with a preliminary rangefinding test.

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

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 fertilization period

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

significant difference (PMSD) value <25.

Copper chloride Reference Toxicant Testing:

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 the 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 (TUc) values.

Results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollutant 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; results are reported as "Pass" if a sample is considered non-toxic at the IWC according to the TST calculation, or "Fail" if considered toxic at the IWC according to the TST. As the TST is not included in the CDP permit at this time, the TST analysis was performed for comparison purposes only.

Client: IDE Americas, Inc. Test ID: 1709-S036

Sample ID: M-001 Sample Date: September 5, 2017

### **RESULTS**

A Statistically significant decrease in fertilization rate was observed at 10 and 15 percent effluent concentrations compared to the lab control. The NOEC is reported as 6.06 and the TUc is equal to 16.5, which meets the maximum effluent limitation of 16.5 for this permit. None of the effluent concentrations were significantly reduced according to the TST analysis. Statistical results 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 copies of the chain-ofcustody form are in Appendices B and C, respectively.

Table 4. Statistical Results for the Purple Urchin Fertilization Testing

Sample I D	NOEC LOEC (% sample)		EC <sub>50</sub> (% sample)		TST Result (Pass/Fail)	Percent Effect at IWC	
M-001	6.06	10	>15	16.5	Pass	3.2	

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<sub>c</sub> = Chronic Toxic Unit: 100÷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 (% Sample)	Mean Percent Fertilization						
Lab Control	94.6						
2.5	95.0						
5.0	94.2						
6.06	91.6						
10	88.6*						
15	77.4*						

<sup>\*</sup>An asterisk indicates a statistically significant decrease compared to the lab control

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Sample Date: September 5, 2017

### **QUALITY ASSURANCE**

The sample was received on the day of collection and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. The PMSD value, which is a measure of test variability, was within the acceptable limits. Statistical analyses followed USEPA flowchart selections and doseresponse relationships were reviewed to ensure the reliability of the data. Based on the dose responses observed during testing, the calculated effect concentrations reported are deemed reliable. 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 met all test acceptability criteria. The median effect (EC50) value calculated for this test was within two standard deviations (2SD) of the historical mean for our laboratory, indicating organisms were of typical sensitivity to copper. Results for the reference toxicant test are summarized in Table 6 and presented in full in Appendix D. A list of qualifier codes can be found in Appendix E.

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC <sub>50</sub> (μg/L Copper)	Historical Mean EC <sub>50</sub> ±2 SD (µg/L Copper)	CV (%)
9/5/17	37.4	49.5 ± 34.2	34.5

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

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Client: IDE Americas, Inc. Test ID: 1709-S036 Sample ID: M-001 Sample Date: September 5, 2017

### REFERENCES

California Regional Water Quality Control Board Region 9, San Diego (RWQCB) 2006. Waste Discharge Requirements for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project, Discharge to the Pacific Ocean via the Encina Power Station Discharge Channel. Order No. R9-2006-0065, NPDES No. CA109223. June 2006.

- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- Tidepool Scientific Software. 2000-2013. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20
- 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:

11 Sep-17 11:12 (p 1 of 1)

Test Code:

1709-S036 | 01-3175-6211

								it oodo.		000010	1 0170 021
Echinoid Spe	rm Cell Fertiliza	tion Tes	t 15C						Nautilu	s Environr	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	12-7452-3096 05 Sep-17 17:0 05 Sep-17 17:4 40m	7 P 7 S	est Type: Protocol: Species: Source:	Fertilization EPA/600/R-95/136 (1995) Strongylocentrotus purpuratus Pt. Loma				alyst: uent: Laboratory Seawater ne: Not Applicable e:			
· ·	13-0420-6614 05 Sep-17 08:0 : 05 Sep-17 12:1 9h (3°C)	0 N 6 S	Code: flaterial: cource: station:	17-0974 Facility Effluent IDE Americas, Inc. M-001 (Daily) WELLO				ient: IDE oject: Carlsbad Desal Plant			
Comparison S	Summary					j					
Analysis ID	Endpoint	and the second s			TOEL	PMSD	TU	Method			
20-7682-4076	The second secon		6.06	10	7.785	4.21%	16.5	Dunnett M	lultiple Com	parison Te	st
Point Estimate	e Summary										
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Method			
03-7044-8438	•			>15 >15	N/A N/A	N/A N/A	<6.667 <6.667	Linear Inte	Linear Interpolation (ICPIN)		
Test Acceptab	oility										
Analysis ID	Endpoint		Attribu	ute	Test Stat	TAC Limi	ts	Overlap	Decision		
03-7044-8438	Fertilization Rat	e	Contro	ol Resp	0.946	0.7 - NL		Yes		cceptability	Criteria
20-7682-4076	Fertilization Rat		Contro	l Resp	0.946	0.7 - NL		Yes	Passes Acceptability Criteria		
20-7682-4076	Fertilization Rat	e	PMSD		0.04214	NL - 0.25		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	0.946	0.9272	0.9648	0.93	0.97	0.006782	0.01517	1.6%	0.0%
2.5		5	0.95	0.9138	0.9862	0.91	0.98	0.01304	0.02915	3.07%	-0.42%
5		5	0.942	0.9075	0.9765	0.9	0.97	0.01241	0.02775	2.95%	0.42%
6.06		5	0.916	0.869	0.963	0.88	0.96	0.01691	0.03782	4.13%	3.17%
10					0.0404	0.04	0.0	0.01166	0.02608	2.94%	6.34%
1 =		5	0.886	0.8536	0.9184	0.84	0.9	0.01100			
15		5 5	0.886 0.774	0.8536 0.7416	0.9184 0.8064	0.84	0.9	0.01166	0.02608	3.37%	18.18%
Fertilization R	Rate Detail										
	Rate Detail Control Type										
Fertilization R		5	0.774	0.7416	0.8064	0.75					
Fertilization R	Control Type	5 Rep 1	0.774 Rep 2	0.7416 Rep 3	0.8064 Rep 4	0.75 Rep 5					
Fertilization R C-%	Control Type	<b>Rep 1</b> 0.94	0.774 Rep 2 0.94	0.7416 <b>Rep 3</b> 0.95	0.8064 Rep 4 0.93	0.75 <b>Rep 5</b> 0.97					
Fertilization R C-% 0 2.5	Control Type	<b>Rep 1</b> 0.94 0.97	0.774  Rep 2  0.94  0.96	0.7416  Rep 3 0.95 0.93	0.8064 Rep 4 0.93 0.91	0.75  Rep 5 0.97 0.98					
Fertilization R C-% 0 2.5 5	Control Type	<b>Rep 1</b> 0.94 0.97 0.96	0.774  Rep 2 0.94 0.96 0.9	0.7416  Rep 3 0.95 0.93 0.95	0.8064 Rep 4 0.93 0.91 0.93	0.75  Rep 5 0.97 0.98 0.97					

Report Date:

11 Sep-17 11:11 (p 1 of 2)

Test Code:

