

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

Sample ID: M-001 (Daily/Weekly) Sample Collection Date: September 14, 2017

Prepared for: IDE AMERICAS, Inc.

4590 Carlsbad Boulevard Carlsbad, CA 92008

Prepared by: Nautilus Environmental

Submitted: October 5, 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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San Diego, California 92120 858.587.7333 fax: 858.587.3961 Results verified by: Adrienne libor

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT - SEPTEMBER 2017
ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: September 14, 2017

<u>Test Date:</u> September 14, 2017

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

Effluent Limitation: 16.5 TU_c

Results Summary:

	Effluent Te	st Results	Effluent Limitation
Bioassay Type:	NOEC	TU_c	Met? (Yes/No)
Urchin Fertilization	2.5	40	No

Client: IDE Americas, Inc. Test ID: 1709-S129 Sample ID: M-001

Sample Date: September 14, 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 14, 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 (off-spec period & weekly sample)
Monitoring Period:	September 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	9/14/17, 08:00
Sample Receipt Date, Time:	9/14/17, 12:52

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 CaCO₃)	(mg/L)
9/14/17	7.73	8.7	4.5	32.9	111	0.03

TOXICITY SUMMARY REPORT

Client: IDE Americas, Inc. Test ID: 1709-S129 Sample ID: M-001

Sample Date: September 14, 2017

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times: 9/14/17, 15:24 through 16:04

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:

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 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.

Test ID: 1709-S129

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RESULTS

A statistically significant decrease in fertilization rate was observed in the 5, 6.06, 10, and 15 percent effluent concentrations compared to the lab control. The NOEC is reported as 2.5 and the TU_c is equal to 40, which is above the maximum effluent limitation of 16.5 for this permit. According to the TST analysis no significant effect was observed at any of the effluent concentrations tested when compared to lab control. 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-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for Purple Urchin Fertilization Testing

Sample I D	NOEC (% sample)	LOEC (% sample)	EC ₅₀ (% sample)		TST Result (Pass/Fail)	Percent Effect at IWC
M-001	2.5	5	>15	40	Pass	6.1

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 (% Sample)	Mean Percent Fertilization
Lab Control	92.2
2.5	90.8
5.0	84.0*
6.06	86.6*
10	75.0*
15	74.0*

^{*}An asterisk indicates a statistically significant decrease compared to the lab control

Client: IDE Americas, Inc. Test ID: 1709-S129 Sample ID: M-001

Sample Date: September 14, 2017

QUALITY ASSURANCE

The sample was received on the day it was collected 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 dose-response 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 ₅₀ (µg/L Copper)	Historical Mean EC ₅₀ ±2 SD (µg/L Copper)	CV (%)
9/14/17	34.2	51.2 ± 32.7	31.9

EC₅₀ = Concentration expected to cause an adverse effect to 50 percent of the test organisms Historical Mean EC₅₀ \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: 1709-S129 Sample ID: M-001

Sample Date: September 14, 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:

19 Sep-17 15:17 (p 1 of 1)

Test Code:

1709-S129 | 04-7331-4478

Echinoid Sperm Cell Fertilization Test 15C	Nautil	us Environ	mental (CA
Potest ID: 02.0200.2550 Total Total Total			•
	aboratory Se Not Applicable		
	DE Carlsbad Desa	al Plant	
Comparison Summary			
Analysis ID Endpoint NOEL LOEL TOEL PMSD TU Method	d		
	t Multiple Cor	mparison Te	est
Point Estimate Summary			
Analysis ID Endpoint Level % 95% LCL 95% UCL TU Method	d		
	Interpolation ((ICPIN)	
Test Acceptability			
Analysis ID Endpoint Attribute Test Stat TAC Limits Overland	p Decisior	n	
09-1999-1776 Fertilization Rate Control Resp 0.922 0.7 - NL Yes		Acceptability	v Criteria
18-9449-2802 Fertilization Rate Control Resp 0.922 0.7 - NL Yes		Acceptability	•
18-9449-2802 Fertilization Rate PMSD 0.0378 NL - 0.25 No	Passes A	Acceptability	y Criteria
Fertilization Rate Summary			
C-% Control Type Count Mean 95% LCL 95% UCL Min Max Std Err	Std Dev	CV%	%Effect
0 Lab Control 5 0.922 0.9058 0.9382 0.91 0.94 0.00583	31 0.01304	1.41%	0.0%
2.5 5 0.908 0.8918 0.9242 0.89 0.92 0.00583	31 0.01304	1.44%	1.52%
5 0.84 0.7761 0.9039 0.76 0.89 0.02302	2 0.05148	6.13%	8.89%
6.06 5 0.866 0.8452 0.8868 0.85 0.89 0.00748	33 0.01673	1.93%	6.07%
10 5 0.75 0.7376 0.7624 0.74 0.76 0.00447		1.33%	18.66%
15 5 0.74 0.6798 0.8002 0.7 0.81 0.02168	0.04848	6.55%	19.74%
Fertilization Rate Detail			
C-% Control Type Rep 1 Rep 2 Rep 3 Rep 4 Rep 5			
0 Lab Control 0.93 0.94 0.91 0.92 0.91			
2.5 0.91 0.89 0.9 0.92 0.92			
5 0.88 0.89 0.84 0.83 0.76			
6.06 0.85 0.87 0.87 0.89 0.85			
10 0.76 0.74 0.75 0.76 0.74			
15 0.7 0.72 0.77 0.7 0.81			

Report Date:

19 Sep-17 15:17 (p 1 of 2)

Test Code:

