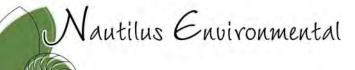


# **Appendix I Brine Dilution Salinity Tolerance**

Renewal of NPDES CA0109223 Carlsbad Desalination Project



# Poseidon Brine Dilution Pump Study **Final Toxicity Test Results**

Prepared for:

Poseidon Water 5780 Fleet Street. Suite 140 Carlsbad, CA 92008

Prepared by:

Nautilus Environmental 4340 Vandever Avenue San Diego, CA 92120

**Report Submitted:** July 24, 2015

#### Data Quality Assurance:

- Nautilus Environmental is accredited in accordance with NELAP by the State of 0 Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053-001). It is also certified by the State of California Water Resources Control Board Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552). Specific fields of testing applicable to each accreditation are available upon request.
- All data have been reviewed and verified. 0
- All test results have met minimum test acceptability criteria under their respective 0 EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements. 0

Results verified by: \_\_\_\_\_ Reducine Cibor

**British** Columbia 8664 Commerce Court Burnaby, British Columbia V5A 4N7 604-420-8773 fax: 604-603-9381

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California

858 587 7333 fax: 858.587.3961

#### INTRODUCTION

In response to the amendments made to the Ocean Plan by the California State Water Quality Control Board (SWQCB), Poseidon Resources [Channelside] LP (Poseidon) contracted with Nautilus Environmental (Nautilus) to assess the potential effects of varying salinity levels on sensitive larval-stage marine organisms. This study was designed and conducted for internal research and development purposes to support Poseidon's effort to formulate a plan to comply with the Ocean Plan requirements to minimize mortality of marine life. The study design was focused on potential effects due to salinity fluctuations on organisms travelling into the intake from ambient seawater salinity in the receiving environment, through the brine dilution systems of the Carlsbad desalination plant, and then being discharged back into the receiving water. Species and endpoints evaluated for this study included red abalone (Haliotis rufescens) development and purple sea urchin (Strongylocentrotus purpuratus) development. These species and endpoints were identified as two of the most sensitive to elevated salinity levels relative to other accepted monitoring species in the Ocean Plan, based on previous studies using standard EPA whole effluent toxicity (WET) tests (Philips et al., 2012). However, standard EPA WET tests were designed to expose organisms to a given test concentration for the entire duration of the exposure, which is between 48 hours and 7 days, depending on the test protocol. Conversely, organisms would be exposed to salinity fluctuations over considerably shorter durations based on modeling of the plant's operational characteristics (Jenkins 2015 and Alden 2015).

Because the goal of this study was to determine a scenario that would result in no salinityinduced adverse effects to these organisms as they travel through the brine dilution system, an exposure system was designed to assess several potential scenarios involving differing salinity levels and residence times that were within the plant's operational capabilities. On the basis of a preliminary simulation test, procedures were established to simulate the salinity fluctuations an organism might experience as it moves through the brine dilution system, encountering elevated salinity as the brine discharge is mixed with seawater from the flow augmentation system then a reduction in salinity to 35.5 ppt as it travels through the discharge system to the edge of the brine mixing (BMZ), and finally a reduction from 35.5 ppt to ambient salinity. Using these procedures, this report describes the methods and results of this study, including an operating scenario that is expected to result in no salinity-related adverse effects to organisms passing through the system. Note, the results of the study should be interpreted only to include parameters and conditions tested and cannot be extrapolated to interpret water quality parameters, durations, or other test conditions beyond those reported herein.

#### MATERIALS AND METHODS

#### **Exposure Scenarios**

Three scenarios to be tested were provided by Poseidon and included the expected salinity and duration at each point in the intake/brine dilution system based on brine dilution models (Jenkins 2014).

There were three distinct phases common to each exposure scenario; only the maximum salinity and duration of each phase were varied:

- Phase 1 consisted of simulation of initial brine mixing with seawater from the flow augmentation system. The salinity was raised from ambient seawater (33.5 ppt) by adding 67 ppt brine at a rate calculated to reach the desired salinity within approximately one minute, and then held there for a specified amount of time depending on the scenario being tested.
- Phase 2 involved simulation of the dilution that occurs in the BMZ technology. Continuous addition of ambient seawater at a rate calculated to reach 35.5 ppt within a specified period.
- Phase 3 represents the return to ambient seawater salinity from 35.5 ppt, with the rate of return varied according to specification.

All scenarios assumed that transitions between salinity levels were linear. The tests were conducted in a step-wise manner, starting with the lowest salinity and duration, and then testing at increased salinity and duration if no effects were observed. The various scenarios tested, as well as species tested and test dates, are described in Table 1.

Exposure Scenario	Species; Test Date	Phase 1	Phase 2	Phase 3
1	Abalone; 02/06/15 Urchin; 02/17/15	33.5 to 44 ppt in one minute, hold for 2.8 minutes	44 to 35.5 ppt in 39 minutes	35.5 to 33.5 ppt in 30 minutes
2	Abalone; 01/30/15	33.5 to 42 ppt in one minute, hold for 2.2 minutes	42 to 35.5 ppt in 36 minutes	35.5 to 33.5 ppt in 30 minutes
3	Abalone; 01/22/15	33.5 to 40 ppt in one minute, hold for 1.7 minutes	40 to 35.5 ppt in 34 minutes	35.5 to 33.5 ppt in 30 minutes

Table 1, Exr	osure Scenarios	and Test Date	es for the Pum	n/Brine Dilutio	n Study
					locuay

#### Water Quality

All water used in this study was natural seawater collected off the Scripps Institution of Oceanography (SIO) pier in La Jolla, California. Hypersaline seawater (brine), derived from freezing natural seawater, was added to ambient seawater to increase the salinity to the appropriate level. A static brine control was also tested at ambient salinity by mixing brine with deionized water and seawater. The brine control incorporated the highest percentage brine used in the exposure and was tested to ensure that effects observed in the bench-top exposures were not attributable to the brine itself.

Water quality parameters (i.e., pH, dissolved oxygen (DO), salinity, temperature) were measured on both the brine and ambient seawater prior to test initiation, and once daily for the duration of the test in a surrogate test chamber. Salinity and temperature were measured throughout the scenarios in both the control and exposure systems after the first minute of Phase 1, at the end of Phase 1, and every 5 to 15 minutes in Phases 2 and 3. A Hach SensION5 salinity meter calibrated daily to a certified 35.0 ppt standard solution was used for all salinity readings throughout the study.

#### Bench-Top Pump/Brine Dilution System

The bench-top system consisted of a shallow plastic bin fitted with drains containing 4 liters of seawater. Cylindrical plexiglass screen tubes with  $35-\mu m$  screens on the bottom to retain organisms were inserted into the plastic bin. A pump was placed in the exposure bin, with a flow set at approximately six liters per minute to mix the input water with the existing water in the exposure bin. Input water (brine or seawater, depending on which phase of testing) was delivered to the bin via gravity through a 1/8" plastic tube with flow rates adjusted to achieve the desired salinity levels in the required amount of time. Water was delivered into the bin directly adjacent to the intake for the pump. Additional mixing was accomplished by manually raising, lowering, and rotating the screen tubes containing the test organisms in the bin (while keeping organisms below the water line) periodically throughout the entire exposure scenario to ensure that there was constant equilibration of salinity inside the screen tubes, as well as inside the exposure bin. The entire bench top system was contained within an environmental chamber to maintain a temperature of  $15 \pm 1$  degrees Celsius (°C) throughout the tests.

In addition to the bench-top exposures to salinity fluctuations, a control was conducted in parallel for each scenario. The control setup was identical to the bench-top exposure setup and experienced the same volume inputs and physical movements. However, the exposure setup experienced salinity changes in accordance with the scenario parameters, whereas the control had inputs consisting of only ambient seawater. This procedure allowed for the isolation of salinity as the only factor that varied between the control and exposure.

While the proportion of normal development after the conclusion of all three exposure phases was the endpoint of interest, a subset of replicates was removed after each phase to evaluate any phase-specific adverse effects to the test organisms. This step was important to determine at which point the embryos were most sensitive and allowed for appropriate adjustment in salinity and/or duration in subsequent scenarios. Whether removed intermittently or at the

conclusion of the exposure scenario (i.e., at the end of Phase 3), all replicates were placed in a separate bin containing seawater at ambient salinity for the remainder of the test duration.

#### **Toxicity Testing Conditions**

Test methods generally followed those described in *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). However, due to the nature of the study some modifications, such as test chamber size and water volume per replicate, were made to the methods to accommodate exposure types. General test methods for each species are provided in Tables 2 and 3.

Table 2. Neu Abaione Development Te	st opeenieddons
Test Organism; age:	Red Abalone ( <i>Haliotis rufescens</i> ); newly fertilized embryos (within one hour of fertilization)
Test Duration and Endpoint:	48-hour normal development rate
Test Organism Source:	American Abalone (Davenport, CA)
Number of Organisms per Replicate:	~200
Test Chamber:	plexiglass screen tubes with 35- $\mu$ m screens
Number of Replicates:	Between 10 and 12 total per scenario, with two to five replicates per test phase, including one water quality surrogate.
Test Temperature:	15 ± 1 °C
Test Acceptability Criteria:	Mean normal shell development in the lab control must be $\ge$ 80 percent. Must have a statistically significant effect at 56 µg/L zinc; must achieve a MSD of <20%.
Concurrent Reference Toxicant Test:	Zinc Sulfate

#### Table 2. Red Abalone Development Test Specifications

Test Organism; age:	Purple sea urchin ( <i>Strongylocentrotus purpuratus</i> ); newly fertilized embryos (within one hour of fertilization)
Test Duration and Endpoint:	72-hour normal development rate
Test Organism Source:	Field collected off Point Loma, San Diego, CA
Number of Organisms per Replicate:	~250
Test Chamber:	plexiglass screen tubes with 35- $\mu$ m screens
Number of Replicates:	Three replicates per phase, and one water quality surrogate.
Test Temperature:	15 ± 1 °C
Test Acceptability Criteria:	Mean normal development in the lab control must be $\geq$ 80 percent; must achieve a MSD of <25%
Concurrent Reference Toxicant Test:	Copper chloride

#### Table 3. Purple Urchin Development Test Specifications

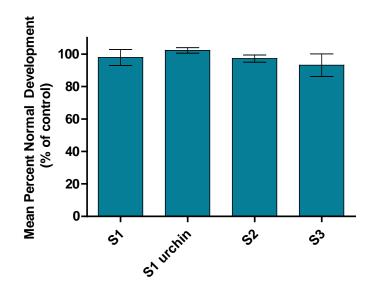
As noted above, each exposure period lasted approximately one to two hours including the initial brine spike, followed by an incremental return to ambient salinity. The embryos were then incubated in ambient seawater for the remainder of the protocol prescribed testing period (i.e., 48 hr for abalone, 72 hr for urchins). After the grow-out, all embryos were transferred to 30-mL glass shell vials, fixed with a 10% formalin solution buffered in seawater, and 100 embryos were scored per the EPA 1995 protocol guidelines as normal or abnormal. All exposure scenarios were evaluated with red abalone, but purple sea urchins were tested only with Scenario #1 to provide confirmation of results with a second species.

#### **Statistical Evaluation**

Normal development data, expressed as a proportion, were arcsin square-root transformed prior to analysis to normalize the distribution of the data and satisfy statistical assumptions for analysis. Following transformation, homogeneity of variance was evaluated using the F test. Unpaired one-tailed Student's t-tests were performed between the control and brine for each phase where mean normal development in the brine test was less than that in the control. If variances were significantly different, the t-test was performed using Welch's correction (Zar 1984). Statistical analyses were performed using GraphPad Prism software, Version 4.02. Tests were considered statistically significant when p values were less than or equal to 0.05. Statistical analyses of reference toxicant data followed standard USEPA flow chart methods specified for these test types using the Comprehensive Environmental Toxicity Information System<sup>™</sup> (CETIS) program by Tidepool Scientific Software.

#### **RESULTS AND DISCUSSION**

A summary of the results for normal development at the end of Phase 3 for all Exposure Scenarios and species is shown in Figure 1. Results for all species and exposure scenarios are presented in Table 4. Full test results, including all water quality measurements and summary tables, are presented in Appendix A.



**Figure 1.** Mean Normal Development after Completion of Phase 3 for all four Scenarios (S1-S3). Scenario #1: P1 44 ppt for 2.8 min, P2: 39 min, P3: 30 min; Scenario #2: P1 42 ppt for 2.2 min, P2: 36 min, P3: 30 min; Scenario #3: P1 40 ppt for 1.7 min, P2: 34 min, P3: 30 min.

None of the three scenarios described in this report resulted in statistically significant effects after Phase 3 compared to the control exposure (p<0.05). In all exposure scenarios, replicates were terminated after each of the phases. There was one statistically significant effect (p<0.05) that was detected in Phase 1 of Exposure Scenario #2. However, the effect was small (8.5 percent compared to the Phase 1 control results), and there were no statistically significant effect, we conclude that this finding was not due to the treatment itself.

Although urchins were tested only with Scenario #1, the similarity of results to those obtained for abalone suggests that the abalone results should be predictive of those obtained with echinoderms.

#### Table 4. Summary of Results for Bench-top Exposure Scenarios

Scenario	Scenario Description	Test date	Species Tested	Mean	Normal De	velopment	
#	Scenario Description	Test date	Species rested	Sample	Phase 1	Phase 2	Phase 3
1	P1: 44 ppt for 2.8 minutes;	2/6/15	Abalone	Control	83.8	77.7	80.5
I	P2: 39 min.; P3: 30 min.	Development	Brine Exposure	76.7*	79.1	78.8	
1	P1: 44 ppt for 2.8 min.;	2/17/15	Urchin	Control	93.7	92.0	89.3
I	P2: 39 min.; P3: 30 min.	2/17/13	Development	Brine Exposure	91.3	90.3	91.3
2	P1: 42 ppt for 2.2 min.;	1/30/15	Abalone	Control	94.0	93.7	94.3
2	P2: 36 min.; P3: 30 min.	1/30/13	Development	Brine Exposure	95.7	92.7	91.7
2	P1: 40 ppt for 1.7 min.;	1/22/15	Abalone	Control <sup>a</sup>	66.0	61.0	67.3
<sup>3</sup> P2: 34 min.; P3: 30 min.	1/22/15	<sup>5</sup> Development <sup>a</sup>	Brine Exposure	68.5	67.0	60.3	

P1, P2, P3 = Phase 1, 2, and 3

\* An asterisk indicates a statistically significant decrease compared to the control (p < 0.05) a The abalone test Scenario #3 conducted on January 22 did not meet the 80% test acceptability criterion for normal development in the control; see QA section.

#### QUALITY ASSURANCE

Each exposure was timed so that the embryos were added within 1-hour of fertilization at the beginning of Phase 1, except for the abalone test on Exposure Scenario #3 in which the embryos were added 1 hour and 12 minutes after fertilization. However, this test also did not meet control test acceptability with regards to normal development. Both the control for bench-top exposure and the control in the concurrent reference toxicant test were below 80 percent mean normal development. Although this test failed to meet this acceptability criterion, there was no significant difference between the treatment and control exposures. Therefore, the data are reported herein because the response relative to control was considered valuable in deciding which scenario to use in the subsequent test. In fact, the follow-up test (Exposure Scenario #2), initiated on January 30, 2015 at a higher salinity rate and duration resulted in no statistically significant effects treatment compared to the control.

All nominal salinity targets were met within plus or minus 1 ppt. Where there were any exceedances of the nominal targeted salinity, they were usually above the required level, which would result in a more conservative assessment of the effects. Regardless, any differences between target and measured salinity levels were not likely to impact the final test results.

#### **REFERENCE TOXICANT TESTING**

Concurrent reference toxicant tests were conducted with all exposure scenarios. All reference toxicant tests met the minimum test acceptability criteria, except for the abalone development test initiated on January 22, 2015 with Scenario #3. The lab control for this test did not meet the lab control acceptability criterion of 80 percent; however, all other test acceptability requirements were met. Additionally, a typical dose response was observed and the median effect concentration ( $EC_{50}$ ) value was within two standard deviations of the historical mean. The calculated  $EC_{50}$  values for all other tests were also within two standard deviations of the internal control chart means, indicating test organism sensitivity was typical. Reference toxicant results are provided in full in Appendix B. A glossary of qualifier codes is provided in Appendix C.