1709-S036 | 01-3175-6211

Echinoid En											
Echinola Sp	erm Cell Fertiliz	ation Test	15C					2104	Nautilus	Environ	mental (CA)
Analysis ID:	20-7682-4076	En	idpoint: Fer	tilization Ra	te		CET	IS Version:	CETISv1.	8.7	
Analyzed:	11 Sep-17 11:	11 An	alysis: Par	rametric-Cor	trol vs Trea	tments	Offic	ial Results:	Yes		
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA	C > T	NA	NA		4.21%	6.06	10	7.785	16.5
Dunnett Mul	tiple Compariso	n Test									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(	α:5%)		
Lab Control	2.5	***********************	-0.434	2.362	0.079 8	0.9304	CDF		ficant Effect		
	5		0.1686	2.362	0.079 8	0.7787	CDF	•	ficant Effect		
	6.06		1.657	2.362	0.079 8	0.1769	CDF	Ū	icant Effect		
	10*		3.307	2.362	0.079 8	0.0063	CDF	Significant			
15*			7.854	2.362	0.079 8	< 0.0001	CDF	Significant			
ANOVA Tabl	e										
Source	Sum Squares Mean			ıare	DF	F Stat	P-Value	Decision(	α:5%)		
Between	0.275337		0.0550674	11	5	19.73	<0.0001	Significant	Effect		
Error	0.066989	34	0.0027912	223	24			J			
Total	0.342326		29	_							
Distribution	al Tests					A					
Attribute	Test						Doginian	a.10/\			
		i est Stat	Cillical	P-Value	Decision(	u. 1 /0/					
Variances	Bartlett E	Equality of \	/ariance				Decision( Equal Var			***************************************	
Variances Distribution		Equality of \		4.208 0.9717	15.09 0.9031	0.5198 0.5864	***************************************	iances			
Distribution				4.208	15.09	0.5198	Equal Var	iances			
Distribution	Shapiro-			4.208	15.09	0.5198	Equal Var	iances	Std Err	CV%	%Effect
Distribution Fertilization C-%	Shapiro- Rate Summary	Wilk W Nor	mality	4.208 0.9717	15.09 0.9031	0.5198 0.5864	Equal Var Normal Di	iances stribution	<b>Std Err</b> 0.006782	CV% 1.6%	%Effect
Distribution Fertilization C-% 0	Shapiro- Rate Summary Control Type	Wilk W Nor	mality  Mean	4.208 0.9717 95% LCL	15.09 0.9031 <b>95% UCL</b>	0.5198 0.5864 Median	Equal Var Normal Di	iances stribution Max			~
Distribution  Fertilization  C-%  0 2.5	Shapiro- Rate Summary Control Type	Wilk W Nor  Count  5	Mean 0.946	4.208 0.9717 95% LCL 0.9272	15.09 0.9031 <b>95% UCL</b> 0.9648	0.5198 0.5864 <b>Median</b> 0.94	Equal Var Normal Di Min 0.93	iances stribution Max 0.97	0.006782	1.6%	0.0% -0.42%
Distribution  Fertilization  C-%  0  2.5  5	Shapiro- Rate Summary Control Type	Count 5 5	Mean 0.946 0.95	4.208 0.9717 <b>95% LCL</b> 0.9272 0.9138	15.09 0.9031 <b>95% UCL</b> 0.9648 0.9862	0.5198 0.5864 <b>Median</b> 0.94 0.96	Equal Var Normal Di Min 0.93 0.91	Max 0.97 0.98	0.006782 0.01304	1.6% 3.07%	0.0% -0.42% 0.42%
Distribution Fertilization	Shapiro- Rate Summary Control Type	Count 5 5 5	Mean 0.946 0.95 0.942	4.208 0.9717 <b>95% LCL</b> 0.9272 0.9138 0.9075	15.09 0.9031 <b>95% UCL</b> 0.9648 0.9862 0.9765	0.5198 0.5864 Median 0.94 0.96 0.95 0.91	Min 0.93 0.91 0.9	Max 0.97 0.98 0.97	0.006782 0.01304 0.01241	1.6% 3.07% 2.95%	0.0% -0.42%
Fertilization C-% 0 2.5 5 6.06	Shapiro- Rate Summary Control Type	Count 5 5 5 5	Mean 0.946 0.95 0.942 0.916	4.208 0.9717 <b>95% LCL</b> 0.9272 0.9138 0.9075 0.869	15.09 0.9031 <b>95% UCL</b> 0.9648 0.9862 0.9765 0.963	0.5198 0.5864 Median 0.94 0.96 0.95	Equal Var Normal Di Min 0.93 0.91 0.9 0.88	Max 0.97 0.98 0.97 0.96	0.006782 0.01304 0.01241 0.01691	1.6% 3.07% 2.95% 4.13%	0.0% -0.42% 0.42% 3.17%
Fertilization C-% 0 2.5 5 6.06 10 15	Shapiro- Rate Summary Control Type	Count 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.946 0.95 0.942 0.916 0.886 0.774	4.208 0.9717 95% LCL 0.9272 0.9138 0.9075 0.869 0.8536	95% UCL 0.9648 0.9862 0.9765 0.963 0.9184	0.5198 0.5864 Median 0.94 0.96 0.95 0.91 0.9	Equal Var Normal Di Min 0.93 0.91 0.9 0.88 0.84	Max 0.97 0.98 0.97 0.96 0.9	0.006782 0.01304 0.01241 0.01691 0.01166	1.6% 3.07% 2.95% 4.13% 2.94%	0.0% -0.42% 0.42% 3.17% 6.34%
Pistribution  Fertilization  C-%  0  2.5  5  6.06  10  15  Angular (Con	Shapiro- Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.946 0.95 0.942 0.916 0.886 0.774	4.208 0.9717 95% LCL 0.9272 0.9138 0.9075 0.869 0.8536	95% UCL 0.9648 0.9862 0.9765 0.963 0.9184	0.5198 0.5864 Median 0.94 0.96 0.95 0.91 0.9	Equal Var Normal Di Min 0.93 0.91 0.9 0.88 0.84	Max 0.97 0.98 0.97 0.96 0.9	0.006782 0.01304 0.01241 0.01691 0.01166	1.6% 3.07% 2.95% 4.13% 2.94%	0.0% -0.42% 0.42% 3.17% 6.34%
Fertilization C-% 0 2.5 5 6.06 10 15	Shapiro- Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 7 med Sumr	Mean 0.946 0.95 0.942 0.916 0.886 0.774	4.208 0.9717 <b>95% LCL</b> 0.9272 0.9138 0.9075 0.869 0.8536 0.7416	95% UCL 0.9648 0.9862 0.9765 0.963 0.9184 0.8064	0.5198 0.5864 Median 0.94 0.96 0.95 0.91 0.9 0.77	Min 0.93 0.91 0.9 0.88 0.84 0.75	Max 0.97 0.98 0.97 0.96 0.9 0.81	0.006782 0.01304 0.01241 0.01691 0.01166 0.01166	1.6% 3.07% 2.95% 4.13% 2.94% 3.37%	0.0% -0.42% 0.42% 3.17% 6.34% 18.18%
Distribution  Fertilization C-% 0 2.5 5 6.06 10 15  Angular (Cor	Shapiro- Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 7 Treed Sumr	Mean 0.946 0.95 0.942 0.916 0.886 0.774 mary Mean	4.208 0.9717 95% LCL 0.9272 0.9138 0.9075 0.869 0.8536 0.7416	95% UCL 0.9648 0.9862 0.9765 0.963 0.9184 0.8064	0.5198 0.5864 Median 0.94 0.96 0.95 0.91 0.9 0.77	Min 0.93 0.91 0.9 0.88 0.84 0.75	Max 0.97 0.98 0.97 0.96 0.9 0.81	0.006782 0.01304 0.01241 0.01691 0.01166 0.01166	1.6% 3.07% 2.95% 4.13% 2.94% 3.37% CV% 2.68%	0.0% -0.42% 0.42% 3.17% 6.34% 18.18% %Effect 0.0%
Distribution  Fertilization C-% 0 2.5 5 6.06 10 15  Angular (Cor C-% 0 2.5	Shapiro- Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 cmed Sumr Count 5	Mean 0.946 0.95 0.942 0.916 0.886 0.774 mary Mean 1.338	4.208 0.9717 95% LCL 0.9272 0.9138 0.9075 0.869 0.8536 0.7416 95% LCL 1.294	95% UCL 0.9648 0.9862 0.9765 0.963 0.9184 0.8064 95% UCL 1.383	0.5198 0.5864 Median 0.94 0.96 0.95 0.91 0.9 0.77 Median 1.323 1.369	Min 0.93 0.91 0.9 0.88 0.84 0.75	Max 0.97 0.98 0.97 0.96 0.9 0.81  Max 1.397 1.429	0.006782 0.01304 0.01241 0.01691 0.01166 0.01166 Std Err 0.01605 0.02999	1.6% 3.07% 2.95% 4.13% 2.94% 3.37% CV% 2.68% 4.96%	0.0% -0.42% 0.42% 3.17% 6.34% 18.18% %Effect 0.0% -1.08%
Distribution  Fertilization C-% 0 2.5 5 6.06 10 15  Angular (Cor C-% 0 2.5 5	Shapiro- Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 Count Count Count 5 5 5 5 5 5 5 6 6 6 6 6 6 6 7 6 7 7 7 8 7 8 7 8 8 7 8 8 8 8	Mean 0.946 0.95 0.942 0.916 0.886 0.774  mary  Mean 1.338 1.353	4.208 0.9717 95% LCL 0.9272 0.9138 0.9075 0.869 0.8536 0.7416 95% LCL 1.294 1.27 1.261	95% UCL 0.9648 0.9662 0.9765 0.963 0.9184 0.8064 95% UCL 1.383 1.436 1.405	0.5198 0.5864 Median 0.94 0.96 0.95 0.91 0.9 0.77 Median 1.323 1.369 1.345	Min 0.93 0.91 0.9 0.88 0.84 0.75  Min 1.303 1.266 1.249	Max 0.97 0.98 0.97 0.96 0.9 0.81  Max 1.397 1.429 1.397	0.006782 0.01304 0.01241 0.01691 0.01166 0.01166 Std Err 0.01605 0.02999 0.02597	1.6% 3.07% 2.95% 4.13% 2.94% 3.37% CV% 2.68% 4.96% 4.36%	0.0% -0.42% 0.42% 3.17% 6.34% 18.18% %Effect 0.0% -1.08% 0.42%
Distribution  Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Shapiro- Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count  5 5 5 5 Count  Count  Count  5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.946 0.95 0.942 0.916 0.886 0.774  mary  Mean 1.338 1.353 1.333	4.208 0.9717 95% LCL 0.9272 0.9138 0.9075 0.869 0.8536 0.7416 95% LCL 1.294 1.27	95% UCL 0.9648 0.9662 0.9765 0.963 0.9184 0.8064 95% UCL 1.383 1.436	0.5198 0.5864 Median 0.94 0.96 0.95 0.91 0.9 0.77 Median 1.323 1.369	Min 0.93 0.91 0.9 0.88 0.84 0.75  Min 1.303 1.266	Max 0.97 0.98 0.97 0.96 0.9 0.81  Max 1.397 1.429	0.006782 0.01304 0.01241 0.01691 0.01166 0.01166 Std Err 0.01605 0.02999	1.6% 3.07% 2.95% 4.13% 2.94% 3.37% CV% 2.68% 4.96%	0.0% -0.42% 0.42% 3.17% 6.34% 18.18% %Effect 0.0% -1.08%