1709-S129 | 04-7331-4478

2.5 5 1.263 1.235 1.291 1.266 1.233 1.284 0.01001 1.77% 1.97% 5 1.163 1.077 1.248 1.159 1.059 1.233 0.03078 5.92% 9.76% 6.06 5 1.197 1.166 1.227 1.202 1.173 1.233 0.01111 2.08% 7.14% 10 5 1.047 1.033 1.062 1.047 1.036 1.059 0.005164 1.1% 18.72%								1631	Code:	170	3-3123 0	14-7331-4478
Analyzer: 19 Sp-17 (15 !-5) Analyser: Parametricorrows (17 start) Tible Sp-17 (15 !-5) Alt Hyp Trials Sector PMSD NOEL LOEL TOE TUD Angular (Corrows) Na 2 s 2 Na	Echinoid Sp	erm Cell Fertiliz	ation Test	15C						Nautilus	Environ	mental (CA)
Angular (Corrected) NA	•			=			ıtments				.8.7	
May May	Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Control vs C-% Test Stat Critical MSD DF P-Value p-Type Decision:cs-%; Lab Control 2.5 0.9916 2.382 0.000 8 0.0001 CDF Non-Significant Effect Significant Effect 6.06° 3.6 2.362 0.060 8 0.0001 CDF Significant Effect 10° 9.445 2.362 0.060 8 0.0001 CDF Significant Effect Significant Effect To 10° 9.445 2.362 0.060 8 0.0001 CDF Significant Effect	Angular (Cor	rected)	NA	C > T	NA	NA		-				
	Dunnett Mul	tiple Compariso	n Test									
Part	Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(α:5%)		
	Lab Control	2.5		0.9916	2.362	0.060 8	0.4237		Non-Signif	icant Effect		
		5*		4.924	2.362	0.060 8	0.0001		J			
10° 9.45 2.362 0.060 8 0.0001 CDF Significant Effect		6.06*		3.6	2.362	0.060 8	0.0031	CDF	•			
Note		10*		9.445	2.362	0.060 8	<0.0001					
Source Sum Squata Mean Squata DF F Stat P-Value Decision(c:5%) Significant Effect State of Control (1.5%) Significant Effect State of Control (1.5%) Significant Effect State of Control (1.5%) State of Control (1.5%) Significant Effect State of Control (1.5%) State of Contro	1	15*		9.84	2.362	0.060 8	<0.0001		•			
Between	ANOVA Tabl	е										
First 0.03915038 0.001631266 24 29 29 29 20 20 20 20 20	Source	Sum Squ	uares	Mean Squ	ıare	DF	F Stat	P-Value	Decision(α:5%)		
Error 0.0016326 24 Total 0.3196326 29 Distribution: Tests Test Stat Critical P-Value Decision(c:1%) Attribute Test State	Between	0.280482	22	0.0560964	13	5	34.39	<0.0001	Significant	Effect		E
Distributional Tests Test Test Stat Critical P-Value Decision(α:1%)	Error	0.039150	38	0.0016312	266	24			9			
Attribute Test State of the properties of t	Total	0.319632	?6			29	-					
Variances Bartlett Equality of Variance 14.22 15.09 0.0143 Equal Variances Shapiro-Wilk W Normality 0.9625 0.9031 0.3580 Normal Distribution	Distributiona	al Tests										
Distribution Shapiro-Wilk W Normality D.9625 D.9031 D.3580 Normal Distribution	Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
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C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 0.922 0.9058 0.9382 0.92 0.91 0.94 0.005831 1.41% 0.0% 2.5 5 0.908 0.8918 0.9242 0.91 0.89 0.92 0.005831 1.44% 1.52% 5 0.84 0.7761 0.9039 0.84 0.76 0.89 0.02302 6.13% 8.89% 6.06 5 0.866 0.8452 0.8868 0.87 0.85 0.89 0.007483 1.93% 6.07% 10 5 0.75 0.7376 0.7624 0.75 0.74 0.76 0.004473 1.33% 18.66% 15 0.74 0.6798 0.8002 0.72 0.7 0.81 0.02168 6.55% 19.74% C-% Control Type Count Mean	Distribution	Shapiro-	Wilk W No	rmality	0.9625		0.3580	Normal Di	istribution			
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6.06 5 0.866 0.8452 0.8868 0.87 0.85 0.89 0.007483 1.93% 6.07% 10 5 0.75 0.7376 0.7624 0.75 0.74 0.76 0.004473 1.33% 18.66% 15 5 0.74 0.6798 0.8002 0.72 0.7 0.81 0.02168 6.55% 19.74% Angular (Corrected) Transformed Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 1.289 1.258 1.319 1.284 1.266 1.323 0.01106 1.92% 0.0% 2.5 5 1.263 1.235 1.291 1.266 1.233 1.284 0.01001 1.77% 1.97% 5 1.163 1.077 1.248 1.159 1.059 1.233 0.01111 2.08% 7.14% 6.06 5 1.197 1.166 1.227 1.202 1.173 1.233 0.01111 <td>5</td> <td></td> <td>5</td> <td>0.84</td> <td>0.7761</td> <td>0.9039</td> <td>0.84</td> <td>0.76</td> <td></td> <td>0.02302</td> <td></td> <td></td>	5		5	0.84	0.7761	0.9039	0.84	0.76		0.02302		
15	6.06		5	0.866	0.8452	0.8868	0.87	0.85	0.89	0.007483	1.93%	
Angular (Corrected) Transformed Summary C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Lab Control 5 1.289 1.258 1.319 1.284 1.266 1.323 0.01106 1.92% 0.0% 2.5 5 1.263 1.235 1.291 1.266 1.233 1.284 0.01001 1.77% 1.97% 5 1.163 1.077 1.248 1.159 1.059 1.233 0.03078 5.92% 9.76% 6.06 5 1.197 1.166 1.227 1.202 1.173 1.233 0.01111 2.08% 7.14% 10 5 1.047 1.033 1.062 1.047 1.036 1.059 0.005164 1.1% 18.72%	10		5	0.75	0.7376	0.7624	0.75	0.74	0.76	0.004473	1.33%	18.66%
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5 1.163 1.077 1.248 1.159 1.059 1.233 0.03078 5.92% 9.76% 6.06 5 1.197 1.166 1.227 1.202 1.173 1.233 0.01111 2.08% 7.14% 10 5 1.047 1.033 1.062 1.047 1.036 1.059 0.005164 1.1% 18.72%	2.5		5	1.263	1.235	1.291	1.266	1.233	1.284	0.01001	1.77%	1.97%
6.06 5 1.197 1.166 1.227 1.202 1.173 1.233 0.01111 2.08% 7.14% 10 5 1.047 1.033 1.062 1.047 1.036 1.059 0.005164 1.1% 18.72%	5		5	1.163	1.077	1.248	1.159	1.059	1.233	0.03078	5.92%	
10 5 1.047 1.033 1.062 1.047 1.036 1.059 0.005164 1.1% 18.72%	6.06		5	1.197	1.166	1.227	1.202	1.173	1.233	0.01111		
	10		5	1.047	1.033	1.062	1.047	1.036	1.059	0.005164	1.1%	18.72%
1.0170	15		5	1.037	0.9671	1.107	1.013	0.9912	1.12	0.02525	5.44%	19.51%

Analyst: QA: AC 10/5/17

Report Date: Test Code: 19 Sep-17 15:17 (p 2 of 2) 1709-S129 | 04-7331-4478

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA) Analysis ID: 18-9449-2802 Endpoint: Fertilization Rate CETISv1.8.7 **CETIS Version:** Analyzed: 19 Sep-17 15:15 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 1.0 F 0.10 0,08 Reject Null 0.06 0.04 0.7 0.02 0.6 0.00 0.5 -0.02 0.4 -0.04 0.3 -0.06 0.2 0.1 -0.10 0.0 -0.12 2.5 6.06 10 15 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 C-% Rankits

Analyst: QA: ACIDS117

Report Date:

19 Sep-17 15:17 (p 1 of 1)

Test Code:

1709-S129 | 04-7331-4478

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

09-1999-1776 Analysis ID: Analyzed:

19 Sep-17 15:16

Endpoint: Fertilization Rate

Analysis: Linear Interpolation (ICPIN)

CETIS Version: Official Results: Yes

CETISv1.8.7

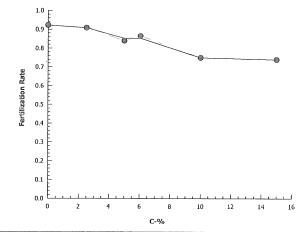
Linear Interpol	lation Options				
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	421281	1000	Yes	Two-Point Interpolation