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### **APPENDIX A**

Brine Exposure Scenarios Raw Data and Statistical Analyses Scenario #1 48-hour Red Abalone Larval Development Test Initiation Date: February 6, 2015

## Summary of Toxicity Test Results Poseidon - Brine Dilution Pump Study (Scenario #1) 48-hour Abalone (*Haliotis rufescens*) Development Test Test Initiation Date: 02/06/15

	Mear	Mean Percent Normal Development					
	Phase 1	Phase 2	Phase 3				
Sample ID <sup>a</sup>	Ambient to 44 ppt in 2.8 minutes <sup>b</sup>	44 to 35.5 ppt in 39 minutes	35.5 ppt to ambient salinity in 30 minutes				
Control	83.8	77.7	80.5				
Brine Exposure	76.7*	79.1	78.8				

<sup>a</sup> The control and brine exposure were each assigned 10 replicates. Rep A was used a water quality surrogate; reps B, C, and D = phase 1; reps E, F, and G = phase 2; reps H, I, and J = phase 3 (full treatment). Phase 1 and phase 2 treatments were moved to ambient seawater immediately after treatment for the duration of the 48-hour exposure.

<sup>b</sup> Rise from 33.5 to 44 in one minute, held for an additional 1.8 minutes.

Note: Control replicates underwent all the same physical movements as brine replicates, but with ambient seawater only. Ambient salinity =  $\sim$  33.5 ppt.

\*An asterisk indicates a statistically significant decrease compared to the control (p < 0.05).

## Summary of Toxicity Test Results Poseidon - Brine Dilution Pump Study (Scenario #1) 48-hour Abalone (*Haliotis rufescens*) Development Test Test Initiation Date: 02/06/15

Sample ID	Rep	# Normal	# Counted	Percent Normal	Mean Percent Normal	Standard Deviation
Control (WQ surrogate)	А					
	В	68	80	85.0		
Control (Phase 1)	С	76	89	85.4	83.8	2.4
	D	77	95	81.1		
	Е	68	81	84.0		
Control (Phase 2)	F	56	85	65.9	77.7	10
	G	69	83	83.1		
	Н	68	84	81.0		
Control (Phase 3)	I	67	82	81.7	80.5	1.5
	J	67	85	78.8		
Brine Exposure (WQ surrogate)	А					
	В	58	77	75.3	76.7	
Brine Exposure (Phase 1)	С	44	59	74.6		3.1
	D	53	66	80.3		
	Е	74	93	79.6		
Brine Exposure (Phase 2)	F	62	80	77.5	79.1	1.4
	G	57	71	80.3		
	Н	46	57	80.7		
Brine Exposure (Phase 3)		66	89	74.2	78.8	4.0
	J	57	70	81.4		
	Α	53	70	75.7		
	В	58	69	84.1		
Brine Control <sup>a</sup>	С	79	99	79.8	79.9	4.2
	D	63	85	74.1		
	E	63	76	82.9		

<sup>a</sup> The control and brine exposure (Phases 1, 2, and 3) were tested in screen tubes. The brine control was tested in 5 replicate vials to ensure no adverse effects from the brine water used.

## **Embryo Larval Bioassay**

## **48-hour Development**

Client: Poseidon

Project ID: Pump brine dilution study

Test Species: *H. rufescens* 

**Start Date/Time:** 2/6/2015 (333)

End Date/Time: 2/8/2015 1300

Sample	Rep	Number Counted	Number Normal	Technician Initials
Lab Control	A	Surrogate	16-10	
	В	80	68	CH 2/9/15
	С	89	76	CH
	D	95	77	СН
	E	-81	68	CH
	F	85	56	CH
	G	83	69	CH
	Н	<del>5</del> 4	68	CH
		82	67	CH
	J	85	67	CH
Brine dilution	Α	Surrogate		
	B	77	58	CH
	C	59	44	СН
	D	66	53	CH
	E	93	74	CH
	F	80	62	CH
	G	71	57	CH
	Н	57	46	CH
		<del>3</del> 9	66	CH
	J	70	57	CH V
Brine Control	A	70	53	
	B	69	58	
	C	99	79	
	D	85	63	
	E	76	63	Ý

Comments: Reps B, C, and D ended after phase 1 (67 to 44 ppt) Reps E, F, G ended after phase 2 (44 to 35.5 ppt) Reps H, I, J ended after phase 3 (35.5 to 33.5 ppt)

QC Check:

AC 2/9/15 Final Review: 179 2/10/15

# Abalone Development Test Date: 2/6/15 t-test results

Parameter	Value
Table Analyzed	Scenario 2/6/15 P1
Column A	Control
VS	vs
Column B	Brine
Unpaired t test P value P value summary Are means signif. different? (P < 0.05) One- or two-tailed P value? t, df	0.0178 * Yes One-tailed t=3.119 df=4
How big is the difference? Mean ± SEM of column A Mean ± SEM of column B Difference between means 95% confidence interval R squared	1.157 ± 0.01875 N=3 1.068 ± 0.02166 N=3 0.08933 ± 0.02864 0.009819 to 0.1688 0.7086
F test to compare variances F,DFn, Dfd P value P value summary Are variances significantly different?	1.334, 2, 2 0.8567 ns No

Parameter	Value
Table Analyzed	Scenario 2/6/15 P3
Column A	Control
VS	VS
Column B	Brine
Unpaired t test	
P value	0.2620
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
t, df	t=0.6974 df=4
How big is the difference? Mean ± SEM of column A Mean ± SEM of column B Difference between means 95% confidence interval R squared	1.114 ± 0.01073 N=3 1.093 ± 0.02762 N=3 0.02067 ± 0.02963 -0.06159 to 0.1029 0.1084
F test to compare variances F,DFn, Dfd P value P value summary Are variances significantly different?	6.628, 2, 2 0.2622 ns No

# -AC 7/24/15

## Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study

Sample ID: Lab Control

Test No.: 1502-S029

Test Species:*H. rufescens*Start Date/Time:2/6/2015End Date/Time:2/8/2015\300

Total Treatment Duration	NA	NA 2.8 minutes			39 m	inutes		30 minutes		
Brine/ seawater addition	ambient	ambient pient seawater addition			bient seav	water addi	tion	ambient	t seawater	addition
Time Between Readings	TO	+1 min.	+ 1 min. 48 sec.		+ 10 min.	+ 10 min.	0 %5)61 <sup>9</sup> + 14 min.	+ 5 min.	+ 10 min.	+ 15 min.
Actual Time	1347 ABH8-	1348	13:49:48	1354	1404	1414	1424	1429	1439	A-++++- 1454
Salinity (ppt)	33.5	33.5	33.5	33.5	33.4	33.5	33.5	33.5	37.5	99.5
Temp (∘C)	15.0	15.0	15.0	15.0	15.0	14.7	14.6	14.6	14,7	14.5

**Technician Initials:** 

WQ Readings: UPP Dilutions made by: ACAPA

Comments:

0 hrs: ambient seawater salinity 33.5 ppt @ preak 2/6/15

\_\_\_\_\_

24 hrs: \_\_\_\_\_\_ 48 hrs: \_\_\_\_\_

QC Check:

Final Review: 3-6/15/15

## Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study

Sample ID: Brine Mixture

Test No.: 1502-S029

 Test Species:
 H. rufescens

 Start Date/Time:
 2/6/2015
 1333

 End Date/Time:
 2/8/2015
 \3t\)

Total Treatment Duration	NA	2.8 m	2.8 minutes		39 minutes			30 minutes		
Brine/ seawater addition	ambient	67 ppt addition to final salinity 44 ppt		seawate	r addition i p	to final sal pt	linity 35.5	Securit 33.5 p AC Q18 210 sa	er et addition linity 3 <del>5.</del> 5	to final opt
Time Detucer Deedinge	то		+ 1 min.		1. 10 min	10 min	1.14 min			1 15 min
Time Between Readings	ТО	+1 min.	48 sec.	+ 5 min.	<u> + 10 min.</u>		+ 14 min.	+ 5 min.	+ 10 min.	
Actual Time	1339	1340	13:41:48	1346	1350	1406	1420	1425	1435	1450
Salinity (ppt)	33.5	42.9	44.8	41.9	38.8	37.0	35.5	35.0	34.4	33.6
Temp (∘C)	14.9	15.2	15.2	15.2	15.1	15.2	15.1	15.1	14.9	14.9

Technician Initials:	WQ Readings: KFP Dilutions made by: Ac/PA	
Comments:	0 hrs: <u>@knp alg 2/6/15 ° 42.8ppt@ 134</u> 24 hrs: 48 hrs:	14 °
QC Check:	Ac 2/9/15	Final Review: KTP 2/10/15

Client: Poseidon

Sample ID: Pump Brine Dilution Study

Sample Log No.: --

Test No.: 1502-S029

## Water Quality Measurements

Test Species:	H. rufesce	ns
Start Date/Time:	2/6/2015	1333
End Date/Time:	2/8/2015	1300

Concentration (ppt)		Salinity (ppt)	nin (Kolink in Anthrop yn ar neg yw annar an an	T	emperatu (°C)	re	Diss	olved Ox (mg/L)	ygen		pH (pH units	;)
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	33.4			15.4			8.3			8.08 <sub>.</sub>		
67 ppt	67.2			15.2			7.9			7.79		
Lab Control Surrogate	33.0	331	33.0	15.0	15.7	15.6	8.3	83	8.1	7.98	7.85	7.93
Brine Dilution Surrogate	33.3	33.2	33.0	15.1	15.2	15.4	8.2	7.8	7.9	7.99	7.94	8,02
Brine Control	33.5	337	33.4	15.4	15.0	15.3	8.3	7:9	7.9	7.95	7:96	8.05
-												

	0 24	48	
Technician Initials:	WQ Readings: NCR/AP AB	BK	
	Dilutions made by:		
Comments:	0 hrs: <u>Day 0 surrogate readings reco</u>	rded after final mixing	
	24 hrs:		
	48 hrs:		
QC Check:	AC 2/9/15	Final Review: KEP 2/10/15	

#### **Abalone Embryo-Larval Development**

Client:	Poseidun	brine pumpfdilut	IM StudyTest Spec	cies: <u>Haliotis rufes</u>	cens
Sample ID:	Frozen bri	ne (Nautilus)	Start Date/T	ime: 2/10/15	1333
Test No.:	1502-5	5029	End Date/T	ime: <u>21815</u>	1300
Animal Source/Da	te Received:	American f	balone	2/3/15	

Number of abalone and condition upon receipt/holding:

Males:

Females:

4 good 4 good \_\_\_\_\_

	Ma	les:	Females:
Tris & peroxide addition time	10	OS	0925
Spawn time	10	200	1292
Number of spawners		4	Ц
Condition of spawn (light, moderate, heavy)	hear	м	heavy
Fertilization time		2:5	2

Embryo counts (per 0.5 ml)					
1	164				
2	144				
3	132				
Mean	147				

Time of test Initiation: 1333

Technician Initials: \_\_\_\_\_AC/ IP4

48 hr. QC (54/94 = 8990

Comments:

QC Check:

2

Final Review: 479 2/10/15

Scenario #1 72-hour Purple Urchin Larval Development Test Initiation Date: February 17, 2015

## Summary of Toxicity Test Results Poseidon - Brine Dilution Pump Study (Scenario #1) 72-hour Purple Urchin (*Strongylocentrotus purpuratus*) Development Test Test Initiation Date: 02/17/15

	Mean Percent Normal Development					
	Phase 1	Phase 2	Phase 3			
Sample ID <sup>a</sup>	Ambient to 44 ppt in 2.8 minutes <sup>b</sup>	44 to 35.5 ppt in 39 <sup>c</sup> minutes	35.5 ppt to ambient salinity in 30 minutes			
Control	93.7	92.0	89.3			
Brine Exposure	91.3	90.3	91.3			

<sup>a</sup> The control and brine exposure were each assigned 10 replicates. Rep A was used a water quality surrogate; reps B, C, and D = phase 1; reps E, F, and G = phase 2; reps H, I, and J = phase 3 (full treatment). Phase 1 and phase 2 treatments were moved to ambient seawater immediately after treatment for the duration of the 72-hour exposure.

<sup>b</sup> Rise from 33.5 to 44 in one minute, held for an additional 1.8 minutes.

<sup>c</sup> The time duration for phase 2 was intended to be 39 minutes, but actual time was 38 minutes 12 seconds due to rounding error.

Note: Control replicates underwent all the same physical movements as brine exposure replicates, but with ambient seawater only. Ambient salinity =  $\sim$  33.5 ppt.

## Detailed Summary of Toxicity Test Results Poseidon - Brine Dilution Pump Study (Scenario #1) 72-hour Purple Urchin (*Strongylocentrotus purpuratus*) Development Test Test Initiation Date: 02/17/15

		#	#	Percent	Mean Percent	Standard
Sample ID	Rep	Normal	Counted	Normal	Normal	Deviation
Control (WQ surrogate)	А					
	В	94	100	94.0		
Control (Phase 1)	С	96	100	96.0	93.7	2.5
	D	91	100	91.0		
	Е	96	100	96.0		
Control (Phase 2)	F	93	100	93.0	92.0	4.6
	G	87	100	87.0		
	Н	89	100	89.0		
Control (Phase 3)	I	92	100	92.0	89.3	2.5
	J	87	100	87.0		
Brine Exposure (WQ surrogate)	А					
	В	88	100	88.0		
Brine Exposure (Phase 1)	С	91	100	91.0	91.3	3.5
	D	95	100	95.0		
	Е	86	100	86.0		
Brine Exposure (Phase 2)	F	93	100	93.0	90.3	3.8
	G	92	100	92.0		
	Н	93	100	93.0		
Brine Exposure (Phase 3)		90	100	90.0	91.3	1.5
	J	91	100	91.0		
	Α	96	100	96.0		
	В	94	100	94.0		
Brine Control <sup>a</sup>	С	93	100	93.0	94.8	1.6
	D	97	100	97.0		
<sup>a</sup> The sector lead being one course (Db	Е	94	100	94.0		

<sup>a</sup> The control and brine exposure (Phases 1, 2, and 3) were tested in screen tubes. The brine control was tested in 5 replicate vials to ensure no adverse effects from the brine water used.

## **Embryo Larval Bioassay**

ーファ Ac **48-hour Development** イ<sub>23</sub>月代

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Client: Poseidon

Project ID: Pump brine dilution study

Test Species: <u>S. purpuratus</u>

Start Date/Time: 2/17/2015 512

End Date/Time: 2/20/2015 1530

Sample	Rep	Number Counted	Number Normal	Technician Initials
Lab Control	A	Surrogate		
	В	(00	94	S
	С	ĵ	96	
	D		91	
	E		96	
	F		93	
	G		87	
r	Н		89	
			92	
	J	Y	87	Ŷ
Brine dilution	Α	Surrogate	100 m	
	В	100	88	15
	C	]	91	
	D		ä 5	
	E		86	
	F		93	
	G		92	
	Н		93	
			90	
	J	1	91	Ŵ
Brine Control	A 5	106	96	F
	B		94	Í
	0		93	
	D		97	
	Ê	¥	94	of
		,		and the second
	_			

2/24/15

Comments: Reps B, C, and D ended after phase 1 (33.5 to 44 ppt) Reps E, F, G ended after phase 2 (44 to 35.5 ppt) Reps H, I, J ended after phase 3 (35.5 to 33.5 ppt)

AC 2/24/15

QC Check:

Final Review: 47 2 2 4 15

# Urchin Development Test Date: 2/17/15 t-test results

Parameter	Value
Table Analyzed	Scenario 2/17/15 spdev P1
Column A	Control
VS	vs
Column B	Brine
Unpaired t test P value P value summary Are means signif. different? (P < 0.05) One- or two-tailed P value? t, df	0.2076 ns No One-tailed t=0.9080 df=4
How big is the difference? Mean ± SEM of column A Mean ± SEM of column B Difference between means 95% confidence interval R squared	1.319 ± 0.02979 N=3 1.276 ± 0.03729 N=3 0.04333 ± 0.04773 -0.08915 to 0.1758 0.1709
F test to compare variances F,DFn, Dfd P value P value summary Are variances significantly different?	1.567, 2, 2 0.7792 ns No

Parameter	Value
Table Analyzed	Scenario 2/17/15 spdev P2
Column A	Control
vs	vs
Column B	Brine
Unpaired t test	
P value	0.3052
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
t, df	t=0.5519 df=4
How big is the difference?	
Mean ± SEM of column A	1.291 ± 0.04856 N=3
Mean ± SEM of column B	1.258 ± 0.03592 N=3
Difference between means	$0.03333 \pm 0.06040$
95% confidence interval	-0.1343 to 0.2010
R squared	0.07075
F test to compare variances	
F,DFn, Dfd	1.828, 2, 2
P value	0.7073
P value summary	ns
Are variances significantly different?	No