Report Date: Test Code: 11 Sep-17 11:12 (p 2 of 2) 1709-S036 | 01-3175-6211

**Echinoid Sperm Cell Fertilization Test 15C** Nautilus Environmental (CA) Analysis ID: 20-7682-4076 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 11 Sep-17 11:11 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 1.0 0.105 0.9 0.084 0.8 0.063 -Fertilization Rate 0.042 0.6 0.021 0.5 0,000 -0.021 0.3 -0.042 0.2 -0.063 0.1 -0.084 0.0 -0.105 0 LC 2.5 6.06 10 15 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 2.5 0.5 1.0 1.5 2.0 C-% Rankits

Report Date:

11 Sep-17 11:12 (p 1 of 1)

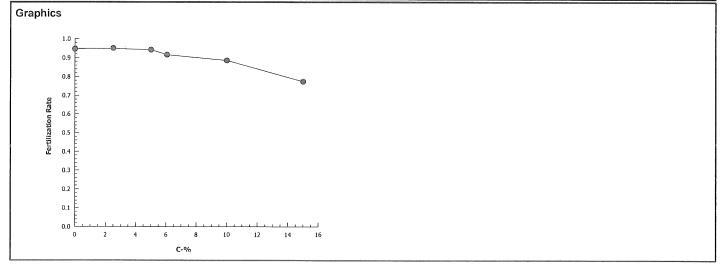
Test Code:

1709-S036 | 01-3175-6211

Echinoid Spe	rm Cell Fertilization 1	est 15C			Nautilus Environmental (CA)
Analysis ID:	03-7044-8438	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	11 Sep-17 11:11	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear	Linear Interpolation Options											
X Trans	sform	Y Transform	Seed	i	Resamples	Exp 95% CL	Method					
Linear		Linear	1887	'196	1000	Yes	Two-Point Interpolation					
Point E	stimates	>										
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										
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.946	0.93	0.97	0.006782	0.01517	1.6%	0.0%	473	500
2.5		5	0.95	0.91	0.98	0.01304	0.02915	3.07%	-0.42%	475	500
5		5	0.942	0.9	0.97	0.01241	0.02775	2.95%	0.42%	471	500
6.06		5	0.916	0.88	0.96	0.01691	0.03782	4.13%	3.17%	458	500
10		5	0.886	0.84	0.9	0.01166	0.02608	2.94%	6.34%	443	500
15		5	0.774	0.75	0.81	0.01166	0.02608	3.37%	18.18%	387	500