Point Estimates

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

Fertilizat	tion Rate Summary				Calc	ulated Varia	te(A/B)				
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.922	0.91	0.94	0.005831	0.01304	1.41%	0.0%	461	500
2.5		5	0.908	0.89	0.92	0.005831	0.01304	1.44%	1.52%	454	500
5		5	0.84	0.76	0.89	0.02302	0.05148	6.13%	8.89%	420	500
6.06		5	0.866	0.85	0.89	0.007483	0.01673	1.93%	6.07%	433	500
10		5	0.75	0.74	0.76	0.004473	0.01	1.33%	18.66%	375	500
15		5	0.74	0.7	0.81	0.02168	0.04848	6.55%	19.74%	370	500

Graphics



TST

Report Date: Test Code: 19 Sep-17 15:17 (p 1 of 1) 1709-S129 | 04-7331-4478

			10	1			1631	Coae:		9-5129 0	
Echinoid Sp	erm Cell Fertiliz	ation Test	15C						Nautilus	Environ	mental (CA)
Analysis ID: Analyzed:	04-2701-4770 19 Sep-17 15:		•	tilization Ra		-Two Samp		IS Version:		.8.7	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	rected)	NA	C*b < T	NA	NA	0.75	3.53%	15	>15	NA	6.667
TST-Welch's	t Test										
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision((α:5%)		
Lab Control	2.5*		22.82	1.895	0.025 7	<0.0001	CDF		ficant Effect		
	5*		6.16	2.132	0.068 4	0.0018	CDF		ficant Effect		
	6.06*		16.6	1.895	0.026 7	<0.0001	CDF	•	ficant Effect		
	10*		8.275	1.943	0.019 6	<0.0001	CDF	•	ficant Effect		
	15*		2.663	2.132	0.057 4	0.0281	CDF	•	ficant Effect		
ANOVA Tabi	e										
Source	Sum Squ	ares	Mean Squ	iare	DF	F Stat	P-Value	Decision(α:5%)		
Between	0.280482	2	0.0560964	3	5	34.39	<0.0001	Significant	t Effect		
Error	0.039150	38	0.0016312	:66	24			- 13			
Total	0.319632	6			29						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett E	quality of	Variance	14.22	15.09	0.0143		Equal Variances			
Distribution	Shapiro-	Wilk W No	rmality	0.9625	0.9031	0.3580	Normal Di				
Distribution	Shapiro- Rate Summary	Wilk W No	rmality	0.9625	0.9031	0.3580					
Distribution		Wilk W No	rmality Mean	0.9625 95% LCL	0.9031 95% UCL	0.3580 Median			Std Err	CV%	%Effect
Distribution Fertilization	Rate Summary						Normal Di	stribution	Std Err 0.005831	CV%	%Effect 0.0%
Distribution Fertilization C-%	Rate Summary Control Type	Count	Mean	95% LCL	95% UCL	Median	Normal Di	stribution Max	0.005831		
Distribution Fertilization C-% 0	Rate Summary Control Type	Count 5	Mean 0.922	95% LCL 0.9058	95% UCL 0.9382	Median 0.92	Min 0.91	Max 0.94		1.41%	0.0%
Fertilization C-% 0 2.5 5	Rate Summary Control Type	Count 5 5	Mean 0.922 0.908	95% LCL 0.9058 0.8918	95% UCL 0.9382 0.9242	Median 0.92 0.91	Min 0.91 0.89	Max 0.94 0.92	0.005831 0.005831	1.41% 1.44%	0.0% 1.52% 8.89%
Distribution Fertilization C-% 0 2.5	Rate Summary Control Type	Count 5 5 5	Mean 0.922 0.908 0.84	95% LCL 0.9058 0.8918 0.7761	95% UCL 0.9382 0.9242 0.9039	Median 0.92 0.91 0.84	Min 0.91 0.89 0.76	Max 0.94 0.92 0.89	0.005831 0.005831 0.02302	1.41% 1.44% 6.13%	0.0% 1.52%
Fertilization C-% 0 2.5 5 6.06	Rate Summary Control Type	Count 5 5 5 5 5 5	Mean 0.922 0.908 0.84 0.866	95% LCL 0.9058 0.8918 0.7761 0.8452	95% UCL 0.9382 0.9242 0.9039 0.8868	Median 0.92 0.91 0.84 0.87	Min 0.91 0.89 0.76 0.85	Max 0.94 0.92 0.89 0.89	0.005831 0.005831 0.02302 0.007483	1.41% 1.44% 6.13% 1.93%	0.0% 1.52% 8.89% 6.07%
Fertilization C-% 0 2.5 5 6.06 10 15	Rate Summary Control Type	Count 5 5 5 5 5 5 5 5 5	Mean 0.922 0.908 0.84 0.866 0.75 0.74	95% LCL 0.9058 0.8918 0.7761 0.8452 0.7376	95% UCL 0.9382 0.9242 0.9039 0.8868 0.7624	Median 0.92 0.91 0.84 0.87 0.75	Min 0.91 0.89 0.76 0.85 0.74	Max 0.94 0.92 0.89 0.89 0.76	0.005831 0.005831 0.02302 0.007483 0.004473	1.41% 1.44% 6.13% 1.93% 1.33%	0.0% 1.52% 8.89% 6.07% 18.66%
Fertilization C-% 0 2.5 5 6.06 10 15	Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 5 5 5	Mean 0.922 0.908 0.84 0.866 0.75 0.74	95% LCL 0.9058 0.8918 0.7761 0.8452 0.7376	95% UCL 0.9382 0.9242 0.9039 0.8868 0.7624	Median 0.92 0.91 0.84 0.87 0.75	Min 0.91 0.89 0.76 0.85 0.74	Max 0.94 0.92 0.89 0.89 0.76	0.005831 0.005831 0.02302 0.007483 0.004473	1.41% 1.44% 6.13% 1.93% 1.33%	0.0% 1.52% 8.89% 6.07% 18.66%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor	Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 5 med Sum	Mean 0.922 0.908 0.84 0.866 0.75 0.74	95% LCL 0.9058 0.8918 0.7761 0.8452 0.7376 0.6798	95% UCL 0.9382 0.9242 0.9039 0.8868 0.7624 0.8002	Median 0.92 0.91 0.84 0.87 0.75 0.72	Min 0.91 0.89 0.76 0.85 0.74 0.7	Max 0.94 0.92 0.89 0.89 0.76 0.81	0.005831 0.005831 0.02302 0.007483 0.004473 0.02168	1.41% 1.44% 6.13% 1.93% 1.33% 6.55%	0.0% 1.52% 8.89% 6.07% 18.66% 19.74%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0	Rate Summary Control Type Lab Control rected) Transfor Control Type	Count 5 5 5 5 5 5 med Sum Count	Mean 0.922 0.908 0.84 0.866 0.75 0.74 mary Mean	95% LCL 0.9058 0.8918 0.7761 0.8452 0.7376 0.6798	95% UCL 0.9382 0.9242 0.9039 0.8868 0.7624 0.8002	Median 0.92 0.91 0.84 0.87 0.75 0.72	Min 0.91 0.89 0.76 0.85 0.74 0.7	Max 0.94 0.92 0.89 0.76 0.81	0.005831 0.005831 0.02302 0.007483 0.004473 0.02168	1.41% 1.44% 6.13% 1.93% 1.33% 6.55%	0.0% 1.52% 8.89% 6.07% 18.66% 19.74%
Distribution Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Rate Summary Control Type Lab Control rected) Transfor Control Type	Count 5 5 5 5 5 med Sum Count 5	Mean 0.922 0.908 0.84 0.866 0.75 0.74 mary Mean 1.289	95% LCL 0.9058 0.8918 0.7761 0.8452 0.7376 0.6798 95% LCL 1.258	95% UCL 0.9382 0.9242 0.9039 0.8868 0.7624 0.8002 95% UCL 1.319	Median 0.92 0.91 0.84 0.87 0.75 0.72 Median 1.284	Min 0.91 0.89 0.76 0.85 0.74 0.7	Max 0.94 0.92 0.89 0.76 0.81 Max 1.323	0.005831 0.005831 0.02302 0.007483 0.004473 0.02168 Std Err 0.01106	1.41% 1.44% 6.13% 1.93% 1.33% 6.55% CV% 1.92%	0.0% 1.52% 8.89% 6.07% 18.66% 19.74% %Effect 0.0%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor	Rate Summary Control Type Lab Control rected) Transfor Control Type	Count 5 5 5 5 5 med Sum Count 5 5	Mean 0.922 0.908 0.84 0.866 0.75 0.74 mary Mean 1.289 1.263	95% LCL 0.9058 0.8918 0.7761 0.8452 0.7376 0.6798 95% LCL 1.258 1.235	95% UCL 0.9382 0.9242 0.9039 0.8868 0.7624 0.8002 95% UCL 1.319 1.291	Median 0.92 0.91 0.84 0.87 0.75 0.72 Median 1.284 1.266	Min 0.91 0.89 0.76 0.85 0.74 0.7 Min 1.266 1.233	Max 0.94 0.92 0.89 0.76 0.81 Max 1.323 1.284	0.005831 0.005831 0.02302 0.007483 0.004473 0.02168 Std Err 0.01106 0.01001	1.41% 1.44% 6.13% 1.93% 1.33% 6.55% CV% 1.92% 1.77%	0.0% 1.52% 8.89% 6.07% 18.66% 19.74% %Effect 0.0% 1.97%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5	Rate Summary Control Type Lab Control rected) Transfor Control Type	Count 5 5 5 5 5 med Sum Count 5 5 5	Mean 0.922 0.908 0.84 0.866 0.75 0.74 mary Mean 1.289 1.263 1.163	95% LCL 0.9058 0.8918 0.7761 0.8452 0.7376 0.6798 95% LCL 1.258 1.235 1.077	95% UCL 0.9382 0.9242 0.9039 0.8868 0.7624 0.8002 95% UCL 1.319 1.291 1.248	Median 0.92 0.91 0.84 0.87 0.75 0.72 Median 1.284 1.266 1.159	Min 0.91 0.89 0.76 0.85 0.74 0.7 Min 1.266 1.233 1.059	Max 0.94 0.92 0.89 0.76 0.81 Max 1.323 1.284 1.233	0.005831 0.005831 0.02302 0.007483 0.004473 0.02168 Std Err 0.01106 0.01001 0.03078	1.41% 1.44% 6.13% 1.93% 1.33% 6.55% CV% 1.92% 1.77% 5.92%	0.0% 1.52% 8.89% 6.07% 18.66% 19.74% %Effect 0.0% 1.97% 9.76%