BC = AC 7/24/15

# Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study

Sample ID: Lab Control

Test No.: 1507 - 5053

Test Species:	S. purpuratus
Start Date/Time:	2/17/2015 459 1512
End Date/Time:	2/20/15 1530

Total Treatment Duration	NA	2.8 m	inutes	38 <b>39 minutes</b> ۵۷ ۵۷ کاکلار				30 minutes			
Brine/ seawater addition	ambient	amt seawatei	oient addition	am	bient seav		tion	ambient	t seawater	addition	
Time Between Readings	то	+1 min.	+ 1 min. 48 sec.	+ Smin.	+ 10 min.	+ 10 min.	+ 14 min.	+ 5 min.	+ 10 min.	+ 15 min.	
Actual Time	15.24:60	15:27:00	15:28:48	1533	1543	1553	1607	1612	1622	1637	
Salinity (ppt)	33.4	33.4	33.6	33.6	33.4	33,5	33.4	33.5	33.5	33.5	
Temp (∘C)	14.6	14.5	14.6	14.4	14.5	14.2	14.2	14.4	14,5	14.5	

**Technician Initials:** 

WQ Readings:	KFP
Dilutions made by:	AC

Comments:	0 hrs: am	nbient seawater salinity 33.5 ppt	A PAP Q18 2/17/15 - Hach Sension 5 Salintz
	24 hrs:		meter used for all
	48 hrs:		Salinity measurements
		6KAP2/24/15	
QC Check:	AC 2124	115	Final Review: bpp 2/24/15

## Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study

Sample ID: Brine Mixture

Test No.: 1502-S053

Test Species:S. purpuratusStart Date/Time:2/17/2015End Date/Time:2/20/15

Total Treatment Duration	NA	2.8 m	inutes			inutes	30 minutes			
Brine/ seawater addition	ambient	final sa	67 ppt addition to final salinity 44 ppt			क्षाऽ to final sal pt	inity 35.5	eaw B 33.5 p sa	ater pt addition linity 35.5 33.5	to final opt
Time Between Readings	то	+1 min.	+ 1 min. 48 sec.	+ 5 min. + 10 min. + 10 min. + 14 min.			+ 5 min.	+ 10 min.	+ 15 min.	
Actual Time	15:16:00	15:17:00	15:18:48	1523	1533	1543	1557	1602	1612	1627
Salinity (ppt)	33.5	44.8	45.0	41.9	38.9	37.1	35.4	35.1	34.2	335
Temp (∘C)	14.5	14.7	14.7	14.7	14.7	14.9	14.7	14-7	14.5	14.5

**Technician Initials:** 

WQ Readings: KAPP Dilutions made by: AC

Comments:

Ohrs: A 107 Q18 2/17/15 BAC Q18 2/17/15 - Hach Sension 5 motes used 24 hrs: For measuremente 48 hrs: AC 018,2/23/15 Final Review: KAP2/24/15 24/15

QC Check:

## Water Quality Measurements

Client: Poseidon											Test S	pecies	: S. purp	ouratus		
Sample ID:	Sample ID: Pump Brine Dilution Study									S		-	: 2/17/20		15/2	
Sample Log No.:						_						2/20/				
Concentration (ppt)		(p	linity opt)			-	erature °C)		D	issolve (m	d Oxyg g/L)	jen	pH (pH units)			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24		
Lab Control	33.9	33.4			15.0				8.6				8.13			
67 ppt	67.2				16.0			l	8.1				7.63			
Lab Control Surrogate	33.5	33,4	33.4	33.4	14,5	15.4	14,9	(5,1	9.4	7.4	7.6	7.9	8.02	7.93	8.04	6,62
Brine Dilution Surrogate		33.5	39.5	33.5	14.6	15.4	14,6	14,8	9.4	7.4	7.6	8.2	7.97	7.96	8.04	6.62 867
Brine Control	<u>†</u>	33.5	33.5	33.6	15.8	15.6	15,6	14.7	8.3	7.6	7,7	8.4	8.00	7.9.1	8.02	6.67
	33.6 Q18 VER 217/15															
												:				
Technician Initials:			adings: ade by:	0 Juripe AC	24 AG	48 ビム	<b>72</b> EG									
Comments:	0 hrs: 24 hrs:	Day 0 s	surrogat	e readin	igs reco	rded aft	ter final ı	mixing -	- Szl med	inite	) meter	er Ha	ch Se 1 seli	insion nit n	<u>- 5</u> Nasur	ements
	48 hrs: 72 hrs:												****			

-

QC Check: ACHA415

Final Review: 177 2/2415

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

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#### **Echinoderm Larval Development Worksheet**

Client: Sample ID: Test No.: Tech initials: Injection Time:	Poseid brine ( 1502 AC 1430	un-PVmp/J Nautilistin -SOS3	ilution Study <u>220n Seaub</u> ter	Start Date/Time: <u>2/17/15 1 1512</u> End Date/Time: <u>2/20/15 1 1530</u> Species: <u>5. provratus</u> Date Collected: <u>2/4/15</u>
Sperm Absorbance at 40	00 nm:	<u>.</u> (targe	et range of 0.8 - 1.0 for density o	4x10 <sup>6</sup> sperm/ml)
Eggs Counted:	25 19 18 22 23	(target coun	1.9 X 50 = $1070ts of 20 eggs per vertical pass on Shal density of 1000 eggs/ml)$	
Initial density: Final density:	107 <i>0</i> 1000	eggs/ml = eggs/ml	1.07 dilution factor -1.0, part egg stock parts seawater	egg stock <u>ZUÜ</u> ml seawater <u> </u>

Prepare the egg 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).

Add 100 µL sperm stock per 100mL of egg stock. For example, if you have 60mL of egg stock, add 60µL sperm stock.

Embryo Stock Fertilization Checks (Initiate test only when fertilization is ≥90%):

5 minutes (1st fert.) 10 minutes (2nd fert. If		Time	No   	. <u>Unfert.</u>	% 96	
Fernization	time:	1445				
Test Initiation Time:	(B)+50	01512	Emb	oryo Stock Added:	0.25 ml	
Test initiation must be w	vithin 1 hour	of fertilization	time.			
Test Termination:						
	No.	No.	%			
72-hour QC check 1 <sup>a</sup> QC check 2	Normal 94	Abnormal	Normal 94			
Comments:	<sup>a</sup> If the em	bryo developn	nent does not	meet the mean te	st acceptability	criterion of 80% normally
(	developed BQ21 test	AC 2/17/15	"Did no			limity in 2.8 min. re-Susped n same stock.
QC Check:	AC 2/2	-415	_		·	Final Review: 1779 2/24/15

Scenario #2 48-hour Red Abalone Larval Development Test Initiation Date: January 30, 2015

## Summary of Toxicity Test Results Poseidon - Brine Dilution Pump Study (Scenario #2) 48-hour Abalone (*Haliotis rufescens*) Development Test Test Initiation Date: 01/30/15

	Mean	Percent Normal Devel	opment
	Phase 1	Phase 2	Phase 3
Sample ID <sup>a</sup>	Ambient to 42 ppt in 2.2 minutes <sup>b</sup>	42 to 35.5 ppt in 36 minutes	35.5 ppt to ambient salinity in 30 minutes
Control	94.0	93.7	94.3
Brine Exposure	95.7	92.7	91.7

<sup>a</sup> The control and brine exposure were each assigned 10 replicates. Rep A was used a water quality surrogate; reps B, C, and D = phase 1; reps E, F, and G = phase 2; reps H, I, and J = phase 3 (full treatment). Phase 1 and phase 2 treatments were moved to ambient seawater immediately after treatment for the duration of the 48-hour exposure. No statistically significant effects were calculated (p < 0.05).

<sup>b</sup> Rise from 33.5 to 42 in one minute, held for an additional 1.2 minutes.

Note: Lab control replicates underwent all the same physical movements as brine replicates, but with ambient seawater only. Ambient salinity =  $\sim$  33.5 ppt.

## Summary of Toxicity Test Results Poseidon - Brine Dilution Pump Study (Scenario #2) 48-hour Abalone (*Haliotis rufescens*) Development Test Test Initiation Date: 01/30/15

					Mean	
		#	#	Percent	Percent	Standard
Sample ID	Rep	Normal	Counted	Normal	Normal	Deviation
Control (WQ surrogate)	А					
	В	91	100	91.0		
Control (Phase 1)	С	95	100	95.0	94.0	2.6
	D	96	100	96.0		
	Е	95	100	95.0		
Control (Phase 2)	F	94	100	94.0	93.7	2.0
	G	92	100	92.0		
	Н	96	100	96.0		
Control (Phase 3)	I	92	100	92.0	94.3	2.1
	J	95	100	95.0		
Brine Exposure (WQ surrogate)	Α					
	В	96	100	96.0		
Brine Exposure (Phase 1)	С	93	100	93.0	95.7	2.5
	D	98	100	98.0		
	Е	89	100	89.0		
Brine Exposure (Phase 2)	F	94	100	94.0	92.7	0.58
	G	95	100	95.0		
	Н	94	100	94.0		
Brine Exposure (Phase 3)	I	91	100	91.0	91.7	2.1
	J	90	100	90.0		
	Α	91	100	91.0		
	В	94	100	94.0		
Brine Control <sup>a</sup>	С	92	100	92.0	92.8	2.2
	D	91	100	91.0		
	Е	96	100	96.0		

<sup>a</sup> The control and brine exposure (Phases 1, 2, and 3) were tested in screen tubes. The brine control was tested in 5 replicate vials to ensure no adverse effects from the brine water used.

## **48-hour Development**

Client: Poseidon

Project ID: Pump brine dilution study

Test NO: 1501-5098

**Test Species:** *H. rufescens* 

1522 Start Date/Time: 1/30/2015

End Date/Time: 2/1/201

015	4	15

Sample	Rep	Number Counted	Number Normal	Technician Initials		
Lab Control	Α	Surrogate				
	В	100	91	СН		
PI }	С	100	95	СН		
<	D	106	96	Clt		
C	E	100	95	CH		
P2 5	F	100	94	CH		
	G	100	92	СН		
(	Н	160	96	CH		
P35	I	100	92	CH		
	J	100	95	CH		
Brine dilution	Α	Surrogate				
(	В	100	96	CH		
p1)	С	100	93	CH		
<u> </u>	D	100	98	CH		
(	E	100	89	CH		
P27	F	100	94	CH		
	G	100	95	CH		
(	Н	100	94	CH		
P35		100	91	CH		
<u>\</u>	J	100	90	CH		
Brine Control	A	100	91	CH		
	В	100	94	CH		
	0	100	92	CH		
	D	100	<u>a1</u>	CH		
	E	100	96	CH		

Comments: Reps B, C, and D ended after phase 1 (67 to 42 ppt) Reps E, F, G ended after phase 2 (42 to 35.5 ppt) Reps H, I, J ended after phase 3 (35.5 to 33.5 ppt)

AC 2/3/15

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QC Check:

Final Review: 1479 6/15/15

# Abalone Development Test Date: 1/30/15 t-test results

Parameter	Value			
Table Analyzed	Scenario 1/30/15 P2			
Column A	Control			
VS	vs			
Column B	Brine			
Unpaired t test				
P value	0.3413			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
One- or two-tailed P value?	One-tailed			
t, df	t=0.4401 df=4			
How big is the difference? Mean ± SEM of column A Mean ± SEM of column B Difference between means 95% confidence interval R squared	1.317 ± 0.01784 N=3 1.300 ± 0.03426 N=3 0.01700 ± 0.03862 -0.09022 to 0.1242 0.04619			
F test to compare variances F,DFn, Dfd P value P value summary Are variances significantly different?	3.690, 2, 2 0.4265 ns No			

Parameter	Value			
Table Analyzed	Scenario 1/30/15 P3			
Column A	Control			
VS	VS			
Column B	Brine			
Unpaired t test				
P value	0.0947			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
One- or two-tailed P value?	One-tailed			
t, df	t=1.579 df=4			
How big is the difference? Mean ± SEM of column A Mean ± SEM of column B Difference between means 95% confidence interval R squared	1.333 ± 0.02530 N=3 1.279 ± 0.02238 N=3 0.05333 ± 0.03378 -0.04043 to 0.1471 0.3840			
F test to compare variances F,DFn, Dfd P value P value summary Are variances significantly different?	1.278, 2, 2 0.8779 ns No			

QC=AC7/24/15

## Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study

Sample ID: Brine Mixture

Test No.: 1501-S098

 Test Species:
 *H. rufescens* 

 Start Date/Time:
 1/30/2015
 1522

 End Date/Time:
 2/1/2015
 14/5

Total Treatment Duration	NA	2.2 minutes		36 minutes		30 minutes					
		67 ppt addition to final salinity 42		seawater addition to final salinity 35.5			salinity 35.5 ppt				
Brine/ seawater addition	ambient	ppt		ppt		33.5					
			+ 1 min.								
Time Between Readings	<u> </u>	+1 min.	12 sec.	+ 6 min.	+ 10 min.	+ 10 min.	+ 10 min.	+ 5 min.	+ 10 min.	+ 15 min.	
Actual Time	1535	15:36:00	15:37:12	1543	1553	1603	1613	1618	1628	1633	1/30/15
Salinity (ppt)	33.6	41.5	42.1	39.4	31.2	36.4	35.le	34.9	34.0	33.6	
Temp (∘C)	15.1	14.8	14.9	14.8	14.9	14.8	14.8	14.8	14.7	14.6	

Technician Initials:	WQ Readings: KPP Dilutions made by: PA/AC	INCR
Comments:	@Q181c6/sh5 0 hrs: <u>Remove 3</u> 24 hrs: 48 hrs:	reps after each phase (noted by red box)
QC Check:	Acal315	Final Review: prp 6 15 15

## Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study

đ

Sample ID: Lab Control

Test No.: 1501-S098

 Test Species:
 *H. rufescens* 

 Start Date/Time:
 1/30/2015
 1522

 End Date/Time:
 2/1/2015
 1405

Total Treatment Duration	NA	2.2 m	inutes	36 minutes				30 minutes		
Brine/ seawater addition	ambient	ambient seawater addition		ambient ambient seawater addition		ambient seawater addition		addition		
Time Between Readings	то	+1 min.	+ 1 min. 12 sec.	+ 6 min.	+ 10 min	+ 10 min	+ 10 min.	+ 5 min.	1. 10 min	L 1E min
Actual Time		15:26:00	12 300.	1533	1543	1653	1603	1608	+ 10 min. 1618	+ 15 min. 1633
Salinity (ppt)	33.6	33.6	33.6	33.6	33.6	33.6	33.U	33.4	33.6	33.6
Temp (∘C)	15.1	15.2	15.0	15.1	14.9	14.8	14.5	14.5	14.6	14.7

**Technician Initials:** 

WQ Readings: MP Dilutions made by: PAIAC

Comments:

0 hrs: ambient seawater salinity 33.5 ppt ,	Remove 3 reps	after	each	phase (	red box
24 hrs:					/

48 hrs:

QC Check:

AC 2/3/15

Final Review: AP 6 5 5

ο.