TST

Report Date: Test Code: 11 Sep-17 11:12 (p 1 of 1) 1709-S036 | 01-3175-6211

				<b>9</b>			Test	Code:	170	9-S036   0	1-3175-621	
Echinoid Sp	erm Cell Fertiliz	ation Test	15C						Nautilus	Environi	mental (CA)	
Analysis ID:	05-2467-0604	Er	ndpoint: f	Fertilization Rat	te		CET	IS Version	: CETISv1	.8.7		
Analyzed:	11 Sep-17 11	:11 <b>A</b> r	nalysis: [	Parametric Bioe	equivalence	-Two Samp	le Offic	cial Results	s: Yes	IAME TO A		
Data Transfe	orm	Zeta	Alt Hy	p Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU	
Angular (Cor	rected)	NA	C*b < T	NA	NA	0.75	1.68%	15	>15	NA	6.667	
TST-Welch's	s t Test											
Control	vs C-%		Test St	at Critical	MSD DF P-Value		P-Type	Decision	η(α:5%)			
Lab Control	2.5*		10.8	2.015	0.065 5	<0.0001	CDF	Non-Sigr	nificant Effect			
	5*		11.49	2.015	0.058 5	<0.0001	CDF	Non-Sigr	nificant Effect			
	6.06*		8.193	2.015	0.069 5	0.0002	CDF	Non-Sigr	nificant Effect			
	10*		10.58	1.895	0.040 7	<0.0001	CDF	Non-Sigr	nificant Effect			
15*			3.898	1.895	0.035 7	0.0030	CDF	Non-Sigr	nificant Effect			
ANOVA Tabl	le											
Source				Square	DF	F Stat	P-Value	Decision	n(α:5%)			
Between	0.275337	6741	5	19.73	<0.0001	Significa	nt Effect					
Error	0.066989	934	0.00279	91223	24							
Total	0.342326		29									
Distribution	al Tests				TO SECURE A							
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)				
Variances	Bartlett	Equality of \	Variance	4.208	15.09	0.5198	Equal Var	iances				
Distribution	Shapiro-	-Wilk W No	rmality	0.9717	0.9031	0.5864	Normal D	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	0.946	0.9272	0.9648	0.94	0.93	0.97	0.006782	1.6%	0.0%	
2.5		5	0.95	0.9138	0.9862	0.96	0.91	0.98	0.01304	3.07%	-0.42%	
5		5	0.942	0.9075	0.9765	0.95	0.9	0.97	0.01241	2.95%	0.42%	
6.06		5	0.916	0.869	0.963	0.91	0.88	0.96	0.01691	4.13%	3.17%	
10		5 .	0.886	0.8536	0.9184	0.9	0.84	0.9	0.01166	2.94%	6.34%	
15		5	0.774	0.7416	0.8064	0.77	0.75	0.81	0.01166	3.37%	18.18%	
Angular (Co	rrected) Transfo	rmed Sumi	mary									
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	1.338	1.294	1.383	1.323	1.303	1.397	0.01605	2.68%	0.0%	
2.5		5	1.353	1.27	1.436	1.369	1.266	1.429	0.02999	4.96%	-1.08%	
5		5	1.333	1.261	1.405	1.345	1.249	1.397	0.02597	4.36%	0.42%	
		_	4 000	4 404	4 272	1.266	1.217	1.369	0.03189	5.56%	4.14%	
6.06		5	1.283	1.194	1.372	1.200	1.217	1.503	0.03109	5.56%	4.14/0	
6.06 10 15		5 5 5	1.283	1.194	1.276	1.249	1.159	1.249	0.03789	3.17%	8.26%	

Analyst: QA: K9 M 17

Report Date:

01 Sep-17 09:25 (p 1 of 1)

Test Code: 1709-5086 01-3175-6211/7DA70B3

### **Echinoid Sperm Cell Fertilization Test 15C**

Nautilus Environmental (CA)

Start Date: End Date:

05 Sep-17 05 Sep-17

Species: Strongylocentrotus purpuratus

Sample Code: 17- 0974

ample Date: 05 Sep-17  C-% Code Rep Pos					al: Facility Eff	uent	Sample Station: M-001 (Daily) (9/5 Sam			samp
C-%	Code	Rep		# Counted	# Fertilized		Notes			
			61	loù	96	918/17	1			
			62		89		1			
			63		98					
			64		97					
			65		89					
			66		91					
			67		97					
			68		81					
			69		90					
			70		95	778.4.1811				
			71		96		****			
			72		97				70.	2 1 Mary 2000 - 11
			73		79					000
			74		<u> 15</u>			*****	78.4	
			75		75					
			76		95	WWW.	74			
			77		90	014101 0161				
	-		78 79		90-90	Q18 PL 9/8/17				
			80		93					***************************************
			81		96					
			82		88	***************************************	10°485aaa			
	-		83		88					
			84							
			85		94					
			86							
			87		93				***************************************	
			88		94	A				
			89		93					
			90		95					

Report Date:

01 Sep-17 09:25 (p 1 of 1)

Test Code: 1709-5036 01-3175-6211/7DA70B3

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

05 Sep-17 Start Date: Species: Strongylocentrotus purpuratus Sample Code: 17- 0974 End Date: 05 Sep-17 Protocol: EPA/600/R-95/136 (1995) Sample Source: IDE Americas, Inc.

Sample Date: 05 Sep-17 Sample Station: M-001 (Daily) (9/5 Sample) Material: Facility Effluent

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	84			
0	LC	2	88			
0	LC	3	70			
0	LC	4	79			
0	LC	5	64	100	96	m 95/17
2.5		1	72			
2.5		2	80			
2.5		3	87			
2.5		4	66			
2.5		5	63	$C(\mathcal{O})$	90	
5		1	61			
5		2	78			
5		3	90			
5		4	89			
5		5	67	$Co_L$	97	
6.06		1	76			
6.06		2	81			
6.06		3	82			
6.06		4	71			
6.06		5	83	100	90	
10		1	65			
10		2	69	200000000		
10		3	86			
10		4	77			
10		5	62	100	90	
15		1	75	`		
15		2	85			
15		3	73			
15		4	68			/
15		5	74	100	79	V

QU.CG

# **Marine Chronic Bioassay**

# **Water Quality Measurements**

Client :	IDE	Test Species: S. purpuratus
Sample ID:	M-001 Daily (9/5 sample)	Start Date/Time: 9/5/2017
Sample Log No.:	17-0974	End Date/Time: 9/5/2017
Dilutions made by:	AD	Test No: 1709 - 3036

	Analyst:						
	Initial Readings						
Concentration	DO	рН	Salinity	Temperature			
%	(mg/L)	(units)	(ppt)	(°C)			
Lab Control	8.4	8.07	394	14.7			
2.5	8.3	8.00	33.7	15.4			
5.0	83	8.0e	33.7	15.4			
6.06	8.4	8.00	33.7	15.4			
10	8.5	8.00	33.5	15.1			
15	8.5	8.06	33.7	15.0			

Comments:		
OC Charles	1/9/10/17	Final Review:
QC Check:	70 11011	rinai Review:

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

Marine Chronic Bio	passay	Echinoderm Sperm-Cell Fertilization Workshee
Client: Sample ID: Test No.:	1DE Daily M-001 (9/5 sample) 1709-s	Start Date/Time: 9/5/2017 / 1707 End Date/Time: 9/5/2017 / 1744 Species: S. purpuratus
Tech initials: Injection Time:	(G/A)	Animal Source: Pt. Lown Date Collected: 3 22 12
Sperm Absorbance at	400 nm: 0.923 (target range of 0.8 -	1.0 for density of 4x10 <sup>6</sup> sperm/ml)
Eggs Counted:	Mean: $115.6$ X 50 = $10.5$ (target counts of 80 eggs per ve Rafter slide for a final density of	rtical pass on Sedgwick-
Initial density: Final density:	124   126   5780 eggs/ml =   145 dilution f   4000 eggs/ml	stock seawater (9 39 45 ml
Prenare the embryo sto	ock according to the calculated dilution factor. For	example if the dilution factor is 2.25, use 100 ml of

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 1625 1644	<u>Ra</u>	ingefinder Ra SOLI 100 LI 100 LI 100 LI 100 LI	<u> </u>	52 1,88 12 1399 247			

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).