Report Date:

14 Sep-17 10:40 (p 1 of 1)

Test Code: 1709-5129 04-7331-4478/1C3634AE

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: End Date:

14 Sep-17 14 Sep-17

Sample Date: 14 Sep-17

Species: Strongylocentrotus purpuratus

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

Material: Facility Effluent

Sample Code: 17- 1020 Sample Source: IDE Americas, Inc.

Sample Station: M-001 (Daily) (9/14 Sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	\00	74 77	9/19/17
			62	100	77	1,17,17
			63	100	89	
			64	/00	89 76 92	
			65	160	92	
			66	100		
			67	00/.	76 74 76 76 70 90 88 85 89 91 91 81 70 79	
			68	100	94	
			69	100	76	
			70	100	70	
			71	100	90	
			72	/00	88	
			73	100	85	
			74	1.00	81	
			75	100	71	
			76 77	100	<u> </u>	
			78	100	$-\mathcal{I}_{\Omega}^{O}$	
			79	160	79	
			80	/00	75 92 92	
			81	100	76	
			82	100	91	
	1		83	100	22	
			84	100	83	
			85	100	92	
			86	100	93 89 72	
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			87	100	72	
			88	100	27	
			89	100	87 85 84	
			90	100	24	

@ 9/19/17 SG

CETIS Test Data Worksheet

Report Date:

14 Sep-17 10:40 (p 1 of 1)

Test Code: 1709-S12904-7331-4478/1C3634AE

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 14 Sep-17 Species: Strongylocentrotus purpuratus End Date: 14 Sep-17 Protocol: EPA/600/R-95/136 (1995)

Sample Code: 17- 1020 Sample Source: IDE Americas, Inc.

Sample Date: 14 Sep-17 Material: Facility Effluent Sample Station: M-001 (Daily) (9/14 Sample)

ple Dat					il: Facility Eff	iueiit		Sample Station: M-001 (Daily) (9/14 Sample)
C-%	Code	Rep	Pos	# Counted	# Fertilized			Notes
0	LC	1	85	100	94	RH	9/14/17	
0	LC	2	68					
0	LC	3	84					
0	LC	4	65					
0	LC	5	82					
2.5		1	75	100	93	RH	9/14/17	
2.5		2	86					
2.5		3	71					The state of the s
2.5		4	81					
2.5		5	80					
5		1	72	100	93	RH	9/14/17	
5		2	74		1 111 111 1111 1111			
5		3	90					
5		4	83					
5		5	64					
6.06		1	73	100	74	RH	9/14/17	
6.06		2	88				1	
6.06		3	67					
6.06		4	63					
6.06		5	89					
10		1	69	100	73	RH	9/14/17	
10		2	61			,	,	
10		3	79					
10		4	66					
10		5	78					
15		1	77	100	65	RH	9/14/17	
15		2	87		36%			
15		3	62					
15		4	70		A THE STATE OF THE			
15		5	76				V 2007-00-00-00-00-00-00-00-00-00-00-00-00-	

QU: (G

Marine Chronic Bioassay

Water Quality Measurements

Client:

IDE

Test Species: S. purpuratus

Sample ID:

M-001 (9/14 sample)

Start Date/Time: 9/14/2017 1524

Sample Log No.: 17- 10 10

End Date/Time: 9/14/2017 | 6 0 9

Dilutions made by:

Test No: 1709-S12-9

			Analyst:	(6
Concentration	DO		eadings	T
%	(mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	6.12	33.5	15.2
2.5	8.5	8.11	33.7	15.5
5.0	8.6	8.10	.33.6	15.1
6.06	8.5	8,10	33.7	95.515.1
10	8.5	8.09	33.6	15.0
15	8. b	8.08	33.6	14.9

Com	ന്നവ	PA FE	
~~**		8 8 6 530	

@ CGQ189/14/17

QC Check:

Final Review: 18 10/5/17

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

Marine Chronic Bio	assay			Ech	inodern	n Sperm-Cell	Fertilizatio	on Worksheet
Client:	IDE					Start Date/Ti	me: 9/14/201	7/1524
Sample ID:	Drubert We	ekhi M.	-001 (9	114 san	nale)		me: 9/14/201	
Test No.:	1700	1-5120	7	17.17 3261.			ies: S. purpu	
100(110).		1 3 10	I				rce: Pt. Lu	
Tech initials:	CG						ed: 4/5/1	
Injection Time:	1430					y •		
Sperm Absorbance at 4	1) 00	(ta	rget range of	f 0.8 - 1.0 fo	r density o	of 4x10 ⁶ sperm/	ml)	
Eggs Counted:	78	Mean.		50 = <u>4</u>		eggs/ml		
	88	(target cour	nts of 80 eggs	ner vertical r	ass on Sa	dawick-		
	95		for a final der			agwick-		
	_89			·	,			
	93							
Initial density:	4430 eg	gs/ml =	: 1.12 dil	ution factor		egg stock	/00 m	I
Final density:	4000 eg	gs/ml	- 1.0 pa	rt egg stock rts seawater		seawater	<u>12</u> m	
Prepare the embryo storexisting stock (1 part) are						dilution factor i	s 2.25, use 1	00 ml of
				Sperm:E	gg Ratio			
Rangefinder Test:	<u>2000:1</u>	1600:1	1200:1	<u>800:1</u>	400:1	200:1	100:1	50:1
ml Sperm Stock	50 0.0	40 10	30 20	20 30	10 40	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75
	Time	Rai	ngefinder Ra	tio: Fe	<u>rt.</u> !	Jnfert.		
Sperm Added (100 μl):	1451		50:1	15	74	25,26		
Eggs Added (0.5 ml):	1505	1	oU'.	96,	95 4	1,5		
Test Ended:	1515			$\overline{}$				
NOTE: Choose a spern this range, choose the ra organism health, stage of	atio closest to 90 p	percent unles	s profession					
Definitive Test		Spo	erm:Egg Rati	io Used: 【[1:00			
•	Time			Fe	rt. I	Jnfert.		
Sperm Added (100 µl):	1524	QC	;1	88	9(A) 1	2		
Eggs Added (0.5 ml):	1544	QC		\overline{a}	2	8		
Test Ended:	1604		g Control 1	<u>.</u> 0		100		4

Egg Control 2

QC Check: AC 9/15 17

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

Comments:

(A) Chargaly 12

Final Review: 9 10/6/17

Appendix B

Sample Receipt Information

Nautilus Environmental 4340 Vandever Avenue San Diego, CA 92120

Client:	106			`
Sample ID:	+Daily	M-001	(9)14	sample)
Test ID No(s).:	1700	9-5129		

Sample (A, B, C): Log-in No. (17-xxxx): Sample Receipt Date & Time: Approx. Total Volume Received (L): Check-in Temperature (C): Temperature (C): Temperature (C): DO (mg/L) pH (units) Conductivity (µs/cm) Salinity (ng/L) Alkalinity (mg/L) Hardness (mg/L) Total Chlorine (mg/L) Technician Initials Test Performed: Control/Dilution Water: Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Sal							
Sample Collection Date & Time: Sample Receipt Date & Time: Number of Containers & Container Type: Approx. Total Volume Received (L): Check-in Temperature (°C) Temperature OK? DO (mg/L) pH (units) Conductivity (µS/cm) Salinity (ppt) Alkalinity (mg/L) Hardness (mg/L) Total Chlorine (mg/L) Technician Initials Test Performed: Control/Dilution Water: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Additional Control? Y N =		Sample (A, B, C):	A]
Number of Containers & Container Type:		Log-in No. (17-xxxx);					
Number of Containers & Container Type: Approx. Total Volume Received (L): Check-in Temperature (°C) N	S	ample Collection Date & Time:	913171850	1			
Approx. Total Volume Received (L): Check-in Temperature (°C) Temperature OK? DO (mg/L) pH (units) Conductivity (px/cm) Salinity (ppt) Salinity (ppt) Alkalinity (mg/L) Hardness (mg/L) Technician Initials Total Chiorine (mg/L) Technician Initials Total Chiorine (mg/L) Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: Alkalinity: Hardness or Salinity: Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:		Sample Receipt Date & Time:	9/14/7-1252				1
Check-in Temperature (°C) Temperature OK? DO (mg/L) pH (units) Conductivity (µS/cm) Salinity (ppt) Alkalinity (mg/L) Hardness (mg/L) Technician Initials Technician Initials Test Performed: Control/Dilution Water: Additional Control? Additional Control? Additional Control? Additional Control? Additional Control? Test Performed: Control/Dilution Water: Alkalinity: Hardness or Salinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Alkalinity: Hardness or Salinity: Additional Control? Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Alkalinity: Alkalinity: Hardness or Salinity: Alkalinity: Alka	Number of	Containers & Container Type:	1-41_0031				1
Test Performed: Control/Dilution Water: 8:2 Lab SW Lab ART Other:	Аррг	ox. Total Volume Received (L):	~46				1
DO (mg/L) pH (units) Conductivity (µS/cm) Salinity (ppt) Alkalinity (mg/L)² Hardness (mg/L)²²² Total Chlorine (mg/L) Technician Initials Total Chlorine (mg/L) Technician Initials Control/Dilution Water: 8:2 Lab SW Lab ART Other: Alkalinity: Hardness or Salinity: Hardness or Salinity: Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: Alkalinity: Hardness or Salinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Salinity:		Check-in Temperature (°C)	64745				1
ph (units) Conductivity (µS/cm) Salinity (ppt) Alkalinity (mg/L)² Hardness (mg/L)²³ Total Chlorine (mg/L) Technician Initials Total Chlorine (mg/L) Total Chlorine (mg/L) Technician Initials Total Chlorine (mg/L) Total		Temperature OK?	₩ N	ΥN	YN	Y N	1
Conductivity (µS/cm) Salinity (ppt) Alkalinity (mg/L)² Hardness (mg/L)² Total Chlorine (mg/L) Technician Initials Control/Dilution Water: 8:2 Lab SW Lab ART Other: Alkalinity: / 3c Hardness or Salinity:		DO (mg/L)	8.7				1
Salinity (ppt) Alkalinity (mg/L) ² Hardness (mg/L) ² Total Chlorine (mg/L) Technician Initials Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: H		pH (units)	7.73				1
Alkalinity (mg/L) 2 Hardness (mg/L) 2,3 Total Chlorine (mg/L) Technician Initials Total Chlorine (mg/L) Technician Initials Control/Dilution Water: 8:2 Lab SW Lab ART Other: Alkalinity: Hardness or Salinity: 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: Additional Control? Y N = Alkalinity: Hardness or Salinity: Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Hardness or Salinity: Notes: 1 Temperature of sample should be 0-6°C, if received more than 24 hours past collection time. 2 mg/L as CaCO3, 3 Measured for freshwater samples only, NA = Not Applicable		Conductivity (µS/cm)	all angular and the control of the c				
Hardness (mg/L) 2.3 Total Chlorine (mg/L) Technician Initials Alkalinity: Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Hardness or Salinity: Hardness or Salinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Salinity: Hardness or Salinity: Alkalinity: Hardness or Salinity:		Salinity (ppt)	32.9				1
Total Chlorine (mg/L) Technician Initials Technician Initials Technician Initials Technician Initials Total Chlorine (mg/L) Alkalinity:		Alkalinity (mg/L) 2	111				1
Test Performed: WCA IN Test. Control/Dilution Water: 8:2 Lab SW Lab ART Other: Alkalinity: / 36 Hardness or Salinity: 34 pp t Additional Control? Y N = 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: Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Notes: 1 Temperature of sample should be 0-6°C, if received more than 24 hours past collection time. 2 mg/L as CaCO3, 3 Measured for freshwater samples only, NA = Not Applicable		Hardness (mg/L) ^{2, 3}	-				1
Test Performed: WChin Fert. Control/Dilution Water: 8:2 Lab SW Lab ART Other: Alkalinity: / 3C Hardness or Salinity: 3400 to Hardness or Salinity: Hardness or Salinity: Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: 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: Alkalinity: Hardness or Salinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Notes: Temperature of sample should be 0-6°C, if received more than 24 hours past collection time. Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.		Total Chlorine (mg/L)	0,03				1
Additional Control? Y N =Alkalinity: Hardness or Salinity: Alkalinity: Hardness or Salinity:		Technician Initials	てい	37.0			
Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: Alkalinity: Hardness or Salinity: Additional Control? Y N = Alkalinity: Hardness or Salinity: Motes: 1 Temperature of sample should be 0-6°C, if received more than 24 hours past collection time. 2 mg/L as CaCO3, 3 Measured for freshwater samples only, NA = Not Applicable		Additional Control? Y N	Alkalinity: 136	Hardness or Alkalinity: //ater: 8:2 / Lab	Salinity: 340 Hardness or S	otto	
Alkalinity: Hardness or Salinity: Alkalinity: Alkalinity: Hardness or Salinity: Hardness or Salini		Additional Control? Y N		Alkalinity:	Hardness or S	alinity:	-
² mg/L as CaCO3, ³ Measured for freshwater samples only, NA = Not Applicable			Alkalinity:	Hardness or	Salinity:	_	
ditional Comments: ATWG89(14/17 BOLLO18 9/14/17	Notes:					ie.	
	ditional Comments:	A TNG89/14/17	Bat 218 0	1/14/17			