Client: Poseidon

Sample ID: Pump Brine Dilution Study

Sample Log No.: --

Test No.: 501 - 5098

### Water Quality Measurements

 Test Species:
 *H. rufescens* 

 Start Date/Time:
 1/30/2015
 1522

 End Date/Time:
 2/1/2015
 415

Concentration (ppt)		Salinity (ppt)		Т	emperatu (°C)	re	Diss	olved Ox (mg/L)	ygen		рН (pH units	)
	0	24	48	Q	24	48	0	24	48	0	24	48
Lab Control	33.6			14.7			83			8.03		
67 ppt	67.0			14.7			nm			nm		
Lab Control Surrogate	33.4	33.2	33.2	15.)	(5.6	15.2	8.3	7.4	7.7	7.87	7.92	7.95
Brine Dilution Surrogate	335	33.4	33.3	14.8	14.8	15.0	8.3	7.0	7.0	7.95	8.00	8.02
Brine Control	33.6	33.5	33.4	14.7	14,7	15.0	8.2	7.0	7.5	7.84	7.98	8,03
			-									

		0	24	48
Fechnician Initials:	WQ Readings:	AC	AD	BK
	Dilutions made by:	-AC		

Comments:	0 hrs: Day 0 surrogate readings recorded after final mixing	n m= not measured; tech error
	24 hrs: 48 hrs:	
QC Check:	AC 2/3/15	Final Review: KTP 6/15/15

#### **Abalone Embryo-Larval Development**

Client:	Poser	lon - brine dilut	im Study Test Species: 1	Haliotis rufescens
Sample ID:		brine (Nautilus		1/30/2015 1522
Test No.:	1501-	5098	End Date/Time: 2	2/1/2015 1415
Animal Source/Da	te Received:	American A	balone/ 1/=	28/15

Number of abalone and condition upon receipt/holding:

Males: Females:

4 good, small 4 good

	Males:	Females:
Tris & peroxide addition time	1100	1030
Spawn time	1330	1400
Number of spawners	4	AUS 3
Condition of spawn (light, moderate, heavy)	heavy	moderat
Fertilization time	-+4	03 QZI
	AC 1/20/15	- 142

Embryo counts (per 0.5 ml)					
1	141				
2	195				
3	183				
Mean	173				

Time of test Initiatio	n: 1522	48 hr. QC <u>97</u>
Technician Initials	s: <u>{</u> A	
Comments:	621 - used different batch	of # eggs (more dense) and
QC Check:	AC 2/3/15	Final Review: 1479 6 5

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

Scenario #3 48-hour Red Abalone Larval Development Test Initiation Date: January 22, 2015

## Summary of Toxicity Test Results Poseidon - Brine Dilution Pump Study (Scenario #3) 48-hour Abalone (*Haliotis rufescens*) Development Test Test Initiation Date: 01/22/15

	Mean Percent Normal Development			
	Phase 1	Phase 2	Phase 3	
Sample ID <sup>a</sup>	Ambient to 40 ppt for 1.7 minutes <sup>b</sup>	40 to 35.5 ppt in 34 minutes	35.5 ppt to ambient salinity in 30 minutes	
Control <sup>c</sup>	66.0	61.0	67.3	
Brine Exposure	68.5	67.0	60.3	

<sup>a</sup> The control and brine exposure were each assigned 12 replicates. Rep A was used a water quality surrogate; reps B and C = phase 1; reps H and I = phase 2 {reps D through G ended early in Phase 2 for observation and are not included in the mean}; reps J, K, L = phase 3 (full treatment). Phase 1 and phase 2 treatments were moved to ambient seawater immediately after treatment for the duration of the 48-hour exposure.

<sup>b</sup> Salinity reached 39.2 ppt after the first minute, and remained between between 39 and 40 for at least 5 minutes. See water quality datasheet for detail.

<sup>c</sup> The control did not meet the minimum test acceptability criterion of 80 percent mean normal development. However, this test was experimental and not for compliance, and the data are reported as they are useful for determining next steps for testing.

Note: Control replicates underwent all the same physical movements as brine replicates, but with ambient seawater only. Ambient salinity =  $\sim$  33.5 ppt.

### Summary of Toxicity Test Results Poseidon - Brine Dilution Pump Study (Scenario #3) 48-hour Abalone (*Haliotis rufescens*) Development Test Test Initiation Date: 01/22/15

Sample ID	Rep	# Normal	# Counted	Percent Normal	Mean Percent Normal	Standard Deviation
Control (WQ surrogate)	А					
Control (Phase 1)	В	67	100	67.0	66.0	1.53
Control (Filase T)	С	65	100	65.0	00.0	1.55
	D	64	100	64.0	65.0	3.5
	Е	66	100	66.0	0.00	5.5
Control (Phase 2) <sup>a</sup>	F	73	100	73.0	71.0	4.7
Control (Filase 2)	G	69	100	69.0	71.0	4.7
	Н	62	100	62.0	61.0	3.1
	I	60	100	60.0	01.0	
	J	62	100	62.0		
Control (Phase 3)	K	66	100	66.0	67.3	5.59
	L	74	100	74.0		
Brine Exposure (WQ surrogate)	Α					
Brine Exposure (Phase 1)	В	73	100	73.0	68.5	4.51
Diffe Exposure (i flase i)	С	64	100	64.0	00.5	4.51
	D	68	100	68.0	72.0	4.6
	E	76	100	76.0	72.0	4.0
Brine Exposure (Phase 2)	F	68	100	68.0	68.0	1.2
	G	68	100	68.0	00.0	1.2
	Н	66	100	66.0	67.0	6.2
		68	100	68.0	07.0	0.2
	J	65	100	65.0		
Brine Exposure (Phase 3)	K	56	100	56.0	60.3	4.90
	L	60	100	60.0		

<sup>a</sup> only reps H & I went through the entire P2, other reps were pulled early.

### **Embryo Larval Bioassay**

### **48-hour Development**

UUU

Client: Poseidon

**Project ID:** Pump brine dilution study

Test Species:	H. rufescens
---------------	--------------

Start Date/Time: 1/22/2015

End Date/Time: 1/24/2015

WV

Sample	Rep	Number Counted	Number Normal	Technician Initials
Lab Control	Α	Surrogate	86	1 2
PI S	В	100	67	AC 1/25/15
2	С	)	65	
TID (	D		64	
(min)	Е		66	
P2 505	F		73	
lmin	G		69	
34	Н		62	
Imin	1		(40	
<u> </u>	J		62	
P3 5	K		66	
	L	<u>y</u>	74	
Brine dilution	A	Surrogate		
PI S	B	100	73	
	C	1 418		
jo min	D E		68	
- 102	F		<u> </u>	
120 PZ S	G		<u> </u>	
34	H		1 1	
Imin			<u> </u>	
	J		65	
P3 2	ĸ		56	
	L		60	
		· · · · · · · · · · · · · · · · · · ·	<u>_</u>	
· · ·				

Comments: Reps B, C, and D mixing treament ended after phase 1 (67 to 40 ppt)  $A^{C}$ Reps E, F, G mixing treament ended after phase 2 (40 to 35.5 ppt)

Reps H, I, J, K, L full treament see table in spec sheet

QC Check:

Final Review: 56/15/15

# Abalone Development Test Date: 1/22/15 t-test results

Parameter	Value
Table Analyzed	Scenario 1/22/15 P3
Column A	Control
VS	vs
Column B	Brine
Unpaired t test P value P value summary Are means signif. different? (P < 0.05) One- or two-tailed P value? t, df	0.0940 ns No One-tailed t=1.586 df=4
How big is the difference? Mean ± SEM of column A Mean ± SEM of column B Difference between means 95% confidence interval R squared	0.9637 ± 0.03805 N=3 0.8900 ± 0.02663 N=3 0.07367 ± 0.04645 -0.05527 to 0.2026 0.3861
F test to compare variances F,DFn, Dfd P value P value summary Are variances significantly different?	2.042, 2, 2 0.6576 ns No

Qc = Ac 7/24/15

## Water Quality Measurements

 Test Species:
 *H. rufescens* 

 Start Date/Time:
 1/22/2015

 Ind Date/Time:
 1/24/2015

Total Treatment Duration	NA	1.7 m	inutes	34 minutes				30 minutes		
Brine/ seawater addition	ambient	33.5	5 ppt	33.5 ppt addition				33.5 addition		
Time Between Readings	org to	+1 min.	+42 sec.	+ 5 min.	+ 10 min.	+ 10 min.	+ 9 min.	+ 5 min.	+ 10 min.	+ 15 min.
Actual Time	5 - 144	14:48:00	14:48:42	1454	15,04	1514	1523	1528	1538	1553
Salinity (ppt)	33.4	33.5	33.5	33.5	33-7	33.7	33.7	33.7	33.6	33.6
Temp (∘C)	15.5	15.4	15.4	15.4	15.0	15.0	14.9	14.8	147	14.7
										· · ·

**Technician Initials:** 

WQ Readings: HEP/AC Dilutions made by: PA

Comments:

0 hrs. 1459	reps . DtE pulled, 5.1°C, 33. leppt,	F+6 pulled at 15:03
24 hrs: 48 hrs:	the stand free the f	6/15/15
ACTOMIK		Final Review: A 6/15/15

QC Check:

## Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study

Sample ID: Brine Mixture

Sample Log No.: --

Test No.: 1501-5097

**Total Treatment Duration** NA 1.7 minutes 34 minutes 30 minutes 67 ppt addition to seawater addition to final final salinity 40 seawater addition to final salinity 35.5 ppt salinity 33.5 +50° Brine/ seawater addition ambient ppt Time Between Readings +1 min. +42 sec. + 5 min. T0 + 10 min. + 10 min. + 9 min. + 5 min. + 10 min. + 15 min. 602 501-Actual Time Imp -15.03:00 15:03:42 15:08 1528 1518 1607 1531 1552 1542 33.5 35.5 38,8 39.2 40.1 351 33.6 31.2 Salinity (ppt) 35.7 34.7 14.6 14.5 15.0 14.6 14.9 Temp (∘C) 14.6 14.6 14-7 14:5 14.6

Technician Initials: WQ Readings: Kmp/AC Dilutions made by: 0 hrs: 1513 reps DEE pulled; 37.9 ppt 14.7°C°, Fig pulled at 518 Comments: 24 hrs: 48 hrs: Final Review: 9 6/15/15 QC Check:

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

Test Species: H. rufescens Start Date/Time: 1/22/2015 1444-End Date/Time: 1/24/2015 1500

Client: Poseidon

Sample ID: Pump Brine Dilution Study

Sample Log No.:

Test No.: 1501-5097

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### Water Quality Measurements

 Test Species:
 *H. rufescens* 

 Start Date/Time:
 1/22/2015
 1444

 End Date/Time:
 1/24/2015
 1560

Concentration (ppt)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	33.5			15:4			8.4			8.05		
67 ppt	67.0			159			7.7			7.61		
Lab Control Surrogate	33.6	33.4	33,5	14.8	14.7	14.9	87	7.8	8.3	7.84	8.01	8.03
Brine Dilution Surrogate	33.6	33.5	33.5	14.7	14.3	14.4	59	7.7	8.4	7.97	8.05	8.01

02448Technician Initials:WQ Readings:WADilutions made by:PAWA

Comments:

0 hrs: Day 0 surrogate readings recorded after final mixing

24 hrs:

48 hrs:

QC Check:

Final Review: 3 6/15/15

#### Abalone Embryo-Larval Development

Client:	Poseidon	Test Species: Haliotis rufescens
Sample ID:	Nautilus Frozen brine	Start Date/Time: <u>1/22/2015 다구나</u>
Test No.:	1501-S097	End Date/Time: 1/24/2015 しらしし
Animal Source/Dat	e Received: <u>American</u> Aba	Vone 1/20/15

Number of abalone and condition upon receipt/holding:

Males:

2/400/

Females:

	Males:	Females:
Tris & peroxide addition time	1045	1015
Spawn time	1315	1305
Number of spawners	2	
Condition of spawn (light, moderate, heavy)	Modente	light
Fertilization time	1350	9

Embryo counts (per 0	5 ml)
1	180
2	172
3	184
Mean	

Time of test Initiation:

Technician Initials:  $\_PA$ 

AC 1/251

Comments:

QC Check:

Final Review: 5 6/15/15

48 hr. QC 53

**APPENDIX B** 

**Reference Toxicant Test Data** 

48-hour Red Abalone Larval Development Test Initiation Date: January 22, 2015

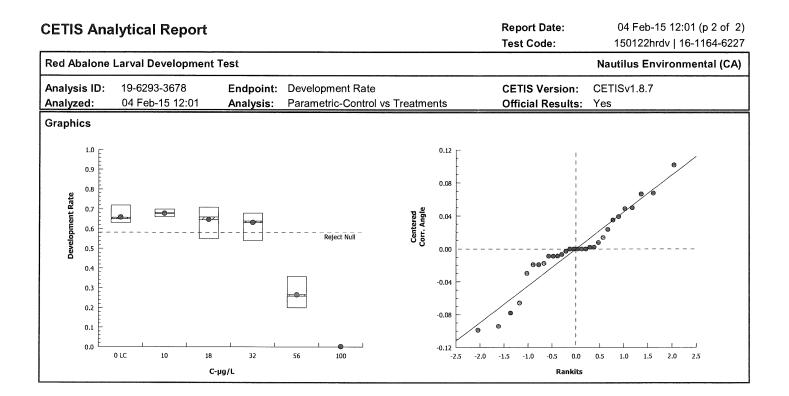
CETIS Sun	nmary Repo	rt						Report Date: Test Code:			1 (p 1 of 1) -1164-6227
Red Abalone	Larval Developm	nent Tes	t						Nautilu	s Environm	ental (CA)
Batch ID: Start Date: Ending Date: Duration:	11-1621-8697 22 Jan-15 14:44 24 Jan-15 15:00 48h	1 P ) S	est Type: rotocol: pecies: ource:	Development EPA/600/R-95 Haliotis rufesc American Aba	ens				ıral Seawatı Applicable	er	
Sample ID: Sample Date: Receive Date: Sample Age:		N	ode: laterial: ource: tation:	150122hrdv Zinc sulfate Reference To: Zinc sulfate	xicant			Client: Inter Project:	nal		
Comparison S Analysis ID 19-6293-3678	Summary Endpoint Development R	ate	NOEL 32	<b>LOEL</b> 56	<b>TOEL</b> 42.33	<b>PMSD</b> 11.6%	τU	Method Dunnett M	lultiple Com	parison Tes	t
Point Estimate Analysis ID 21-0118-1264	e Summary Endpoint Development Ra	ate	Level EC50	μg/L 50.62	<b>95% LCL</b> 49.17	<b>95% UCL</b> 52.11	TU	Method Spearman	-Kärber		
Test Acceptat Analysis ID 19-6293-3678 21-0118-1264 19-6293-3678	Endpoint Development R Development R Development R	ate ate	Contro NOEL	ol Resp ol Resp	0.658 0.658 32	TAC Limi 0.8 - NL 0.8 - NL NL - 56	ts	Overlap Yes Yes No	Below Ac Passes A	ceptability C cceptability	Criteria
19-6293-3678 Development	Development R Rate Summary	ate	PMSE		0.1162	NL - 0.2		No	Passes A	cceptability	Criteria
С-µg/L 0 10 18 32 56 100	Control Type Lab Control	<b>Count</b> 5 5 5 5 5 5 5 5	Mean 0.658 0.678 0.646 0.632 0.268 0	95% LCL 0.6138 0.6596 0.5702 0.5632 0.1841 0	95% UCL 0.7022 0.6964 0.7218 0.7008 0.3519 0	Min 0.63 0.66 0.55 0.54 0.2 0	Max 0.72 0.7 0.68 0.36 0	2 0.01594 0.006633 0.02731 8 0.02478	Std Dev           0.03564           0.01483           0.06107           0.05541           0.0676           0	CV% 5.42% 2.19% 9.45% 8.77% 25.22%	%Effect 0.0% -3.04% 1.82% 3.95% 59.27% 100.0%
Development	Rate Detail Control Type	Pop 1	Rep 2	Rep 3	Rep 4	Rep 5					
С-µg/L 0 10 18 32 56 100	Lab Control	Rep 1           0.63           0.68           0.63           0.64           0.2           0	0.64 0.68 0.71 0.68 0.31 0	0.65 0.66 0.68 0.54 0.26 0	0.65 0.67 0.66 0.63 0.21 0	0.72 0.7 0.55 0.67 0.36 0					