Definitive Test		Sperm:Egg Ratio l	Jsed: 150:\			
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1707 (727	QC1 QC2 Egg Control 1 Egg Control 2	(B) Fert. 9	Unfert. 6 - 9 100 100		4.
Comments:	(AXG 089/5/17	gboda a	15117			
QC Check:	AC 9/10/17			Final Review	v: 3 apm	

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

Appendix B

Sample Receipt Information

Nautilus Environmental 4340 Vandever Avenue San Diego, CA 92120

Test Performed: Urchin Fertilization

Client:	IDE
Project:	CDP Compliance
Test ID No(s).:	1709-5036

Sample ID:	Daily M-001 (9/	5 sample)		
Log-in No. (17-xxxx):	0974	V		
Sample Collection Date & Time:	915/17 0840			
Sample Receipt Date & Time:	7/5/17 1216			
Number of Containers & Container Type:	14-6			
Approx. Total Volume Received (L):	ルリレ			
Check-in Temperature (°C)	3.0			
Temperature OK? 1	N (Ŷ)	Y N	Y N	YN
DO (mg/L)	숭. O			
pH (units)	7.90			
Conductivity (µS/cm)	Curacaji andro			
Salinity (ppt)	33.7			
Alkalinity (mg/L) <sup>2</sup>	122			
Hardness (mg/L) <sup>2, 3</sup>				
Total Chlorine (mg/L)	20.02			
Technician Initials	CG,			

Test Performed:	Urchin Fertilization	Control/Dilution Water:	Lab seawater	
	Additional Control? Y N	Alkalinity: Alkalir		hity:34ppt Hardness or Salinity:
Test Performed:		Control/Dilution Water:	8:2 / Lab SW	/ Lab ART Other:
		Alkalinity:	Hardness or Salin	ity:
	Additional Control? Y N	= Alkalir	nity:	Hardness or Salinity:
Test Performed:		Control/Dilution Water:	8:2 / Lab SW	/ Lab ART Other:
		Alkalinity:	Hardness or Salin	ity:
	Additional Control? Y N	= Alkalir	ity:	Hardness or Salinity:
Notes:	<sup>1</sup> Temperature of sample should	d be 0-6°C, if received more	e than 24 hours pas	st collection time.
	<sup>2</sup> mg/L as CaCO3, <sup>3</sup> Measured	for freshwater samples only	, NA = Not Applica	able
ditional Comments:				

### Sample Check-In Information

A. colorless, cleo	G 10-101 (C)	>2 1 1 2 2 2	
COC Complete (Y/N) A B C	?		
Filtration? Y (N	)		
Pore Size:			
Organisms	or	Debris	
Salinity Adjustment?	YN		
Test:	Source:	Targe	et ppt:
Test:	Source:	Targe	et ppt:
Test:	Source:	Targe	et ppt:
pH Adjustment? Y	(N)		
	A	В	С
Initial pH	:		
Amount of HCI added			
Final pH			
Cl <sub>2</sub> Adjustment? Y(	N		
`	A	В	С
Initial Free Cl₂:			
STS added:			
Final Free Cl <sub>2</sub> :			
Sample Aeration? Y	(N)		
	A	В	С
Initial D.O.			
Duration & Rate			
Final D.O.			
Subsamples for Addi NH3 Othe	tional Chem	nistry Require	d? YN
Tech Initials A	\ B	_c	
	QC Che	eck: <u>A(9</u>	11/17
	Final Revi	iew: & 4/	DIVI

Appendix C

Chain-of-Custody Form



	Turn Around Time
Entahlpy Laboratory:	Normal: <u>X</u>
WECK Laboratory:	RUSH (24 hr):
Nautilus:X	3 Days:
AIM:	5 Days:
Osham	222 0

or many speciments (m. 1921). Speciments and the statement of the statemen	CONTRACTOR CONTRACTOR AND ADVANCED CONTRACTOR CONTRACTO	COLUMN TO THE COLUMN TO THE PROPERTY OF THE PROPERTY OF THE COLUMN TO TH	BUTTO TO SERVICE OF THE PROPERTY OF THE PROPER	- BM-4-70 10 <b>86</b> (31076-7444)		and the state of t	NAME AND ADDRESS OF THE OWNER.	and the same of the same		Othe				rrr Days
Project Name <u>: NPDES Daily / We</u>			t Manager: Peter Sher		Contact Information:_	(76	0) 201-7	777						
Special instruction: Sampled du intervals. Sample collected to f									ANALY:	SES	No. down to the Stay	***************************************		NOTES:
8:00, End: 9/5/17 @ 8:00 VH	•		•	•	, ,, = ,	Fertilizatior								
						rtiliz								
		ic Fe												
	Yes=Y No=N A			Chror										
Drinkin	g Water=DW Seawat	er=SW Soil=S Brine=	В	Pre		Purple Urchin Chronic	,							
Sample ID	Date	Time	Sample	Preservative	Container	e Urc								
			Туре	ive ?	Туре	Purpl								
M-001 (17- 2771)	9/4-5/2017	8:00-8:00	SW	N	4L CUBIE	Х						- III.	1) dr100 . , , , dr. (1) d	TDS - 31.80 ppt, EC - 49.56 mS/cm
												-		155 31.00 ppt, 20 45.50 ms/tm
												-		
														-
										$\dashv$				
Relinquished By:		Date:	Time:		Received By:			<del></del>	- I	ime:	v	10 10 mm	Samp	ole Condition Upon Receipt:
Ken S	1 ~~/	915/17	1045		LMESOLUI	5 V 2	7 5 2 ~		A COUNTY OF THE PARTY OF THE PA	3°. uS	x	Iced	П	Ambient or 3.0 °C
romer didn	t sign re	December 1997 - Constitution of the Constituti			Darl			and the same	Annual Company (Company )	2:16	×	Iced		Ambient or <u>₹</u> 0 °C

915/17

Nawfilus ID# 17-0974

Appendix D

Reference Toxicant Test Data and Statistical Analyses

# **CETIS Summary Report**

Report Date:

11 Sep-17 10:41 (p 1 of 1)

Test Code:

170905sprt | 13-1627-7974

								Test Code	:	1/0	905sprt   13	-1627-7974
Echinoid Spe	rm Cell Fertiliza	tion Test 1	5C							Nautilu	s Environm	ental (CA)
Batch ID: Start Date: Ending Date: Duration:	12-2217-0251 05 Sep-17 17:0 05 Sep-17 17:4 40m	<ul><li>7 Pro</li><li>7 Spe</li></ul>	t Type: tocol: ecies: irce:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma	Analyst: Diluent: Brine: Age:		ural Seawate Applicable	er				
Sample ID: Sample Date: Receive Date: Sample Age:	: 05 Sep-17	Sou	le: erial: irce: tion:	170905sprt Copper chloride Reference Toxi Copper Chlorid	Client: Project:	Inte	rnal					
Comparison S	Summary											
Analysis ID	Endpoint		NOEL	. LOEL	TOEL	PMSD	TU	Met	hod			
18-7744-8472	Fertilization Rat	е	<10	10	NA	5.09%		Dun	nett M	lultiple Com	parison Tes	t
Point Estimat	e Summary											
Analysis ID	Endpoint		Level	μg/L	95% LCL	95% UCL	ΤU	Met	hod			
14-5447-1160	Fertilization Rat	е	EC50	37.36	35.71	39.09		Trim	nmed :	Spearman-k	(ärber	
Test Acceptal	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	ts	Ove	rlap	Decision		
14-5447-1160	Fertilization Rat	е	Contro	ol Resp	0.962	0.7 - NL		Yes	-	Passes A	cceptability	Criteria
18-7744-8472	Fertilization Rat	е	Contro	ol Resp	0.962	0.7 - NL		Yes		Passes A	cceptability	Criteria
18-7744-8472	Fertilization Rat	е	PMSE	)	0.05094	NL - 0.25	~770	No		Passes A	cceptability	Criteria
Fertilization F	Rate Summary											
C-μg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std	Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.962	0.9484	0.9756	0.95	0.98	0.00	4899	0.01096	1.14%	0.0%
10		5	0.898	0.8538	0.9422	0.86	0.94	0.01	594	0.03564	3.97%	6.65%
20		5	0.81	0.6887	0.9313	0.7	0.9	0.04		0.09772	12.06%	15.8%
40		5	0.506	0.4435	0.5685	0.46	0.59			0.0503	9.94%	47.4%
80		5	0.022	0.003583	0.04042	0	0.04		6633	0.01483	67.42%	97.71%
160		5	0	0	0	0	0	0		0		100.0%
Fertilization R	Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2		Rep 4	Rep 5						**
0	Lab Control	0.95	0.96	0.98	0.96	0.96						
10		0.94	0.87	0.93	0.86	0.89						
20		0.9	0.85	0.89	0.71	0.7						
40		0.59	0.48	0.46	0.51	0.49						
80		0.02	0.03	0.04	0.02	0						
160		0	0	0	0	0						

Report Date: Test Code: 11 Sep-17 10:37 (p 1 of 2) 170905sprt | 13-1627-7974

							Test	Code:	1709	905sprt   13	-1627-797
Echinoid Spe	erm Cell Fertiliz	ation Test	15C		***				Nautilus	Environn	nental (CA
Analysis ID:	18-7744-8472	. En	dpoint: Fer	tilization Rat	e		CET	S Version:	CETISv1.	8.7	
Analyzed:	11 Sep-17 10		•	ametric-Cor		tments		ial Results			
Data Transfo	rm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr		NA	C > T	NA	NA	<del>-,</del>	5.09%	<10	10	NA	
Dunnett Mult	iple Compariso	n Test									
Control	vs C-µg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	10*		2.781	2.305	0.105 8	0.0191	CDF	Significan	t Effect		
	20*		5.412	2.305	0.105 8	< 0.0001	CDF	Significan	t Effect		
	40*		12.83	2.305	0.105 8	< 0.0001	CDF	Significan	t Effect		
	80*		27.07	2.305	0.105 8	<0.0001	CDF	Significan	t Effect		
ANOVA Table	9										
Source	Sum Sq	uares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	4.90729		1.226822		4	235.9	<0.0001	Significan	t Effect		
Error	0.10403	18	0.0052015	89	20						
Total	5.01132	1			24						
Distributiona	ıl Tests										90000000000000000000000000000000000000
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)		~	·
Variances	Bartlett	Equality of \	/ariance	7.686	13.28	0.1038	Equal Var	iances			
Distribution	Shapiro	-Wilk W No	rmality	0.9744	0.8877	0.7571	Normal D	stribution			
Fertilization	Rate Summary										
C-μg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.962	0.9484	0.9756	0.96	0.95	0.98	0.004899	1.14%	0.0%
10		5	0.898	0.8538	0.9422	0.89	0.86	0.94	0.01594	3.97%	6.65%
20		5	0.81	0.6887	0.9313	0.85	0.7	0.9	0.0437	12.06%	15.8%
40		5	0.506	0.4435	0.5685	0.49	0.46	0.59	0.02249	9.94%	47.4%
80		5	0.022	0.003583	0.04042	0.02	0	0.04	0.006633	67.42%	97.71%
160		5	0	0	0	0	0	0	0		100.0%
Angular (Cor	rected) Transfo	ormed Sumi	mary								
C-μg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.376	1.338	1.415	1.369	1.345	1.429	0.01391	2.26%	0.0%
10		5	1.25	1.174	1.325	1.233	1.187	1.323	0.02714	4.86%	9.21%
20		5	1.13	0.9748	1.284	1.173	0.9912	1.249	0.05577	11.04%	17.93%
40		5	0.7915	0.7288	0.8542	0.7754	0.7454	0.8759	0.02259	6.38%	42.5%
80		5	0.1419	0.07103	0.2127	0.1419	0.05002	0.2014	0.02551	40.21%	89.69%
400		_	0.0000	0.05004	0.05000	0.05000	0.05000	0.05000	0	0.00/	06 270/

160

5

0.05002

0.05001

0.05003

0.05002

0.05002

0.05002

0

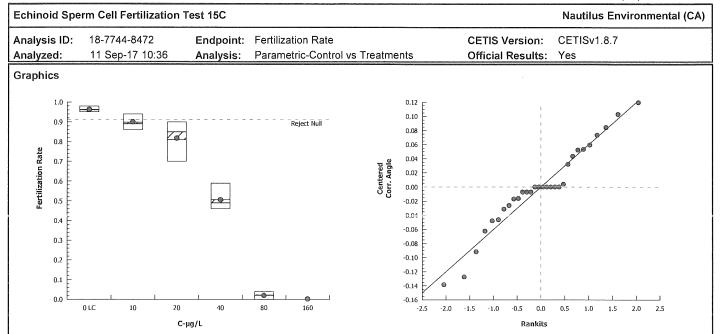
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96.37%

Report Date: Test Code:

11 Sep-17 10:37 (p 2 of 2)

170905sprt | 13-1627-7974



Report Date:

11 Sep-17 10:37 (p 1 of 1)

Test Code:

170905sprt | 13-1627-7974

Echinoid Sperm Cell Fertilization Test 15C	Nautilus Environmental (CA)

Analysis ID: 14-5447-1160 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7

Analyzed: 11 Sep-17 10:37 Analysis: Trimmed Spearman-Kärber Official Results: Yes

Trimmed Spearman-Na	arber Estimates						
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.038	6.65%	1.572	0.00982	37.36	35.71	39.09

Fertilizati	on Rate Summary										
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.962	0.95	0.98	0.004899	0.01096	1.14%	0.0%	481	500
10		5	0.898	0.86	0.94	0.01594	0.03564	3.97%	6.65%	449	500
20		5	0.81	0.7	0.9	0.0437	0.09772	12.06%	15.8%	405	500
40		5	0.506	0.46	0.59	0.02249	0.0503	9.94%	47.4%	252	500
80		5	0.022	0	0.04	0.006633	0.01483	67.42%	97.71%	11	500
160		5	0	0	0	0	0		100.0%	0	500

## Graphics 8.0 0.5 0.3 0.2 0.0 20 40 60 80 100 120 140 160 C-µg/L

**Report Date:** 11 Sep-17 10:39 ( 1 of 1)

### **Echinoid Sperm Cell Fertilization Test 15C**

Sigma:

17.08

CV:

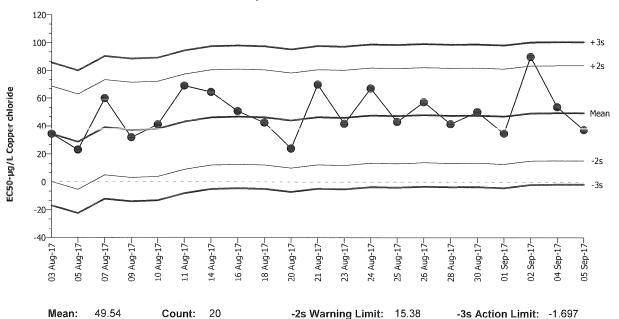
34.50%

### 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**



+2s Warning Limit:

83.7

+3s Action Limit: 100.8

21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	Quality (	Contr	rol Data	3								
5       19:25       23.07       -26.47       -1.55       11-5994-0488       10-6029-2098         7       15:10       59.94       10.4       0.6088       21-2468-7505       14-3489-7019         9       17:08       31.92       -17.62       -1.032       13-6999-3036       11-7131-4234         10       16:51       41.14       -8.404       -0.4921       00-5471-5288       12-0643-2211         11       14:50       69.03       19.49       1.141       04-5796-5476       07-8184-6783         14       14:40       64.51       14.97       0.8762       02-4510-8526       01-5460-0814         16       16:34       50.82       1.277       0.07476       16-3259-1018       06-7497-1035         18       14:09       42.53       -7.009       -0.4104       12-6613-4538       02-2322-5589         20       14:52       24.05       -25.49       -1.492       06-9655-0092       05-8785-3700         21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1	Point Ye	ear N	Month	Day	Time	QC Data	Deita	Sigma	Warning	Action	Test ID	Analysis ID
7       15:10       59.94       10.4       0.6088       21-2468-7505       14-3489-7019         9       17:08       31.92       -17.62       -1.032       13-6999-3036       11-7131-4234         10       16:51       41.14       -8.404       -0.4921       00-5471-5288       12-0643-2211         11       14:50       69.03       19.49       1.141       04-5796-5476       07-8184-6783         14       14:40       64.51       14.97       0.8762       02-4510-8526       01-5460-0814         16       16:34       50.82       1.277       0.07476       16-3259-1018       06-7497-1035         18       14:09       42.53       -7.009       -0.4104       12-6613-4538       02-2322-5589         20       14:52       24.05       -25.49       -1.492       06-9655-0092       05-8785-3700         21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11	1 20	017 <i>A</i>	Aug	3	0:00	34.43	-15.11	-0.8844			02-7356-2235	20-3051-4002
9 17:08 31.92 -17.62 -1.032 13-6999-3036 11-7131-4234 10 16:51 41.14 -8.404 -0.4921 00-5471-5288 12-0643-2211 11 14:50 69.03 19.49 1.141 04-5796-5476 07-8184-6783 14 14:40 64.51 14.97 0.8762 02-4510-8526 01-5460-0814 16 16:34 50.82 1.277 0.07476 16-3259-1018 06-7497-1035 18 14:09 42.53 -7.009 -0.4104 12-6613-4538 02-2322-5589 20 14:52 24.05 -25.49 -1.492 06-9655-0092 05-8785-3700 21 14:46 69.95 20.41 1.195 08-4756-2919 20-2992-4955 23 16:14 41.72 -7.823 -0.458 02-7595-3678 15-3490-2746 24 16:11 67.1 17.56 1.028 04-7651-5518 20-0883-0005 25 14:48 43.11 -6.43 -0.3765 06-8816-1100 09-0830-4014 26 16:00 57.24 7.701 0.4509 10-2039-5656 15-8794-0305 28 14:56 41.55 -7.988 -0.4677 08-1525-2751 10-7829-2432 30 16:38 50.21 0.6719 0.03934 08-1199-3706 11-0543-3886	2			5	19:25	23.07	-26.47	-1.55			11-5994-0488	10-6029-2098
10       16:51       41.14       -8.404       -0.4921       00-5471-5288       12-0643-2211         11       14:50       69.03       19.49       1.141       04-5796-5476       07-8184-6783         14       14:40       64.51       14.97       0.8762       02-4510-8526       01-5460-0814         16       16:34       50.82       1.277       0.07476       16-3259-1018       06-7497-1035         18       14:09       42.53       -7.009       -0.4104       12-6613-4538       02-2322-5589         20       14:52       24.05       -25.49       -1.492       06-9655-0092       05-8785-3700         21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55	3			7	15:10	59.94	10.4	0.6088			21-2468-7505	14-3489-7019
11       14:50       69.03       19.49       1.141       04-5796-5476       07-8184-6783         14       14:40       64.51       14.97       0.8762       02-4510-8526       01-5460-0814         16       16:34       50.82       1.277       0.07476       16-3259-1018       06-7497-1035         18       14:09       42.53       -7.009       -0.4104       12-6613-4538       02-2322-5589         20       14:52       24.05       -25.49       -1.492       06-9655-0092       05-8785-3700         21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21	4			9	17:08	31.92	-17.62	-1.032			13-6999-3036	11-7131-4234
14       14:40       64.51       14.97       0.8762       02-4510-8526       01-5460-0814         16       16:34       50.82       1.277       0.07476       16-3259-1018       06-7497-1035         18       14:09       42.53       -7.009       -0.4104       12-6613-4538       02-2322-5589         20       14:52       24.05       -25.49       -1.492       06-9655-0092       05-8785-3700         21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	5			10	16:51	41.14	-8.404	-0.4921			00-5471-5288	12-0643-2211
16       16:34       50.82       1.277       0.07476       16-3259-1018       06-7497-1035         18       14:09       42.53       -7.009       -0.4104       12-6613-4538       02-2322-5589         20       14:52       24.05       -25.49       -1.492       06-9655-0092       05-8785-3700         21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	6			11	14:50	69.03	19.49	1.141			04-5796-5476	07-8184-6783
18       14:09       42.53       -7.009       -0.4104       12-6613-4538       02-2322-5589         20       14:52       24.05       -25.49       -1.492       06-9655-0092       05-8785-3700         21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	7			14	14:40	64.51	14.97	0.8762			02-4510-8526	01-5460-0814
20       14:52       24.05       -25.49       -1.492       06-9655-0092       05-8785-3700         21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	8			16	16:34	50.82	1.277	0.07476			16-3259-1018	06-7497-1035
21       14:46       69.95       20.41       1.195       08-4756-2919       20-2992-4955         23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	9			18	14:09	42.53	-7.009	-0.4104			12-6613-4538	02-2322-5589
23       16:14       41.72       -7.823       -0.458       02-7595-3678       15-3490-2746         24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	10			20	14:52	24.05	-25.49	-1.492			06-9655-0092	05-8785-3700
24       16:11       67.1       17.56       1.028       04-7651-5518       20-0883-0005         25       14:48       43.11       -6.43       -0.3765       06-8816-1100       09-0830-4014         26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	11			21	14:46	69.95	20.41	1.195			08-4756-2919	20-2992-4955
25     14:48     43.11     -6.43     -0.3765     06-8816-1100     09-0830-4014       26     16:00     57.24     7.701     0.4509     10-2039-5656     15-8794-0305       28     14:56     41.55     -7.988     -0.4677     08-1525-2751     10-7829-2432       30     16:38     50.21     0.6719     0.03934     08-1199-3706     11-0543-3886	12			23	16:14	41.72	-7.823	-0.458			02-7595-3678	15-3490-2746
26       16:00       57.24       7.701       0.4509       10-2039-5656       15-8794-0305         28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	13			24	16:11	67.1	17.56	1.028			04-7651-5518	20-0883-0005
28       14:56       41.55       -7.988       -0.4677       08-1525-2751       10-7829-2432         30       16:38       50.21       0.6719       0.03934       08-1199-3706       11-0543-3886	14			25	14:48	43.11	-6.43	-0.3765			06-8816-1100	09-0830-4014
30 16:38 50.21 0.6719 0.03934 08-1199-3706 11-0543-3886	15			26	16:00	57.24	7.701	0.4509			10-2039-5656	15-8794-0305
	16			28	14:56	41.55	-7.988	-0.4677			08-1525-2751	10-7829-2432
Sep. 1 15:27 34.79 -14.75 -0.8633 13-1244-6646 21-1567-7550	17			30	16:38	50.21	0.6719	0.03934			08-1199-3706	11-0543-3886
000 1 10.27 01.70 11.70 0.0000	18	5	Sep	1	15:27	34.79	-14.75	-0.8633			13-1244-6646	21-1567-7550
2 10:53 89.99 40.45 2.369 (+) 16-4202-9692 18-8681-1855	19			2	10:53	89.99	40.45	2.369	(+)		16-4202-9692	18-8681-1855
4 16:10 53.77 4.232 0.2478 12-2973-1405 10-6032-1229	20			4	16:10	53.77	4.232	0.2478			12-2973-1405	10-6032-1229
5 0:00 37.36 -12.18 -0.7132 13-1627-7974 14-5447-1160	21			5	0:00	37.36	-12.18	-0.7132			13-1627-7974	14-5447-1160