Sample Check-In Information

COLURLASS, CC	EAR, C	Weyess,	NO PES
COC Complete (Y/N)	?		
ABC			
Filtration? Y (N)		
Pore Size:			
Organisms	or	Debris	
Salinity Adjustment?	Y (N)		
Test:	Source:	Targe	et ppt:
Test:	Source:	Targe	et ppt:
Test:	Source:	Targe	et ppt:
pH Adjustment? Y	(M)		
	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/	(N)		
		В	С
Initial D.O.			
Duration & Rate			
Final D.O.			
			(
Subsamples for Addit		nistry Require	d? Y(N)
NH3 Other Tech Initials A			
recii illidais. A	P	,	-11
	QC Ch	eck: AC	9/15/17
	Final Rev	iew:	N/5/07

Appendix C

Chain-of-Custody Form



CDP laoratory:	Turn Around Time
Entahlpy Laboratory:	Normal:x
WECK Laboratory:	RUSH (24 hr):
Nautilus:x	3 Days:
AIM:	5 Days:
Other:	??? Days

Project Name: NPDES	Daily/Weekly Toxicity		Project Manag	er:	Peter Shen		Cont	act Infor	mation:_	(7	60) 201	-7777_	o siglio como contrato.	the state of the s
Special instruction: Sa one hour intervals. Sa									ANA	LYSES			Towns and the	NOTES:
be run unadjusted. St						Purple Urchin Chronic Fertilization								
	G	lass=G Plastic=P				nic Fe								
	Yes=Y No=N	Acid=A Base=B				Chror								
Drii	nking Water=DW Sea	awater=SW Soil=S		Pres		chin								
Sample ID	Date	Tìme	Sample Type	Preservative ?	Container Type	urple Ur				**************************************				
M-001 (17- 2827)	9/13-14/2017	8:00-8:00	SW	N	4L CUBIE	Х								TDS - 31.44 ppt, EC - 48.96 mS/cm
Relinquished By:		Date:	Time:		Received-By:				Date:	Time:			Samp	ole Condition Upon Receipt:
Mayor	<u></u>	9/14/17	1206		F		1/!	4/11/	Be	ر ت	/ x	Iced		Ambient or0C
7		9/14/17	13:50		LI	2	9/14	117	12 3	52		Iced		Ambient or <u>3.00</u> °C

NAUTIUS (D. 19-1020)

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date:

15 Sep-17 14:05 (p 1 of 1)

Test Code:

170914sprt | 11-2846-3680

Echinoid Spe	rm Cell Fertiliza	tion Test 1	5C							Nautilus	s Environm	ental (CA)	
Batch ID: Start Date: Ending Date: Duration:	05-8526-3091 Test Type: Fertilization 14 Sep-17 15:24 Protocol: EPA/600/F : 14 Sep-17 16:04 Species: Strongyloc 40m Source: Pt. Loma				136 (1995) otus purpura	tus		Analyst: Diluent: Brine: Age:		ural Seawati Applicable			
Sample ID: Sample Date: Receive Date: Sample Age:	: 14 Sep-17	Soi	de: terial: urce: tion:	170914sprt Copper chloride Reference Toxi Copper Chlorid	cant			Client: Project:	Inte	rnal			
Comparison S	Summary								**************				
Analysis ID 11-0635-3440	Endpoint Fertilization Rat	te	NOEL <10	LOEL 10	TOEL NA	PMSD 8.57%	TU	Met Dun		lultiple Com	parison Tes	it	
Point Estimat Analysis ID	e Summary Endpoint		Level	μg/L	95% LCL	95% UCL	ΤU	Met	nod				
13-8128-7168	Fertilization Rat	te	EC50	34.24	32.37	36.2		Trim	med :	Spearman-k	(ärber		
Test Acceptat	oility												
Analysis ID 11-0635-3440 13-8128-7168 11-0635-3440	Endpoint Fertilization Rat Fertilization Rat Fertilization Rat	te		ol Resp ol Resp	7est Stat 0.898 0.898 0.08572	0.7 - NL 0.7 - NL NL - 0.25	its	Ove Yes Yes No	Yes Passes Acceptability		cceptability	y Criteria	
Fertilization R	Pate Summary												
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	c Std	Err	Std Dev	CV%	%Effect	
0 10 20 40 80 160	Lab Control	5 5 5 5 5	0.898 0.802 0.618 0.484 0.076	0.8388 0.7273 0.4666 0.4123 0.05717	0.9572 0.8767 0.7694 0.5557 0.09483	0.83 0.74 0.45 0.42 0.06	0.94 0.9 0.75 0.55 0.1	0.02 5 0.05 5 0.02	691 453	0.04764 0.06017 0.1219 0.05771 0.01517	5.31% 7.5% 19.73% 11.92% 19.95%	0.0% 10.69% 31.18% 46.1% 91.54% 100.0%	
Fertilization R	tate Detail								AD AT A COMPANY OF THE A		<u></u>	The state of the s	
C-μg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		***************************************					
0 10 20 40 80 160	Lab Control	0.94 0.78 0.61 0.55 0.07	0.94 0.78 0.56 0.46 0.07	0.91 0.74 0.72 0.42 0.1	0.87 0.81 0.75 0.54 0.06	0.83 0.9 0.45 0.45 0.08							