Q15- Coded as unofficial

Analyst: KB QA: A.C.265115

CETIS Analytical Report								-	ort Date: Code:	04 Feb-15 12:01 (p 1 of 2) 150122hrdv   16-1164-6227		
Red Abalon	e Larva	Developr	nent Te	st	olega derror and a second					Nautilus	Environm	ental (CA)
Analysis ID: Analyzed:		293-3678 eb-15 12:0		•	velopment R ametric-Cor		tments		S Version: al Results:	CETISv1. Yes	8.7	
Data Transfo	orm		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)		NA	C > T	NA	NA		11.6%	32 56 42.33			
Dunnett Mul	ltiple Co	omparison	Test									
Control	vs	C-µg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(	a:5%)		
Lab Control		10		-0.6075	2.305	0.079 8	0.9382	CDF	Non-Signif	icant Effect		9920404040500020 <del>00</del> 0006600000000000000000000000000
		18		0.3524	2.305	0.079 8	0.6675	CDF	Non-Signif	icant Effect		
		32		0.7844	2.305	0.079 8	0.4754	CDF	Non-Signif	icant Effect		
		56*		11.77	2.305	0.079 8	<0.0001	CDF	Significant	Effect		
ANOVA Tabl	le											
Source		Sum Squa	ares	Mean Squ	are	DF	F Stat	P-Value	Decision(	a:5%)		
Between		0.647029		0.1617573		4	54.72	<0.0001	Significant	Effect		
Error		0.0591219	94	0.0029560	97	20						
Total		0.706151				24						
Distribution	al Tests	;										
Attribute		Test			Test Stat	Critical	P-Value	Decision	(a:1%)			
Variances		Bartlett E	quality o	of Variance	7.59	13.28	0.1078	Equal Var	iances			
Distribution		Shapiro-V	Vilk W N	Normality	0.9646	0.8877	0.5135	Normal Di	stribution			
Developmen	nt Rate S	Summary										
C-µg/L	Contr	ol Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab C	ontrol	5		0.0400	0.7000						
		0110101	5	0.658	0.6138	0.7022	0.65	0.63	0.72	0.01594	5.42%	0.0%
10		ontrol	5 5	0.658 0.678	0.6596	0.7022 0.6964	0.65 0.68	0.63 0.66	0.72 0.7	0.01594 0.006633	5.42% 2.19%	0.0% -3.04%
10 18		ontion										
		ontrol	5 5 5	0.678	0.6596	0.6964	0.68	0.66	0.7	0.006633	2.19%	-3.04%
18			5 5	0.678 0.646	0.6596 0.5702	0.6964 0.7218	0.68 0.66	0.66 0.55	0.7 0.71	0.006633 0.02731	2.19% 9.45%	-3.04% 1.82%
18 32			5 5 5	0.678 0.646 0.632	0.6596 0.5702 0.5632	0.6964 0.7218 0.7008	0.68 0.66 0.64	0.66 0.55 0.54	0.7 0.71 0.68	0.006633 0.02731 0.02478	2.19% 9.45% 8.77%	-3.04% 1.82% 3.95%
18 32 56			5 5 5 5 5	0.678 0.646 0.632 0.268 0	0.6596 0.5702 0.5632 0.1841	0.6964 0.7218 0.7008 0.3519	0.68 0.66 0.64 0.26	0.66 0.55 0.54 0.2	0.7 0.71 0.68 0.36	0.006633 0.02731 0.02478 0.03023	2.19% 9.45% 8.77%	-3.04% 1.82% 3.95% 59.27%
18 32 56 100	rrected)		5 5 5 5 med Su	0.678 0.646 0.632 0.268 0 mmary	0.6596 0.5702 0.5632 0.1841	0.6964 0.7218 0.7008 0.3519	0.68 0.66 0.64 0.26	0.66 0.55 0.54 0.2	0.7 0.71 0.68 0.36	0.006633 0.02731 0.02478 0.03023	2.19% 9.45% 8.77%	-3.04% 1.82% 3.95% 59.27%
18 32 56 100 Angular (Con	rrected)	) Transfori ol Type	5 5 5 5 5 med Su	0.678 0.646 0.632 0.268 0 mmary	0.6596 0.5702 0.5632 0.1841 0	0.6964 0.7218 0.7008 0.3519 0	0.68 0.66 0.64 0.26 0	0.66 0.55 0.54 0.2 0	0.7 0.71 0.68 0.36 0	0.006633 0.02731 0.02478 0.03023 0	2.19% 9.45% 8.77% 25.22%	-3.04% 1.82% 3.95% 59.27% 100.0%
18 32 56 100 Аngular (Сон С-µg/L	rrected) Contr	) Transfori ol Type	5 5 5 5 med Su	0.678 0.646 0.632 0.268 0 mmary : Mean	0.6596 0.5702 0.5632 0.1841 0 95% LCL	0.6964 0.7218 0.7008 0.3519 0 <b>95% UCL</b>	0.68 0.66 0.64 0.26 0 Median	0.66 0.55 0.54 0.2 0 Min	0.7 0.71 0.68 0.36 0 <b>Max</b>	0.006633 0.02731 0.02478 0.03023 0 Std Err	2.19% 9.45% 8.77% 25.22%	-3.04% 1.82% 3.95% 59.27% 100.0%
18 32 56 100 Аngular (Сол С-µg/L 0	rrected) Contr	) Transfori ol Type	5 5 5 5 <b>med Su</b> 5 5 5 5 5	0.678 0.646 0.632 0.268 0 mmary : Mean 0.9466	0.6596 0.5702 0.5632 0.1841 0 <b>95% LCL</b> 0.8991	0.6964 0.7218 0.7008 0.3519 0 <b>95% UCL</b> 0.994	0.68 0.66 0.64 0.26 0 <b>Median</b> 0.9377	0.66 0.55 0.54 0.2 0 <b>Min</b> 0.9169	0.7 0.71 0.68 0.36 0 <b>Max</b> 1.013	0.006633 0.02731 0.02478 0.03023 0 <b>Std Err</b> 0.0171	2.19% 9.45% 8.77% 25.22% CV% 4.04%	-3.04% 1.82% 3.95% 59.27% 100.0% %Effect 0.0%
18 32 56 100 Аngular (Сол С-µg/L 0 10	rrected) Contr	) Transfori ol Type	5 5 5 5 <b>med Su</b> 5 5 5 5 5	0.678 0.646 0.632 0.268 0 mmary 	0.6596 0.5702 0.5632 0.1841 0 <b>95% LCL</b> 0.8991 0.9477	0.6964 0.7218 0.7008 0.3519 0 <b>95% UCL</b> 0.994 0.9872	0.68 0.66 0.26 0 <b>Median</b> 0.9377 0.9695	0.66 0.55 0.54 0.2 0 <b>Min</b> 0.9169 0.9483	0.7 0.71 0.68 0.36 0 <b>Max</b> 1.013 0.9912	0.006633 0.02731 0.02478 0.03023 0 <b>Std Err</b> 0.0171 0.007116	2.19% 9.45% 8.77% 25.22% CV% 4.04% 1.65% 6.79% 6.19%	-3.04% 1.82% 3.95% 59.27% 100.0% %Effect 0.0% -2.21% 1.28% 2.85%
18 32 56 100 <b>Angular (Con</b> <b>C-μg/L</b> 0 10 18	rrected) Contr	) Transfori ol Type	5 5 5 5 <b>med Su</b> 5 5 5 5 5	0.678 0.646 0.632 0.268 0 mmary Mean 0.9466 0.9675 0.9345	0.6596 0.5702 0.5632 0.1841 0 95% LCL 0.8991 0.9477 0.8557	0.6964 0.7218 0.7008 0.3519 0 95% UCL 0.994 0.9872 1.013	0.68 0.66 0.64 0.26 0 <b>Median</b> 0.9377 0.9695 0.9483	0.66 0.55 0.54 0.2 0 <b>Min</b> 0.9169 0.9483 0.8355	0.7 0.71 0.68 0.36 0 <b>Max</b> 1.013 0.9912 1.002	0.006633 0.02731 0.02478 0.03023 0 <b>Std Err</b> 0.0171 0.007116 0.02838	2.19% 9.45% 8.77% 25.22% CV% 4.04% 1.65% 6.79%	-3.04% 1.82% 3.95% 59.27% 100.0% %Effect 0.0% -2.21% 1.28%

Analyst:\_<u>VB</u>\_\_\_QA:<u>AC 2/5/15</u>



B QA: AC2/5/15

CETIS Analytical Report Red Abalone Larval Development Test								ort Date: Code:	04 Feb-15 12:01 (p 1 of 1) 150122hrdv   16-1164-6227			
									Nautilus	s Environ	mental (CA)	
-			•	•				CETIS Version: Official Results:		.8.7		
Spearmai	n-Kärber Estimate	es			999933 - 2993 - 1993 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995							
Threshold	d Option	⊺hreshold	Trim	Mu	Sigma		EC50	95% LCL	95% UCL			
Control Th	nreshold	0.342	0.00%	1.704	0.006299		50.62	49.17	52.11			
Developm	nent Rate Summa	ry			Calcu	lated Varia	te(A/B)					
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	А	в	
0	Lab Control	5	0.658	0.63	0.72	0.01594	0.03564	5.42%	0.0%	329	500	
10		5	0.678	0.66	0.7	0.006633	0.01483	2.19%	-3.04%	339	500	
18		5	0.646	0.55	0.71	0.02731	0.06107	9.45%	1.82%	323	500	
32		5	0.632	0.54	0.68	0.02478	0.05541	8.77%	3.95%	316	500	
56		5	0.268	0.2	0.36	0.03023	0.0676	25.22%	59.27%	134	500	
100		5	0	0	0	0	0		100.0%	0	500	
Development Rate	1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1											
(	0 20	40 С-µg/L	60	80 100								

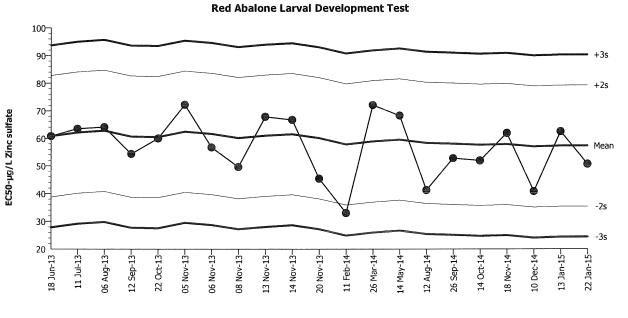
000-089-180-4

CETIS™ v1.8.7.20

Analyst: KB QA: AC 2/4/15

#### **CETIS QC Plot**

Red Abalone Larval Development Test			Nautilus Environmental (CA)
Test Type: Development	Organism: Haliotis rufescens (Red Abalone)	Material:	Zinc sulfate
Protocol: EPA/600/R-95/136 (1995)	Endpoint: Development Rate	Source:	Reference Toxicant-REF



Mean:	57.3	Count:	20	-2s Warning Limit:	35.32	-3s Action Limit:	24.33
Sigma:	10.99	CV:	19.20%	+2s Warning Limit:	79.28	+3s Action Limit:	90.27

**Quality Control Data** 

quum	.,	a or batt	^								
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Jun	18	16:30	60.76	3.461	0.315			15-0077-4900	00-2560-4372
2		Jul	11	17:05	63.48	6.181	0.5624			20-1873-1666	04-6373-4064
3		Aug	6	15:35	64.09	6.786	0.6175			07-0122-1486	08-7879-6283
4		Sep	12	14:40	54.37	-2.929	-0.2666			01-8875-4392	00-0984-0680
5		Oct	22	14:50	59.96	2.657	0.2418			00-1950-7526	03-4814-7235
6		Nov	5	14:25	72.27	14.97	1.362			13-0598-2106	06-6008-6070
7			6	15:30	56.71	-0.5901	-0.05369			17-8546-9636	17-3071-8592
8			8	0:00	49.64	-7.658	-0.6968			20-4825 <b>-</b> 5447	15-3343-8191
9			13	16:00	67.88	10.58	0.9624			01-9285-3290	05-2114-7000
10			14	14:50	66.73	9.429	0.858			12-4955-9047	05-7865-8140
11			20	15:15	45.42	-11.88	-1.081			15-8538-2252	14-5629-7331
12	2014	Feb	11	15:00	33.01	-24.29	-2.21	(-)		00-8191-4476	07-3868-3337
13		Mar	26	15:25	72.1	14.8	1.347			11-0783-9458	08-4579-6000
14		May	14	15:35	68.33	11.03	1.004			14-0092-0578	07-8756-4120
15		Aug	12	15:35	41.25	-16.05	-1.461			09-7316-9900	19-6875-2864
16		Sep	26	16:10	52.78	-4.517	-0.411			12-0077-1970	07-6392-1596
17		Oct	14	15:30	51.91	-5.393	-0.4907			01-1692-6353	05-8596-4968
18		Nov	18	15:05	61.92	4.619	0.4203			12-7477-5365	16-0305-1770
19		Dec	10	15:30	40.8	-16.5	-1.501			18-3651-9027	05-5260-7606
20	2015	Jan	13	14:48	62.52	5.218	0.4748			11-7205-2664	10-2598-5960
21			22	14:44	50.62	-6.679	-0.6077			16-1164-6227	21-0118-1264

Analyst: KB QA: AC 2/5/15

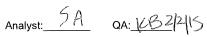
### **CETIS Test Data Worksheet**

Report Date:	20
Test Code:	16-

Red Abalone	e Larva	al Dev	elopn	nent Test				Nautilus Environmental (CA
Start Date:22 Jan-End Date:24 Jan-Sample Date:22 Jan-			5	Protoc	es: Halioti :ol: EPA/6 al: Zinc su	00/R-95/136 (1995) San	Sample Code: Sample Source: Sample Station:	150122hrdv Reference Toxicant Zinc sulfate
C-µg/L	Code	Rep	Pos	# Counted	# Normal		Notes	
			1	(00)	68			
			2	100	67			
			3	100	26	-		
			4	100	Ø			
			5	100	64			
			6	100	Ø			
	-		7	100	63			
	_		8	100	66			
			9	100	20			
			10	100	Ø			
			11	100	67			
			12	100	Ø			
			13	100	63			
			14	100	60			
			15	100	65			
			16	(00	63			
			17	100	31			
			18	100	71			
			19	100	Ø			
			20	100	68			
			21	100	68			
			22	100	64			
			23	100	68			
			24 25	100	65			
			25 26	100	70			
			26	100	54			
			27	100	55			
			28	100	36			
				100	21			
			30	100	72			

000-089-170-2

CETIS™ v1.8.7.20



#### **CETIS Test Data Worksheet**

Report Date:	20 .
Test Code:	16-

Red Abalone	= Larva		eloph	nent lest				Nautilus Environmental (C
Start Date: End Date: Sample Date	24 .	22 Jan-15 24 Jan-15 : 22 Jan-15		Species: Haliotis rufescens Protocol: EPA/600/R-95/136 (1995) Material: Zinc sulfate			Sample Code: Sample Source: Sample Station:	150122hrdv Reference Toxicant Zinc sulfate
C-µg/L	Code	Rep	Pos	# Counted	# Normal		Notes	
0	LC	1	7	(00)	69	xC1/25/15		
0	LC	2	22	100	66			
0	LC	3	15	100	69			
0	LC	4	24	100	68			
0	LC	5	30	100	68			
10		1	20					· · · · · ·
10		2	23					·····
10		3	14					
10		4	2					
10		5	25					
18		1	16					
18		2	18					
18		3	1					· · · · · · · · · · · · · · · · · · ·
18		4	8					
18		5	27					
32		1	5					
32		2	21		· · · · · · · · · · · · · · · · · · ·			
32		3	26			Alter and a second s		
32		4	13					
32		5	11					
56		1	9					
56		2	17					
56		3	3				· · · · · · · · · · · · · · · · · · ·	
56		4	29					1
56		5	28				······	
100		1	12					
100		2	4					
100		3	19					
100		4	10					
100		5	6					

GC=AC

1

### Water Quality Measurements

Client: Internal Sample ID: ZnSO<sub>4</sub> Test ID: 150122hrdv

Test Species: *Haliotis rufescens* Start Date/Time: <u>1/22/2015 ィームム</u> End Date/Time: <u>1/24/2015 ょうのつ</u>

Concentration (µg/L)		Salinity (ppt)			Temperature (°C)			olved Oxyg (mg/L)	gen	pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	32.8	32.8	32.9	15.8	15.0	14.9	8.3	6.3	8.4	7.98	8.01	8.05
10	32.8	32.8	32.9	15.9	14.6	14.9	8.2	4.3	\$.3	7.99	48.0Z	\$.03
18	32.8	32.8	52.9	15.6	14.6	19.5	8.3	8.3	8.3	8.01	8.03	8.03
32	32.8	329	32.8	15.7	14.6	14.9	8.3	4.3	8.3	8-02	8.03	8.0Z
56	32.8	32.4	32.8	15.5	14.7	15.6	8.3	4.3	8.3	8.04	8.03	8.03
100	32.6	32.6	72.7	15.4	14.6	14.9	8.3	4.3	8.3	8.05	8.04	8.01
							-					
	_											
			0	24	48	_	Dilution calc	s. (final vol	ume 500 m	IL):		
Technician Initials:		Readings:		NH			Conc.	10	18	32	56	100
	Dilutions	made by:	AC				Vol. Zn stock (mL):	0.5	0.9	1.6	2.8	5.0
						Zn Stock	Concentratior	ι (μg/L):		/ <	$\eta_{0} \propto$	2
Comments:	0 hrs:										,	
	24 hrs: 48 hrs:				······································							
QC Check:		12/15							al Review:	40	1/5/15	17.1507A.1

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

8

#### **Abalone Embryo-Larval Development**

Client:	Interna	l		Test Species: <u>Haliotis ru</u>	fescens
Sample ID:	2nSay		St	art Date/Time: 1/22/2015	1444
Test No.:	150124	hrdv	E	nd Date/Time: <u>1/24/2015</u>	1500
Animal Source/D	ate Received:	American	Abalone	1/20/15	

Number of abalone and condition upon receipt/holding:

2/4001 2/4001

Males:

Females:

	Males:	Females:
Tris & peroxide addition time	1045	1015
Spawn time	1315	1305
Number of spawners	2	
Condition of spawn (light, moderate, heavy)	Moderate	light
Fertilization time	1350	0

Embryo counts (per 0	5 ml)
1	180
2	172
3	184
Mean	179

Time of test Initiation: 1444

Technician Initials: \_\_\_\_\_PA

48 hr. QC 8370

Comments:

QC Check:

KB 2/2/5

Final Review: <u>AC 245/15</u>

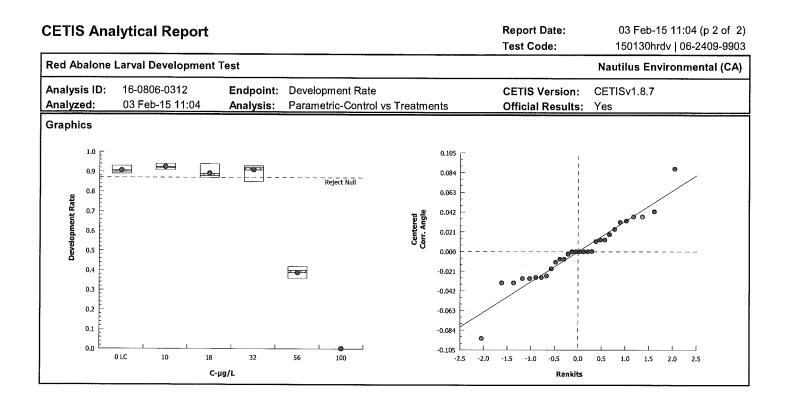
48-hour Red Abalone Larval Development Test Initiation Date: January 30, 2015

CETIS Su	ımmary Re <b>ı</b>	oort						Report Date: Test Code:			:05 (p 1 of 1 06-2409-990:		
Red Abaion	e Larval Develo	pment T	est								mental (CA)		
Batch ID: Start Date: Ending Date Duration:	20-1429-839 30 Jan-15 15 9: 01 Feb-15 14 47h	:22	Test Type: Protocol: Species: Source:	Development EPA/600/R-95 Haliotis rufesc American Aba	ens			Analyst: Diluent: Natural Seawater Brine: Not Applicable Age:					
Sample ID: Sample Date Receive Date Sample Age	e: 30 Jan-15	3	Code: Material: Source: Station:	150130hrdv Zinc sulfate Reference Toxicant Zinc sulfate				Client: Inte Project:	rnal	<u></u>	<del>8 ())</del>		
Comparison	Summary												
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	τU	Method					
16-0806-0312	-0806-0312 Development Rate		32							Iultiple Comparison Test			
Point Estima	ite Summary					The cost of the construction of the cost		<u></u>					
Analysis ID	Endpoint		Level	μg/L	95% LCL	95% UCL	τU	Method					
03-0729-5027	Development	Rate	EC50	52.83	51.42	54.27		Spearman					
Test Accepta	bility								With the second second				
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	ite	Overlap	Decision				
03-0729-5027		Rate		Resp	0.906	0.8 - NL		Yes		cceptability	· Critoria		
16-0806-0312			Contro		0.906	0.8 - NL		Yes		cceptability			
16-0806-0312	Development	Rate	NOEL	·	32	NL - 56		No		cceptability			
16-0806-0312	Development	Rate	PMSD		0.03899	NL - 0.2		No		cceptability			
Development	Rate Summary	,									and a second		
C-µg/L	Control Type	Coun	t Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect		
)	Lab Control	5	0.906	0.8834	0.9286	0.89	0.93		0.01817	2.01%	0.0%		
10		5	0.924	0.9098	0.9382	0.91	0.94	0.005099	0.0114	1.23%	-1.99%		
18		5	0.89	0.8538	0.9262	0.87	0.94	0.01304	0.02915	3.28%	1.77%		
32		5	0.908	0.8664	0.9496	0.85	0.93	0.01497	0.03347	3.69%	-0.22%		
56		5	0.388	0.3547	0.4213	0.36	0.42	0.012	0.02683	6.92%	57.17%		
100		5	0	0	0	0	0	0	0		100.0%		
Development	Rate Detail												
C-μg/L	Control Type	Rep 1		Rep 3	Rep 4	Rep 5							
)	Lab Control	0.92	0.89	0.89	0.9	0.93							
0		0.91	0.92	0.92	0.94	0.93							
8		0.89	0.87	0.88	0.94	0.87							
2		0.85	0.91	0.93	0.93	0.92							
6		0.4	0.36	0.4	0.42	0.36							
00		0	0	0	0	0							

Analyst: AC QA: KB2/11/13

CETIS Analytical Report								•	ort Date: Code:	03 Feb-15 11:04 (p 1 of 2) 150130hrdv   06-2409-9903				
Red Abalon	e Larva	Develop	nent Tes	st						Nautilus Environmental (CA)				
Analysis ID: Analyzed:		806-0312 eb-15 11:0		•	velopment Rate rametric-Control vs Treatments				IS Version: al Results:	CETISv1. Yes	8.7			
Data Transfo	orm		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU		
Angular (Cor	rected)		NA	C > T	NA	NA		3.9%	32	56	42.33			
Dunnett Mul	tiple Co	omparisor	n Test											
Control	vs	C-µg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(a	<b>7:5%</b> )				
Lab Control		10		-1.277	2,305	0.057 8	0.9885	CDF	····· · ·	, icant Effect				
		18		1.001	2.305	0.057 8	0.3804	CDF	5	icant Effect				
		32		-0.2227	2.305	0.057 8	0.8642	CDF	5	icant Effect				
56*				23.62	2.305	0.057 8	<0.0001	CDF	Significant					
ANOVA Tabl	e													
Source		Sum Squ	ares	Mean Squ	are	DF	F Stat	P-Value	Decision(a	<b>7:5%</b> )				
Between	1.405821			0.3514552		4	226.8	<0.0001	Significant	Effect				
Error		0.0309914	13	0.0015495	71	20			U					
Total		1.436812				24	_							
Distributiona	al Tests			28446946 <u>4655550000000000000000000000000000000</u>										
Attribute		Test			Test Stat	Critical	P-Value	Decision(	a:1%)					
Variances		Bartlett E	quality o	f Variance	4.287	13.28	0.3686	Equal Var	iances					
Distribution		Shapiro-V	Vilk W N	ormality	0.9559	0.8877	0.3397	Normal Di	stribution					
Developmen	t Rate \$	Summary								N				
C-µg/L	Contr	ol Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect		
0	Lab C	ontrol	5	0.906	0.8834	0.9286	0.9	0.89	0.93	0.008124	2.01%	0.0%		
10			5	0.924	0.9098	0.9382	0.92	0.91	0.94	0.005099	1.23%	-1.99%		
18			5	0.89	0.8538	0.9262	0.88	0.87	0.94	0.01304	3.28%	1.77%		
32			5	0.908	0.8664	0.9496	0.92	0.85	0.93	0.01497	3.69%	-0.22%		
32 56			5 5	0.908 0.388	0.8664 0.3547	0.9496 0.4213	0.92 0.4	0.85 0.36	0.93 0.42	0.01497 0.012	3.69% 6.92%	-0.22% 57.17%		
			-											
56	rrected)	Transform	5 5	0.388 0	0.3547	0.4213	0.4	0.36	0.42	0.012		57.17%		
56 100	,	Transfori ol Type	5 5	0.388 0	0.3547	0.4213	0.4 0	0.36	0.42	0.012		57.17%		
56 100 Angular (Cor	,	ol Type	5 5 med Sun	0.388 0 nmary	0.3547 0	0.4213 0	0.4 0	0.36 0	0.42 0	0.012 0	6.92%	57.17% 100.0%		
56 100 Angular (Cor C-µg/L	Contr	ol Type	5 5 med Sun Count	0.388 0 nmary Mean	0.3547 0 95% LCL	0.4213 0 95% UCL	0.4 0 Median	0.36 0 Min	0.42 0 Max	0.012 0 Std Err	6.92% CV%	57.17% 100.0% %Effect		
56 100 Angular (Cor C-μg/L 0	Contr	ol Type	5 5 med Sun Count 5	0.388 0 nmary <u>Mean</u> 1.26	0.3547 0 95% LCL 1.221	0.4213 0 95% UCL 1.3	0.4 0 Median 1.249	0.36 0 Min 1.233	0.42 0 Max 1.303	0.012 0 Std Err 0.01421	6.92% CV% 2.52%	57.17% 100.0% %Effect 0.0%		
56 100 <b>Angular (Cor</b> <b>C-μg/L</b> 0 10 18 32	Contr	ol Type	5 5 <b>med Sun</b> 5 5 5 5	0.388 0 mmary Mean 1.26 1.292	0.3547 0 <b>95% LCL</b> 1.221 1.265	0.4213 0 <b>95% UCL</b> 1.3 1.319	0.4 0 Median 1.249 1.284	0.36 0 Min 1.233 1.266	0.42 0 Max 1.303 1.323	0.012 0 Std Err 0.01421 0.009748	6.92% CV% 2.52% 1.69%	57.17% 100.0% %Effect 0.0% -2.52%		
56 100 <b>Angular (Cor</b> <b>C-μg/L</b> 0 10 18	Contr	ol Type	5 5 med Sun Count 5 5 5	0.388 0 mmary Mean 1.26 1.292 1.235	0.3547 0 95% LCL 1.221 1.265 1.172	0.4213 0 <b>95% UCL</b> 1.3 1.319 1.298	0.4 0 Median 1.249 1.284 1.217	0.36 0 Min 1.233 1.266 1.202	0.42 0 Max 1.303 1.323 1.323	0.012 0 <b>Std Err</b> 0.01421 0.009748 0.02271	6.92% CV% 2.52% 1.69% 4.11%	57.17% 100.0% %Effect 0.0% -2.52% 1.98%		

Analyst: AC QA: 18211115



	ETIS Analytical Report								03 Feb-15 11:04 (p 1 of 1) 150130hrdv   06-2409-9903			
Red Abal	one Larval Devel	opment Test							Nautilus	s Enviror	nmental (CA	
-			•					CETIS Version: Official Results:		.8.7		
Spearma	n-Kärber Estimat	es										
Threshold	d Option	Threshold	Trim	Mu	Sigma		EC50	95% LCL	95% UCL			
Control Th	nreshold	0.094	0.00%	1.723	0.005847		52.83	51.42	54.27			
Developn	nent Rate Summa	ıry			Calcu	lated Variat	te(A/B)					
C-µg/L	Control Type	Count	Mean	Min	Мах	Std Err	Std Dev	CV%	%Effect	А	в	
0	Lab Control	5	0.906	0.89	0.93	0.008124	0.01817	2.01%	0.0%	453	500	
10		5	0.924	0.91	0.94	0.005099	0.0114	1.23%	-1.99%	462	500	
18		5	0.89	0.87	0.94	0.01304	0.02915	3.28%	1.77%	445	500	
32		5	0.908	0.85	0.93	0.01497	0.03347	3.69%	-0.22%	454	500	
56		5	0.388	0.36	0.42	0.012	0.02683	6.92%	57.17%	194	500	
100		5	0	0	0	0	0		100.0%	0	500	
Development Rate	1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.0 0.1 0.0 0.0 0.0 0.0 0.0											
ι ι	0 20	40	60 E	0 100								

Analyst: AC QA: KB211/15

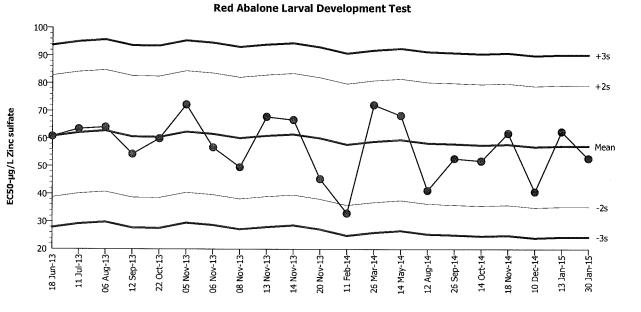
#### **CETIS QC Plot**

Red Abalone Larval Development Test			Nautilus Environmental (CA)
Test Type: Development	Organism: Haliotis rufescens (Red Abalone)	Material:	Zinc sulfate

Endpoint: Development Rate

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

Source: Reference Toxicant-REF



Mean:	57.3	Count:	20	-2s Warning Limit:	35.32	-3s Action Limit:	24.33
Sigma:	10.99	CV:	19.20%	+2s Warning Limit:	79.28	+3s Action Limit:	90.27

**Quality Control Data** 

		11 4 la	<b>D</b>	There a	00 0.4	D 11	<u>.</u>		• ··		
		Month			QC Data		Sigma	Warning	Action	Test ID	Analysis ID
1	2013		18	16:30	60.76	3.461	0.315			15-0077-4900	00-2560-4372
2		Jul	11	17:05	63.48	6.181	0.5624			20-1873-1666	04-6373-4064
3		Aug	6	15:35	64.09	6.786	0.6175			07-0122-1486	08-7879-6283
4		Sep	12	14:40	54.37	-2.929	-0.2666			01-8875-4392	00-0984-0680
5		Oct	22	14:50	59.96	2.657	0.2418			00-1950-7526	03-4814-7235
6		Nov	5	14:25	72.27	14.97	1.362			13-0598-2106	06-6008-6070
7			6	15:30	56.71	-0.5901	-0.05369			17-8546-9636	17-3071-8592
8			8	0:00	49.64	-7.658	-0.6968			20-4825-5447	15-3343-8191
9			13	16:00	67.88	10.58	0.9624			01-9285-3290	05-2114-7000
10			14	14:50	66.73	9.429	0.858			12-4955-9047	05-7865-8140
11			20	15:15	45.42	-11.88	-1.081			15-8538-2252	14-5629-7331
12	2014	Feb	11	15:00	33.01	-24.29	-2.21	(-)		00-8191-4476	07-3868-3337
13		Mar	26	15:25	72.1	14.8	1.347			11-0783-9458	08-4579-6000
14		May	14	15:35	68.33	11.03	1.004			14-0092-0578	07-8756-4120
15		Aug	12	15:35	41.25	-16.05	-1.461			09-7316-9900	19-6875-2864
16		Sep	26	16:10	52.78	-4.517	-0.411			12-0077-1970	07-6392-1596
17		Oct	14	15:30	51.91	-5.393	-0.4907			01-1692-6353	05-8596-4968
18		Nov	18	15:05	61.92	4.619	0.4203			12-7477-5365	16-0305-1770
19		Dec	10	15:30	40.8	-16.5	-1.501			18-3651-9027	05-5260-7606
20	2015	Jan	13	14:48	62.52	5.218	0.4748			11-7205-2664	10-2598-5960
21			30	15:22	52.83	-4.472	-0.4069			06-2409-9903	03-0729-5027

### **CETIS Test Data Worksheet**

Red Abalone	Larva	al Dev	elopn	nent Test		Nautilus Environmental (CA			
Start Date: End Date: Sample Date	01 F	Jan-15 Feb-15 Jan-15	5	Species: Haliotis rufescens Protocol: EPA/600/R-95/136 (1995) Material: Zinc sulfate				Sample Code: Sample Source: Sample Station:	150130hrdv Reference Toxicant Zinc sulfate
C-µg/L	Code	Rep	Pos	# Counted	# Normal			Notes	
			1	100	89	SA 2/2/15			
			2	100	90				
			3	100	92				
			4	100	Ø				
			5	100	0				
			6	100	0 85				
			7	100	29				
			8	100	91				
			9	(00	89		r - 1-25		
			10	100	84	CH 2/301 2/10/1	12/15		
			11	JOU	92		weither day -		
			12	100	42				
			13	100	92				
			14	160	36				
			15	100	47 87				
			16	100	87				·
			17	100	93 (1)	213/15 0.18			
			18 19		93 443				
			20	100	94				
			20	100	91 0				
			21						
			22	100	40 92				
			24	100	93				
			25	100	0				
			26	100	40				
			27	100	94				· · · · · · · · · · · · · · · · · · ·
			28	100	93				arte
			29	100	36				nd add of the
			30	100	0		/		

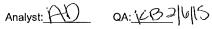
Analyst: SA/CH QA: AC 2/3/15

### **CETIS Test Data Worksheet**

Report Date:	29 Jan-15
Test Code:	06-2409-9

Red Abalone	Larva	al Dev	elopn	nent Test			Nautilus Environmental (C
Start Date: End Date: Sample Date	01 F	Jan-15 Feb-15 Jan-15	5	Protoc	es: Haliotis rufescens col: EPA/600/R-95/136 (19 al: Zinc sulfate	95) Sample Code: Sample Source: Sample Station:	150130hrdv Reference Toxicant Zinc sulfate
C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes	
0	LC	1	11	001	88		
0	LC	2	1	v -			
0	LC	3	7				
0	LC	4	2				
0	LC	5	28				
10		1	20				
10		2	23				
10		3	3	100	85		
10		4	19				
10		5	18				
18		1	9				
18		2	16				
18		3	10				
18		4	27	100	93		
18		5	15				
32		1	6				
32		2	8				
32		3	17	_			
32		4	24	00	93		
32		5	13				
56		1	22			· · · · · · · · · · · · · · · · · · ·	
56		2	14				
56		3	26	001	39		· · · · · · · · · · · · · · · · · · ·
56		4	12		• • • • • • • • • • • • • • • • • • •		
56		5	29				
100		1	21				1997-000
100		2	5				The Manual Annual A
100		3	25				
100		4	30			·	
100		5	4	601	°.		