### **CETIS Test Data Worksheet**

Report Date: Test Code: 01 Sep-17 09:24 (p 1 of 1)

13-1627-7974/170905sprt

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Start Date: 05 End Date: 05

05 Sep-17 05 Sep-17 **Species:** Strongylocentrotus purpuratus **Protocol:** EPA/600/R-95/136 (1995)

Sample Code: 170905sprt
Sample Source: Reference Toxicant

Sample Date: 05 Sep-17

Material: Copper chloride

Sample Station: Copper Chloride

C-µg/L	Code	Ren	Pos	# Counted	# Fertilized	loride Sample Station: Copper Chloride  Notes
μg/L	Code	veb				
			1	100	49	9/8/17
***	-		2		46	
			3		90	
	_		4		93	
			5		87	
			6		70	
			7		96	
			8		51	
			9		O	
			10		89	
			11		89 0 8 3 0	
			12		<b>Ø</b> 3	Q18 RL 9/8/17
			13			
			14		95	
			15		89 .	
			16		0 Ø 3	Q18 pl 918/17
			17		0	
			18		96	
			19		48	
			20		Ó	
			21		4	
			22		94	
			23		9	
			24		0	
····			25		59	
			26		86	
			27		71	
			28		98	
			29		96	
			30		85	

Report Date:

01 Sep-17 09:24 (p 1 of 1)

Test Code:

13-1627-7974/170905sprt

# Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA)

Start Date:05 Sep-17Species:Strongylocentrotus purpuratusSample Code:170905sprtEnd Date:05 Sep-17Protocol:EPA/600/R-95/136 (1995)Sample Source:Reference ToxicantSample Date:05 Sep-17Material:Copper chlorideSample Station:Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	14			
0	LC	2	29			
0	LC	3	28			
0	LC	4	7			
0	LC	5	18	$OO_f$	97	AD 9517
10		1	22			
10		2	5			
10		3	4			
10		4	26			
10		5	10	100	92	
20		1	3	1		
20		2	30			
20		3	15			
20		4	27			
20		5	6	100	59	
40		1	25			
40		2	19			
40		3	2			
40		4	8			
40		5	1	100	30	
80		1	23			
80		2	12			
80		3	21			
80		4	16			
80		5	13	voi	0	
160		1	11			
160		2	24			
160		3	17			
160		4	20			/
160		5	9	100	0	

AC- 66

### Marine Chronic Bioassay

### **Water Quality Measurements**

Analyst:

Client :	Internal	Test Species: S. purpuratus
Sample ID:	CuCl <sub>2</sub>	Start Date/Time: 9/5/2017
Test No:	_170905sprt	End Date/Time: 9/5/2017

Dilutions made by: AD

High conc. made (μg/L): 160

Vol. Cu stock added (mL): 7.8

Final Volume (mL): 500

Cu stock concentration (µg/L): 10200

A00 Initial Readings DO Concentration Salinity Temperature рΗ  $(\mu g/L)$ (mg/L) (units) (ppt) (°C) Lab Control 8.2 804 16.0 8.0 10 8.02 15.6 8:01 20 1.9 8.01 15.9 40 1.9 8.02 15.8 80 160 8.0 8.02 33.2 156

Comments:		
QC Check:	AC 9/11/17	Final Review: VTP 9/20/17

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

# Marine Chronic Bioassay

# **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Sample ID: Test No.:	Ginternal Conces				Start Date/Time: 9/5/2017 / 1707 End Date/Time: 9/5/2017 / 7444  Species: S. purpuratus  Animal Source: Pt, Loma				
Tech initials: Injection Time:	(4/AD					Date Collect			
Sperm Absorbance at	400 nm: <u>0.913</u>	(targ	et range of	0.8 - 1.0 for d	ensity of 4	x10 <sup>6</sup> sperm/i	ml)		
Eggs Counted:	105	target counts	of 80 eggs	50 = 578  per vertical pas sity of 4000 egg	s on Sedgv	gs/ml vick-			
Initial density: Final density:	126 5780 eggs/ 4000 eggs/		1.0par	ition factor t egg stock ts seawater		g stock Awater (a)	-22 \000 59 45 m		
Prepare the embryo sto existing stock (1 part) a	ock according to the cand 125 ml of dilution v	alculated dili vater (1.25 p	ution factor parts).	. For example	e, if the dilu	ution factor is	3 2.25, use 1	00 ml of	
	•			Sperm:Egg	Ratio				
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1 <u>1</u> 50 0.0	<b>600:1</b> 40 10	1200:1 30 20	800:1 20 30	400:1 10 40	200:1 5.0 45	100:1 2.5 47.5	50:1 1.25 48.75	
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1625 1644 1654		efinder Rati	0: Fert. 41 88,85 98,85	Unfe				
NOTE: Choose a sperr this range, choose the organism health, stage	e ratio closest to 90	percent unl	ess profes	een 80 and 90 sional judgme	percent. ent dictate	If more than es considera	one concer tion of othe	ntration is within er factors (e.g.,	
Definitive Test	Sperm:Egg Ratio Used: _\footsymbol{GO:\}								
Sperm Added (100 μl): Eggs Added (0.5 ml): Test Ended:	Time 1707 1727		Control 1 Control 2	B Fert.	Unfe 9 10 10	ert. 9		ā	
Comments:	(A)CG 6487/5/17	BAO	6180	115117					
QC Check:  Nautilus Environmental. 434	AC 91111	T n Diego, CA 9	2120.			Final Reviev	w: Kpal	2017	

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 or ganisms r eceived at a <u>temperature</u> greater than 3°C ou tside the r ecommended t est 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. O rganisms 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. H owever, due t o age -specific pr otocol r equirements and/ or s ample ho lding t ime 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