Analyst: QA: 39/29/1-

Report Date:

15 Sep-17 14:05 (p 1 of 2)

Test Code:

170914sprt | 11-2846-3680

							Test	Code:	1709	314sprt 11	-2846-3680
Echinoid Sp	erm Cell Fertiliz	ation Test	15C						Nautilus	Environn	nental (CA)
Analysis ID:	11-0635-3440	En	dpoint: Fe	rtilization Ra	te		CET	S Version:	CETISv1.	.8.7	
Analyzed:	15 Sep-17 14:		•	rametric-Cor		tments	Offic	ial Results			
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	······································	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	ected)	NA	C > T	NA	NA		8.57%	<10	10	NA	1113
Dunnett Mult	tiple Compariso	n Test								,	
Control	vs C-μg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	10*		2.696	2.305	0.118 8	0.0228	CDF	Significan	t Effect		
	20*		6.723	2.305	0.118 8	<0.0001	CDF	Significan	t Effect		
	40*		9.419	2.305	0.118 8	<0.0001	CDF	Significan	t Effect		
	80*		19	2.305	0.118 8	<0.0001	CDF	Significan	t Effect		
ANOVA Table	е										
Source	Sum Sqւ	iares	Mean Sq	uare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	2.834971		0.708742	8	4	107.9	<0.0001	Significan	t Effect		and the state of the same of t
Error	0.131348		0.006567	398	20						
Total	2.966319				24	_					
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett B	Equality of \	/ariance	7.22	13.28	0.1247	Equal Var	iances			
Distribution	Shapiro-	Wilk W Nor	mality	0.9747	0.8877	0.7637	Normal Di	stribution			
Fertilization	Rate Summary										
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Wax	Std Err	CV%	%Effect
0	Lab Control	5	0.898	0.8388	0.9572	0.91	0.83	0.94	0.02131	5.31%	0.0%
10		5	0.802	0.7273	0.8767	0.78	0.74	0.9	0.02691	7.5%	10.69%
20		5	0.618	0.4666	0.7694	0.61	0.45	0.75	0.05453	19.73%	31.18%
40		5	0.484	0.4123	0.5557	0.46	0.42	0.55	0.02581	11.92%	46.1%
80		5	0.076	0.05717	0.09483	0.07	0.06	0.1	0.006782	19.95%	91.54%
160		5	0	0	0	0	0	0	0		100.0%
Angular (Cor	rected) Transfo	rmed Sumr	mary								
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.252	1.156	1.349	1.266	1.146	1.323	0.03475	6.21%	0.0%
			4 4 4 4	1.013	1.215	1.083	1.036	1.249	0.03631	7.29%	11.03%
10		5	1.114	1.013	1.210				0.0000		
10 20		5 5	0.9075	0.7501	1.065	0.8963	0.7353	1.047	0.0567	13.97%	27.52%
							0.7353 0.7051	1.047 0.8355			
20		5	0.9075	0.7501	1.065	0.8963			0.0567	13.97%	27.52%

Report Date: Test Code: 15 Sep-17 14:05 (p 2 of 2) 170914sprt | 11-2846-3680

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA) Analysis ID: 11-0635-3440 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 15 Sep-17 14:04 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 1.0 0.15 0.9 0.10 Reject Null Fertilization Rate 0.05 0.7 0.6 0.5 -0.05 0.4 0.3 -0.10 0.2 0.1 -0-0.0 10 20 80 160 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 1.0 1.5 2.0 C-µg/L Rankits

Report Date:

15 Sep-17 14:05 (p 1 of 1)

Test Code:

170914sprt | 11-2846-3680

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

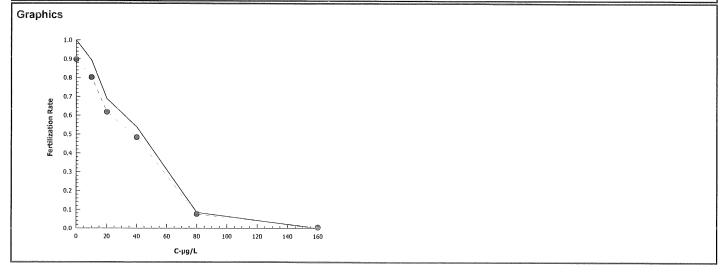
Analysis ID:13-8128-7168Endpoint:Fertilization RateCETIS Version:CETISv1.8.7Analyzed:15 Sep-17 14:04Analysis:Trimmed Spearman-KärberOfficial Results:Yes

Trimmed Spearman-Kärber Estimates

 Threshold Option
 Threshold
 Trim
 Mu
 Sigma
 EC50
 95% LCL
 95% UCL

 Control Threshold
 0.102
 10.69%
 1.534
 0.01213
 34.24
 32.37
 36.2

Fertilization 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	0.898	0.83	0.94	0.02131	0.04764	5.31%	0.0%	449	500
10		5	0.802	0.74	0.9	0.02691	0.06017	7.5%	10.69%	401	500
20		5	0.618	0.45	0.75	0.05453	0.1219	19.73%	31.18%	309	500
40		5	0.484	0.42	0.55	0.02581	0.05771	11.92%	46.1%	242	500
80		5	0.076	0.06	0.1	0.006782	0.01517	19.95%	91.54%	38	500
160		5	0	0	0	0	0		100.0%	0	500



29 Sep-17 09:19 (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 Reference Toxicant-REF Source:

Echinoid Sperm Cell Fertilization Test 15C 120-100-+2s 80-EC50-µg/L Copper chloride 60-Mean 40-20--2s -3s 0 -20-20 Aug-17-25 Aug-17-28 Aug-17-12 Sep-17-14 Sep-17⁻ 01 Sep-17-02 Sep-17-30 Aug-17-04 Sep-17-16 Aug-17-18 Aug-17-21 Aug-17 23 Aug-17-24 Aug-17-26 Aug-17-05 Sep-17-06 Sep-17 08 Sep-17-10 Sep-17-13 Sep-17-Mean: 51.23 Count: 20 -2s Warning Limit: 18.51 -3s Action Limit: 2.147 Sigma: 16.36 CV: 31.90% +2s Warning Limit: 83.95 +3s Action Limit: 100.3