QC=AC



# Water Quality Measurements

Client:	Internal
Sample ID: 2	ZnSO <sub>4</sub>
Test ID:	150130hrdv

Test Species:	Haliotis rufesc	ens
Start Date/Time:	1/30/2015	1522
End Date/Time:	2/1/2015	1415

Concentration (µg/L)		Salinity (ppt) 0 24 48			Temperature (°C) 0 24 48			Dissolved Oxygen (mg/L) 0 24 48			pH (pH units)		
Lab Control	33.6		33.1	14.7	14.q	14.9	8.3	<b>24</b> 7.9	<b>48</b> ଟ.୯	0 8.03	24 7,98	<b>48</b> 8.01	
10	33,5	33.4	33.3	14.4	14.7	14.7	<u> </u>	8.0	8.0	8.04	8.00	8.02	
18	33.6	33.8	33.6	14.6	14.6	14.7	8.3	8.1	8.0	8.04	8.01	8.03	
32	33.6	33.8	33.6	14.6	14.0	14.8	8.3	8.1	7.9	8.05	8.0)	8.01	
56	33.5	337	33.6	14.7	14.6	14.8	8.3	8.1	8.0	8.05	801	8.01	
100	33.3	133. IS	33.5	14.8	14.5	149	8.3	8-1	8.0	8.05	8.01	8,C1	
Technician Initials:		Readings: made by:	0 A.C. A.C	24 AP	48 3K		Dilution calca Conc. Vol. Zn stock (mL):	s. (final vol 10 0 . 25	unie 500 m 18 0,45	32	56 14	100 2 <sub>.</sub> 5	
Comments:	0 hrs: 24 hrs:	-				Zn Stock	Concentration	ι (μg/L):		10,	000		
QC Check:	48 hrs:	13/15						Fina	al Review:	KB	2/5/15	)	

#### **Abalone Embryo-Larval Development**

Client:	Interr	al	Test Species: Haliotis rufescens	
Sample ID:	ZnSOZ	در	Start Date/Time: <u>1/30/2015 1らみ</u> る	<u>}_</u>
Test No.:	501	30hrdv	End Date/Time: 2/1/2015 1415	-
Animal Source/Dat	te Received:	American	Abalone/ 1/28/15	

Number of abalone and condition upon receipt/holding:

Males:

4 good, small 4 good Females:

	Males:	Females:
Tris & peroxide addition time	1100	1030
Spawn time	1330	1400
Number of spawners	4	AUX 3
Condition of spawn (light, moderate, heavy)	heavy	modera*
Fertilization time	44	oiz QZI
	AC ibons	- 144

Embryo counts (per 0	.5 ml)
1	141
2	195
3	183
Mean	173

Time of test Initiation:	1522

Technician Initials:

48 hr. QC  $\frac{97}{200}$ °

621 - used different batch of gegess (more dense) and rew fert. time. AC 2/3/15 Final Review: VB25/5 **Comments:** QC Check:

48-hour Red Abalone Larval Development Test Initiation Date: February 6, 2015

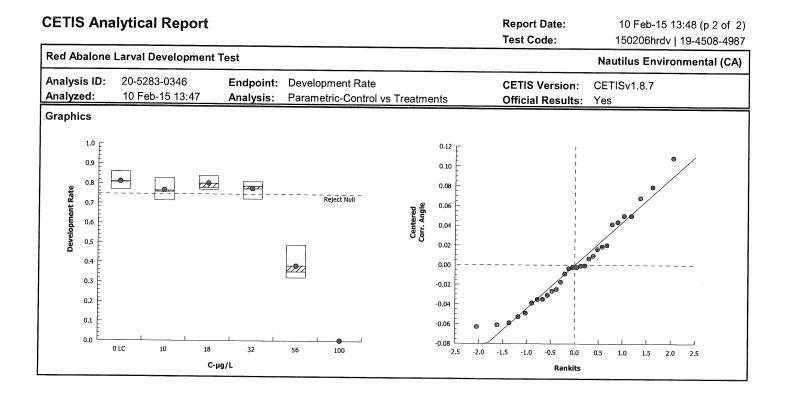
CETIS Summary Report										10 Feb-15 13:48 (p 1 of 1) 150206hrdv   19-4508-4987		
Red Abalone	Larval Developn	nent Te	est		****				Nautilu	s Environm	ental (CA)	
Batch ID: Start Date: Ending Date: Duration:	06 Feb-15 13:33 P 08 Feb-15 13:00 S		Test Type:       Development         Protocol:       EPA/600/R-95/7         Species:       Haliotis rufesce         Source:       American Abale		ens				nt: Natural Seawater			
Sample ID: Sample Date: Receive Date: Sample Age:			Code: Material: Source: Station:	150206hrdv Zinc sulfate Reference Toxicant Zinc sulfate				Client: Internal Project:				
Comparison S	Summary											
Analysis ID	Endpoint		NOEL	. LOEL	TOEL	PMSD	TU	Method				
20-5283-0346	Development R	ate	32	56	42.33	7.73%		Dunnett M	/lultiple Com	parison Tes	st	
Point Estimat Analysis ID	e Summary Endpoint		Level	µg/L	95% LCL	95% UCL	τU	Method				
07-8543-5535	Development R	ate	EC50	54.69	53.03	56.4		Trimmed	Spearman-ł	Kärber		
Test Acceptat	•											
Analysis ID	Endpoint		Attrib			TAC Lim	its					
07-8543-5535	Development R			ol Resp	0.8089	0.8 - NL		Yes				
20-5283-0346 20-5283-0346	Development R Development R			ol Resp	0.8089 32	0.8 - NL NL - 56		Yes	,			
20-5283-0346	Development R			NOEL PMSD		NL - 56 NL - 0.2					otability Criteria	
20 0200 0040			1 1002		0.07735	NL - 0.2		110	1 83363 A	cceptability	Ontena	
Development	Rate Summary											
C-µg/L	Control Type	Coun	t Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect	
0	Lab Control	5	0.808		0.8508	0.77	0.86		0.03382	4.18%	0.0%	
10		5	0.764		0.8264	0.7143	0.82		0.04999	6.54%	5.5%	
18		5	0.800		0.8455	0.77	0.84		0.03611	4.51%	1.02%	
32 56		5 5	0.773		0.8164 0.4655	0.7216 0.3226	0.81 0.49		0.03421 0.06621	4.42% 17.27%	4.32% 52.61%	
100		5	0.565.	0	0.4000	0.3220	0.49	0.02901	0.00021	17.2770	100.0%	
		-	-	-	-	-	-	-	-			
Development												
	Control Type	Rep 1			Rep 4	Rep 5						
	Lab Control	0.817		0.77	0.807	0.8602					:	
10		0.714		0.8	0.8293	0.7582						
18		0.78	0.84	0.84	0.7732	0.77						
32		0.721		0.79	0.76	0.7879						
56 100		0.49	0.4022		0.3488	0.3226						
100		0	0	0	0	0						

Analyst: ig QA: AC2/1415

CETIS Ar	Analytical Report					•	ort Date: Code:		10 Feb-15 13:47 (p 1 of 2) 150206hrdv   19-4508-4987			
Red Abalon	e Larval Develo	oment Test				200-1849.5000000000000000000000000000000000000		an a			mental (CA)	
Analysis ID: Analyzed:	20-5283-0346 10 Feb-15 13	velopment Rate ametric-Control vs Treatments				IS Version		CETISv1.8.7 Yes				
Data Transf	orm	Zeta	Alt Hyp	Trials Seed			PMSD	NOEL	LOEL	LOEL TOEL TU		
Angular (Cor	rrected)	NA	C > T	NA	NA		7.73%	32	56	42.33	10	
Dunnett Mu	Itiple Compariso	on Test					anna an an Anna		<u></u>			
Control	vs C-µg/L		Test Stat	Critical	MSD D	P-Value	P-Type	Decision	(a·5%)			
Lab Control	10		1.618	2.305	0.077 8	0.1665	CDF		ificant Effect			
	18		0.3105	2.305	0.077 8	0.6849	CDF					
	32		1.307	2.305	0.077 8	0.2614	CDF	Non-Significant Effect Non-Significant Effect				
	56*		13.61	2.305	0.077 8	<0.0001	CDF	Significar				
ANOVA Tab	le							~				
Source	Sum Squ	uares	Mean Squ	are	DF	F Stat	P-Value	Decision	(a:5%)			
Between	0.735213	32	0.1838033	}	4	66.49	< 0.0001	Significar			- 04.00	
Error	0.055284	83	0.0027642	41	20	00110	0.0001	orgrinioar				
Total	0.790498	}			24							
Distribution	al Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(	α:1%)				
Variances	Bartlett I	Equality of V	/ariance	1.433	13.28	0.8384	Equal Var			<u>.</u>		
Distribution	Shapiro-	Wilk W No	mality	0.9429	0.8877	0.1727	•	Normal Distribution				
Developmen	it Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Мах	Std Err	CV%	%Effect	
0	Lab Control	5	0.0000	0.7669	0.0500	0.007						
10		0	0.8089	0.7009	0.8508	0.807	0.77	0.8602	0.01512	4.18%	0.0%	
10		5	0.8089	0.7023	0.8508	0.807 0.7582	0.77 0.7143	0.8602 0.8293	0.01512 0.02236	4.18% 6.54%	0.0% 5.5%	
18								0.8602 0.8293 0.84	0.02236	6.54%	5.5%	
18 32		5	0.7644	0.7023	0.8264	0.7582	0.7143	0.8293		6.54% 4.51%	5.5% 1.02%	
18 32 56		5 5	0.7644 0.8006	0.7023 0.7558	0.8264 0.8455	0.7582 0.78	0.7143 0.77	0.8293 0.84	0.02236 0.01615 0.0153	6.54% 4.51% 4.42%	5.5% 1.02% 4.32%	
18 32		5 5 5	0.7644 0.8006 0.7739	0.7023 0.7558 0.7314	0.8264 0.8455 0.8164	0.7582 0.78 0.7879	0.7143 0.77 0.7216	0.8293 0.84 0.81	0.02236 0.01615	6.54% 4.51%	5.5% 1.02%	
18 32 56 100	rected) Transfor	5 5 5 5 5	0.7644 0.8006 0.7739 0.3833 0	0.7023 0.7558 0.7314 0.3011	0.8264 0.8455 0.8164 0.4655	0.7582 0.78 0.7879 0.3529	0.7143 0.77 0.7216 0.3226	0.8293 0.84 0.81 0.49	0.02236 0.01615 0.0153 0.02961	6.54% 4.51% 4.42%	5.5% 1.02% 4.32% 52.61%	
18 32 56 100 Angular (Cor	rected) Transfor Control Type	5 5 5 5 5	0.7644 0.8006 0.7739 0.3833 0	0.7023 0.7558 0.7314 0.3011	0.8264 0.8455 0.8164 0.4655	0.7582 0.78 0.7879 0.3529	0.7143 0.77 0.7216 0.3226	0.8293 0.84 0.81 0.49	0.02236 0.01615 0.0153 0.02961	6.54% 4.51% 4.42%	5.5% 1.02% 4.32% 52.61%	
18 32 56 100 Angular (Cor C-µg/L		5 5 5 5 5 7 med Sumr	0.7644 0.8006 0.7739 0.3833 0 nary	0.7023 0.7558 0.7314 0.3011 0	0.8264 0.8455 0.8164 0.4655 0	0.7582 0.78 0.7879 0.3529 0	0.7143 0.77 0.7216 0.3226 0 Min	0.8293 0.84 0.81 0.49 0 Max	0.02236 0.01615 0.0153 0.02961 0 Std Err	6.54% 4.51% 4.42% 17.27%	5.5% 1.02% 4.32% 52.61% 100.0%	
18 32 56 100 <b>Angular (Cor</b> <b>C-µg/L</b> 0	Control Type	5 5 5 5 7 med Sumr Count	0.7644 0.8006 0.7739 0.3833 0 nary Mean	0.7023 0.7558 0.7314 0.3011 0 <b>95% LCL</b>	0.8264 0.8455 0.8164 0.4655 0 <b>95% UCL</b>	0.7582 0.78 0.7879 0.3529 0 Median	0.7143 0.77 0.7216 0.3226 0 Min 1.071	0.8293 0.84 0.81 0.49 0 <b>Max</b> 1.188	0.02236 0.01615 0.0153 0.02961 0 <b>Std Err</b> 0.01967	6.54% 4.51% 4.42% 17.27% <b>CV%</b> 3.93%	5.5% 1.02% 4.32% 52.61% 100.0% %Effect 0.0%	
18 32 56 100 <b>Angular (Cor</b> <b>C-μg/L</b> 0 10	Control Type	5 5 5 5 med Sumr Count 5	0.7644 0.8006 0.7739 0.3833 0 nary Mean 1.12	0.7023 0.7558 0.7314 0.3011 0 <b>95% LCL</b> 1.065	0.8264 0.8455 0.8164 0.4655 0 <b>95% UCL</b> 1.174	0.7582 0.78 0.7879 0.3529 0 <b>Median</b> 1.116	0.7143 0.77 0.7216 0.3226 0 Min	0.8293 0.84 0.81 0.49 0 Max	0.02236 0.01615 0.0153 0.02961 0 <b>Std Err</b> 0.01967 0.02672	6.54% 4.51% 4.42% 17.27% CV% 3.93% 5.61%	5.5% 1.02% 4.32% 52.61% 100.0% <b>%Effect</b> 0.0% 4.81%	
18 32 56 100 <b>Angular (Cor</b> <b>C-μg/L</b> 0 10 18	Control Type	5 5 5 5 <b>med Sum</b> r Count 5 5	0.7644 0.8006 0.7739 0.3833 0 mary <u>Mean</u> 1.12 1.066	0.7023 0.7558 0.7314 0.3011 0 <b>95% LCL</b> 1.065 0.9916	0.8264 0.8455 0.8164 0.4655 0 <b>95% UCL</b> 1.174 1.14	0.7582 0.78 0.7879 0.3529 0 <b>Median</b> 1.116 1.057	0.7143 0.77 0.7216 0.3226 0 <b>Min</b> 1.071 1.007	0.8293 0.84 0.81 0.49 0 <b>Max</b> 1.188 1.145	0.02236 0.01615 0.0153 0.02961 0 <b>Std Err</b> 0.01967 0.02672 0.02052	6.54% 4.51% 4.42% 17.27% <b>CV%</b> 3.93% 5.61% 4.14%	5.5% 1.02% 4.32% 52.61% 100.0% <b>%Effect</b> 0.0% 4.81% 0.92%	
18 32 56 100	Control Type	5 5 5 5 <b>med Sum</b> r Count 5 5 5	0.7644 0.8006 0.7739 0.3833 0 nary Mean 1.12 1.066 1.109	0.7023 0.7558 0.7314 0.3011 0 <b>95% LCL</b> 1.065 0.9916 1.052	0.8264 0.8455 0.8164 0.4655 0 <b>95% UCL</b> 1.174 1.14 1.166	0.7582 0.78 0.7879 0.3529 0 <b>Median</b> 1.116 1.057 1.083	0.7143 0.77 0.7216 0.3226 0 Min 1.071 1.007 1.071	0.8293 0.84 0.81 0.49 0 <b>Max</b> 1.188 1.145 1.159	0.02236 0.01615 0.0153 0.02961 0 <b>Std Err</b> 0.01967 0.02672	6.54% 4.51% 4.42% 17.27% CV% 3.93% 5.61%	5.5% 1.02% 4.32% 52.61% 100.0% <b>%Effect</b> 0.0% 4.81%	