Qualit	y Con	trol Data	a								
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Aug	14	14:40	64.51	13.28	0.8115			02-4510-8526	01-5460-0814
2			16	16:34	50.82	-0.4131	-0.02525			16-3259-1018	06-7497-1035
3			18	14:09	42.53	-8.699	-0.5317			12-6613-4538	02-2322-5589
4			20	14:52	24.05	-27.18	-1.661			06-9655-0092	05-8785-3700
5			21	14:46	69.95	18.72	1.144			08-4756-2919	20-2992-4955
6			23	16:14	41.72	-9.513	-0.5815			02-7595-3678	15-3490-2746
7			24	16:11	67.1	15.87	0.9702			04-7651-5518	20-0883-0005
8			25	14:48	43.11	-8.12	-0.4964			06-8816-1100	09-0830-4014
9			26	16:00	57.24	6.011	0.3674			10-2039-5656	15-8794-0305
10			28	14:56	41.55	-9.678	-0.5916			08-1525-2751	10-7829-2432
11			30	16:38	50.21	-1.018	-0.06223			08-1199-3706	11-0543-3886
12		Sep	1	15:27	34.79	-16.44	-1.005			13-1244-6646	21-1567-7550
13			2	10:53	89.99	38.76	2.369	(+)		16-4202-9692	18-8681-1855
14			4	16:10	53.77	2.542	0.1554			12-2973-1405	10-6032-1229
15			5	17:07	37.36	-13.87	-0.8478			13-1627-7974	14-5447-1160
16			6	17:15	44.41	-6.817	-0.4167			05-5533-8557	16-8161-1582
17			8	15:48	37.91	-13.32	-0.814			18-6871-7794	04-4479-5076
18			10	14:25	40.4	-10.83	-0.6618			11-6871-9499	08-4248-1228
19			12	15:51	81.07	29.84	1.824			20-0603-9450	06-1182-7961
20			13	19:07	52.04	0.8063	0.04928			01-4575-6189	02-4618-7964
21			14	15:24	34.24	-16.99	-1.039			11-2846-3680	13-8128-7168

CETIS Test Data Worksheet

Report Date:

14 Sep-17 10:33 (p 1 of 1)

Test Code:

11-2846-3680/170914sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: End Date:

14 Sep-17 14 Sep-17

Species: Strongylocentrotus purpuratus

Sample Code:

170914sprt

Sample Date: 14 Sep-17

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

Sample Source: Reference Toxicant

Material: Copper chloride

Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	
			1	100	94	9/15/17
			2		0	
			3		91	
AND MAIN			4		8 55	
			5			
			6		0 72 81	
			7		72	
			8		81	
			9		7	
			10		61	
			11		94	
			12		90	
			13		6	
			14		54	
			15		6 54 7 -23 46 56 42 78 45	
			16		23-46	Q18 9/15/17 JA
			17		56	
			18		42	
			19		78	
			20		.45	·
			21		45	
			22		45 0 0	
			23		0	
			24		83 14 0	
			25			
			26		<u> </u>	
			27		¥ 75	वाह पाड़ा १ तर्थ
	-		28		10 87 78	
	1		29		87	
			30	V	78	V

A QA: 1/29/17

Report Date:

14 Sep-17 10:33 (p 1 of 1)

Test Code:

11-2846-3680/170914sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date:	14 Sep-17	Species:	Strongylocentrotus purpuratus	Sample Code:	170914sprt
End Date:	14 Sep-17	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant
Sample Date:	14 Sep-17	Material:	Copper chloride	Sample Station:	Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	1			
0	LC	2	11	100	93	CG 9/14/17
0	LC	3	3			
0	LC	4	29			
0	LC	5	24			
10		1	19			
10		2	30			
10		3	25			
10		4	8	100	83	CG 9/14/17
10		5	12			T
20		1	10			
20		2	17			
20		3	7			
20		4	27	(00	80	CG 9/14/17
20	and the state of t	5	21			
40		1	5			
40		2	16	100	50	(G 9)14/17
40		3	18			
40		4	14			
40		5	20			3
80		1	9			VALUE
80		2	15			
80		3	28			
80		4	13		anna a	
80		5	4	100	26	CG 9/14/17
160		1	6			
160		2	2			
160		3	26			
160		4	22	100	Ò	CG 9/14/17
160		5	23			

Quich

Marine Chronic Bioassay

Water Quality Measurements

CI	:-	4	-
	ıe	nr	•

Internal

Test Species: *S. purpuratus*

Sample ID:

CuCl₂

Start Date/Time: 9/14/2017 1524

Test No:

170914sprt

End Date/Time: 9/14/2017

Dilutions made by:

(6

High conc. made (μg/L): 160

Vol. Cu stock added (mL): 7 · 8

Final Volume (mL): 500

Cu stock concentration (μg/L):

Analyst:

		Initial D	eadings	(6)
Concentration (µg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.8	8.08	33.6	15.91
10	8.4	8.06	33.6	15.8
20	8.3	8.06	33.6	15.8
40	8.3	8,06	33.5	15.8
80	8.3	8.07	33.4	15.9
160	8.3	8.07	33.2	15.7

Comments:		
QC Check:	Ac 9/15/17	Final Review: 69/19/17

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Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Sample ID: CuCl2 Test No.: $1709145pCC$				
Test No.: 1709145077 Species: S. purpuratus Animal Source: 97.10000 Tech initials: 1430 Species: S. purpuratus Animal Source: 97.10000 Injection Time: 1430 Species: Animal Source: 97.10000 Species: Animal Source: 97.100000 Species: Animal Source: 97.100000 S	Client:	Internal	Start Date/Time: 9/14/2017 / 1524	
Animal Source: Pt. Lowa. Date Collected: 95 PT Injection Time: 1430 Sperm Absorbance at 400 nm: 0.988 (target range of 0.8 - 1.0 for density of 4x10 ⁶ sperm/ml) Eggs Counted: 18 Mean 2443 x 50 = 4430 eggs/ml	Sample ID:	CuC12	End Date/Time: 9/14/2017 / 16の生	
Tech initials: CG Date Collected: GSM Injection Time: SP Date Collected: SP Date Col	Test No.:	170914 SOFT	Species: S. purpuratus	
Injection Time: 1430 Sperm Absorbance at 400 nm: 0.988 (target range of 0.8 - 1.0 for density of 4x10 ⁶ sperm/ml) Eggs Counted: x 50 = 4430 eggs/ml		l .	Animal Source: Pt. Loma.	
Sperm Absorbance at 400 nm: U.988 (target range of 0.8 - 1.0 for density of 4x10 ⁶ sperm/ml) Eggs Counted: X 50 = 4430 eggs/ml	Tech initials:	<u></u>	Date Collected: 4517	
Eggs Counted:	Injection Time:	1430		
Eggs Counted:		.2 0 50		
Eggs Counted:	Sperm Absorbance at 40	(target jarge or o.e	8 - 1.0 for density of 4x10 ⁶ sperm/ml)	
98	Eggs Counted:		= <u>4430</u> eggs/ml	
		<u>88</u>		
(target counts of 80 eggs per vertical pass on Sedgwick-		11 /		
Rafter slide for a final density of 4000 eggs/ml)		Rafter slide for a final density	y of 4000 eggs/ml)	
		89		
<u>93</u>		<u>93</u>		
Initial density: 4430 eggs/ml = 1.12 dilution factor egg stock 150 ml	Initial donaity:	4470 agga/ml = 1.12 dilutio	on factor and attack 1/5/0 ml	
Final density: 4000 eggs/ml - 1.0 part egg stock seawateri mlml	rinai density.	99		

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 145 (1505 1516		ngefinder Ra	ntio: Fe	74 25	fert.		

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 Use	q: <u>[00.]</u>	
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1524 1544 1604	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 3894 92 0	Unfert. 12 -8 -100 -100
Comments:	(ACC) 018914/17			
QC Check:	AC 9/15/17	_		Final Review: 4 9/24/17

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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