000-089-187-5

Analyst: 9 QA: 12/10/15



Analyst: 5 QA: AC 2 (10) 15

	Analytical Re	-	ort Date: Code:	10 Feb-15 13:48 (p 1 of 1) 150206hrdv   19-4508-4987							
Red Abal	one Larval Devel	opment Test							Nautilu	s Enviror	nmental (CA
				evelopment immed Spe	Rate arman-Kärbo	er		IS Version: ial Results:	CETISv1 Yes	.8.7	
Trimmed	Spearman-Kärbe	r Estimates									
Threshold	d Option	Threshold	Trim	Mu	Sigma		EC50	95% LCL	95% UCL		
Control Th	reshold	0.1921	3.17%	1.738	0.006685	;	54.69	53.03	56.4		
Developm	nent Rate Summa	ry			Calc	ulated Varia	te(A/B)				
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	А	в
0	Lab Control	5	0.8089	0.77	0.8602	0.01512	0.03382	4.18%	0.0%	349	432
10		5	0.7644	0.7143	0.8293	0.02236	0.04999	6.54%	5.5%	349	457
18		5	0.8006	0.77	0.84	0.01615	0.03611	4.51%	1.02%	398	497
32		5	0.7739	0.7216	0.81	0.0153	0.03421	4.42%	4.32%	384	496
56		5	0.3833	0.3226	0.49	0.02961	0.06621	17.27%	52.61%	175	456
100		5	0	0	0	0	0		100.0%	0	459
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	6 5 7 4 7 7 7 7										
0.	0 20	40 C-μg/L	50 80	) 100							

CETIS™ v1.8.7.20

Analyst: \_\_\_\_ QA: AC 2/10/15

### **CETIS QC Plot**

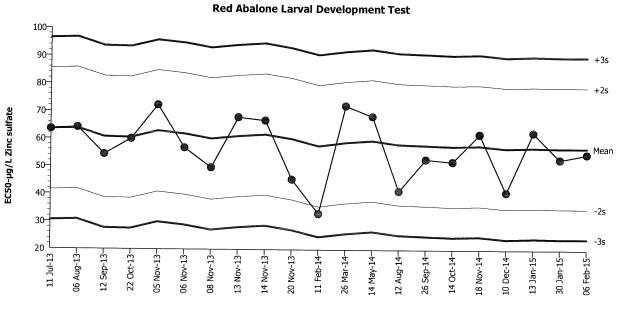
#### Red Abalone Larval Development Test Nautilus Environmental (CA) Test Type: Development **Organism:** Haliotis rufescens (Red Abalone) Material: Zinc sulfate

Endpoint: Development Rate

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

Source:

Reference Toxicant-REF



Mean:	56.9	Count:	20	-2s Warning Limit:	34.9	-3s Action Limit:	23.9
Sigma:	11	CV:	19.30%	+2s Warning Limit:	78.9	+3s Action Limit:	89.9

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Jul	11	17:05	63.48	6.581	0.5983			20-1873-1666	04-6373-4064
2		Aug	6	15:35	64.09	7.186	0.6533			07-0122-1486	08-7879-6283
3		Sep	12	14:40	54.37	-2.529	-0.2299			01-8875-4392	00-0984-0680
4		Oct	22	14:50	59.96	3.057	0.2779			00-1950-7526	03-4814-7235
5		Nov	5	14:25	72.27	15.37	1.397			13-0598-2106	06-6008-6070
6			6	15:30	56.71	-0.1901	-0.01728			17-8546-9636	17-3071-8592
7			8	0:00	49.64	-7.258	-0.6598			20-4825-5447	15-3343-8191
8			13	16:00	67.88	10.98	0.9979			01-9285-3290	05-2114-7000
9			14	14:50	66.73	9.829	0.8936			12-4955-9047	05-7865-8140
10			20	15:15	45.42	-11.48	-1.043			15-8538-2252	14-5629-7331
11	2014	Feb	11	15:00	33.01	-23.89	-2.172	(-)		00-8191-4476	07-3868-3337
12		Mar	26	15:25	72.1	15.2	1.382			11-0783-9458	08-4579-6000
13		May	14	15:35	68.33	11.43	1.039			14-0092-0578	07-8756-4120
14		Aug	12	15:35	41.25	-15.65	-1.423			09-7316-9900	19-6875-2864
15		Sep	26	16:10	52.78	-4.117	-0.3742			12-0077-1970	07-6392-1596
16		Oct	14	15:30	51.91	-4.993	-0.4539			01-1692-6353	05-8596-4968
17		Nov	18	15:05	61.92	5.019	0.4563			12-7477-5365	16-0305-1770
18		Dec	10	15:30	40.8	-16.1	-1.463			18-3651-9027	05-5260-7606
19	2015	Jan	13	14:48	62.52	5.618	0.5107			11-7205-2664	10-2598-5960
20			30	15:22	52.83	-4.072	-0.3702			06-2409-9903	03-0729-5027
21		Feb	6	13:33	54.69	-2.211	-0.201			19-4508-4987	07-8543-5535

CETIS™ v1.8.7.20

Report Date:	10 Feb-15 13:14
Test Code:	19-4508-4987/1

	e Larva	al Dev	elopr	nent Test			Nautilus Environmental (C
Start Date: End Date: Sample Date	08 F 9: 06 F	Feb-18 Feb-18	5 13:3 5 13:0 5	0 Protoc	es: Haliotis rufescens :ol: EPA/600/R-95/136 (1995) al: Zinc sulfate	Sample Code: ) Sample Source: Sample Station:	150206hrdv Reference Toxicant Zinc sulfate
C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes	
			1	97	37		
			2	100	76		
			3	85	30		
			4	93	30 80		
			5	91	69		
			6	94	0		
			7	57	46		
			8	82	67		
			9	93	30		
			10	82	68		
			11		77		
			12	100	77		
			13	100	0		
			14	100	12		
			15	100	81		
			16	100	78		
			17	100	84		
			18 19	97	75		
			20	97	70		
			20	100	79		
			21	66	30		
			23	100	80 74		
				100	74		
				99	78		
			26	100 84	0		
			27		60 49		
					84		
			29		0		
			30	93	0		

Analyst: 0/C14 QA: AC 2/12/15

Report Date:	05 Feb-15 10:03 (p 1 of 1)
Test Code:	19-4508-4987/150206hrdv

Red Abalone	Larva	al Dev	velopr	nent Test		Nautilus	Environmental (CA)
Start Date: End Date: Sample Date	08 F	=eb-1 =eb-1 =eb-1	5	Protoc	es: Halioti col: EPA/6 al: Zinc s	00/R-95/136 (1995) Sample Source: Reference To:	ricant
C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes	
0	LC	1	8				
0	LC	2	23				
0	LC	3	12				
0	LC	4	7				
0	LC	5	4	93	79	AC 2/9/15	
10		1	26		· · · · · · · · · · · · · · · · · · ·		
10		2	14				
10		3	22				
10		4	10			·	
10		5	5	89	69		
18		1	16				
18		2	28				
18		3	17				
18		4	18				
18		5	11	100	80.		
32		1	19				
32		2	15				
32		3	20				
32		4	2	100			
-32		5	24	. 79	79.		
56		1	27	AC Q 18 216			
56		2	1				
56		3	3				
56		4	21				
56		5	9	00	37.	· · · ·	
100		1	30				
100		2	29				
100		3	25				
100		4	6				
100		5	13	100	Q		

QC=VCR

Analyst: AC QA: 10 7/10/15

### Water Quality Measurements

Client: Internal Sample ID: ZnSO<sub>4</sub> Test ID: 150206hrdv 
 Test Species:
 Haliotis rufescens

 Start Date/Time:
 2/6/2015
 \335

 End Date/Time:
 2/8/2015
 \420

Concentration (µg/L)	(μg/L) (ppt)		Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)			
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	32.8	331	32.8	16.0	15.3	15.5	8.2	7.9	7.6	7.99	7.94	7,97
10	33.0	334	33.2	15.8	15.1	15.4	8.1	7.9	7.9	7.98	7.97	8.01
18	32.9	334	33.2	16.0	15.1	15,4	8.1	7.9	7,7	7.99	7.98	8.00
32	32.8	33.4	33.1	16.0	15.0	15.4	8.(	8.0	7.9	8.00	795	8.00
56	32.8	33.3	33.1	16.0	15.0	15.4	8.1	8.0	79	60.8	7.99	8.01
100	32.6	33.2	32,9	16.0	15.0	15.4	8.1	0.8	7.8	7.98	7.98	8,00
									7.8 BK			
		······································										
			0	24	48		Dilution calc	s. (final vol	 ume 500 n	- ο <i>ιε τίφ</i> πL):		
Technician Initials:	WQ	Readings:		AB	BK	]	Conc.	10	18	32	56	100
		s made by:					Vol. Zn stock (mL):	0.25	0.45	01 8	1.4	25
						Zn Stock	Concentratio	 η (μg/L):		10,	000	
Commonto	0 hro											
Comments:	24 hrs											
	48 hrs:		10/2011-0-10-10-10-10-10-10-10-10-10-10-10-1									
QC Check:		2/9/19	5					Fir	nal Review:	¥ z	-/10/15	

#### **Abalone Embryo-Larval Development**

Client:	ntern	al	Test S	Species: Haliotis rufes	scens
Sample ID:	En SO4	,	Start Da	te/Time: <u>2 0 5</u>	1333
Test No.:	15020	6 hrdv	End Da	te/Time: 218115	1300
Animal Source/Dat	e Received:	America	Abalone	2/3/15	

\_\_\_\_\_

\_\_\_\_\_

Number of abalone and condition upon receipt/holding:

4 good 4 good

Males:

Females:

	Males:	Females:
Tris & peroxide addition time	1005	0925
Spawn time	1200	1235
Number of spawners	4	Ц
Condition of spawn (light, moderate, heavy)	heavy	heavy
Fertilization time	12:5	52 '

Embryo counts (per 0	5 ml)
1	164
2	144
3	132
Mean	147

Time of test Initiation: 135

Technician Initials: AC/PA

48 hr. QC  $\frac{64/94}{9}$ 

Comments:

QC Check:

21 19

Final Review: 2/10/15

72-hour Purple Urchin Larval Development Test Initiation Date: February 17, 2015

### **CETIS Summary Report**

16 Mar-15 09:54 (p 1 of 1) 150217spdv | 12-8622-3584

							and the second se		and the second se			
Echinoid Emb	oryo-Larval Dev	elopment	t Test							Nautilus	s Environm	ental (CA)
Batch ID: Start Date: Ending Date: Duration:	01-1508-0642 17 Feb-15 15:1 20 Feb-15 15:3 72h	2 P 30 S	est Type: rotocol: pecies: ource:	Development EPA/600/R-95/ Strongylocentro Pt. Loma		iluent: Natural Seawater rine: Not Applicable						
Sample ID: Sample Date: Receive Date:		M	ode: aterial: ource:	150217spdv Copper chloride Reference Toxi	Client: Project:	Internal		<u> </u>				
Sample Age:			tation:	Copper Chlorid								
Comparison S	Summary			· · · · · · · · · · · · · · · · · · ·								
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	τυ	Metho	bd			
17-2897-7271	Development F	Rate	5	10	7.071	3.74%		Dunne	ett Multi	ple Com	parison Tes	t
Point Estimat	e Summary											
Analysis ID	Endpoint		Level	μg/L	95% LCL	95% UCL	τυ	Metho	bd			
03-8986-1540	Development F	Rate	EC50	12.77	12.46	13.08		Trimm	ned Spe	arman-K	ärber	
Test Acceptat	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	ts	Overla	ap D	ecision		
03-8986-1540	Development R	late	Contro	Resp	0.948 0.8 - NL			Yes	Pa	asses Ad	ceptability	Criteria
17-2897-7271	Development R		Contro	ol Resp	0.948	0.8 - NL		Yes	Pa	asses Ad	ceptability	Criteria
17-2897-7271	Development R	late	PMSD		0.03742	NL - 0.25		No	Pa	asses Ao	ceptability	Criteria
Development	Rate Summary					<u>*************************************</u>						
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std E	rr Si	td Dev	CV%	%Effect
0	Lab Control	5	0.948	0.9114	0.9846	0.91	0.99	0.013	19 0.	0295	3.11%	0.0%
2.5		5	0.94	0.9224	0.9576	0.93	0.96	0.0063	325 0.	01414	1.5%	0.84%
5		5	0.95	0.9324	0.9676	0.93	0.97	0.0063	325 0	01414	1.49%	-0.21%
		0	0.00				0.37	0.000	JEO 0.			
		5	0.794	0.747	0.841	0.76	0.84			03782	4.76%	16.24%
20				0.747 0	0.841 0.03856				91 0.	03782 01817	4.76% 113.5%	98.31%
10 20 40		5	0.794			0.76	0.84	0,0169	91 0.			
20 40		5 5	0.794 0.016	0	0.03856	0.76 0	0.84 0.04	0.0169 0.0081	91 0. 124 0.			98.31%
20	Rate Detail	5 5 5	0.794 0.016 0	0 0	0.03856 0	0.76 0 0	0.84 0.04 0	0.0169 0.0087 0	91 0. 124 0. 0			98.31% 100.0%
20 40 80 Development C-µg/L	Control Type	5 5 5 5 Rep 1	0.794 0.016 0 0 <b>Rep 2</b>	0 0 0 <b>Rep 3</b>	0.03856 0 0 <b>Rep 4</b>	0.76 0 0 0 <b>Rep 5</b>	0.84 0.04 0	0.0169 0.0087 0	91 0. 124 0. 0			98.31% 100.0%
20 40 80 Development C-µg/L 0		5 5 5 5 <b>Rep 1</b> 0.94	0.794 0.016 0 0	0 0 0	0.03856 0 0 <b>Rep 4</b> 0.94	0.76 0 0 0	0.84 0.04 0	0.0169 0.0087 0	91 0. 124 0. 0			98.31% 100.0%
20 40 80 Development C-µg/L 0	Control Type	5 5 5 5 Rep 1	0.794 0.016 0 0 <b>Rep 2</b>	0 0 0 <b>Rep 3</b>	0.03856 0 0 <b>Rep 4</b>	0.76 0 0 0 <b>Rep 5</b>	0.84 0.04 0	0.0169 0.0087 0	91 0. 124 0. 0			98.31% 100.0%
20 40 80 Development C-µg/L 0	Control Type	5 5 5 5 <b>Rep 1</b> 0.94	0.794 0.016 0 0 <b>Rep 2</b> 0.91	0 0 0 <b>Rep 3</b> 0.96	0.03856 0 0 <b>Rep 4</b> 0.94	0.76 0 0 0 0 8 <b>Rep 5</b> 0.99	0.84 0.04 0	0.0169 0.0087 0	91 0. 124 0. 0			98.31% 100.0%
20 40 80 <b>Development</b> <b>C-µg/L</b> 0 2.5 5	Control Type	5 5 5 <b>Rep 1</b> 0.94 0.93	0.794 0.016 0 <b>Rep 2</b> 0.91 0.95	0 0 <b>Rep 3</b> 0.96 0.93	0.03856 0 0 <b>Rep 4</b> 0.94 0.93	0.76 0 0 0 0 8 <b>Rep 5</b> 0.99 0.99	0.84 0.04 0	0.0169 0.0087 0	91 0. 124 0. 0			98.31% 100.0%
20 40 80 <b>Development</b> <b>C-µg/L</b> 0 2.5 5 10	Control Type	5 5 5 <b>Rep 1</b> 0.94 0.93 0.95	0.794 0.016 0 0 <b>Rep 2</b> 0.91 0.95 0.93	0 0 <b>Rep 3</b> 0.96 0.93 0.95	0.03856 0 0 <b>Rep 4</b> 0.94 0.93 0.95	0.76 0 0 0 0 0 8 <b>Rep 5</b> 0.99 0.96 0.97	0.84 0.04 0	0.0169 0.0087 0	91 0. 124 0. 0			98.31% 100.0%
20 40 80 <b>Development</b> <b>C-μg/L</b> 0 2.5	Control Type	5 5 5 <b>Rep 1</b> 0.94 0.93 0.95 0.77	0.794 0.016 0 <b>Rep 2</b> 0.91 0.95 0.93 0.76	0 0 <b>Rep 3</b> 0.96 0.93 0.95 0.84	0.03856 0 0 <b>Rep 4</b> 0.94 0.93 0.95 0.77	0.76 0 0 0 0 0 8 <b>Rep 5</b> 0.99 0.99 0.96 0.97 0.83	0.84 0.04 0	0.0169 0.0087 0	91 0. 124 0. 0			98.31% 100.0%

Analyst: KB QA: <u>¥53||6</u>|15

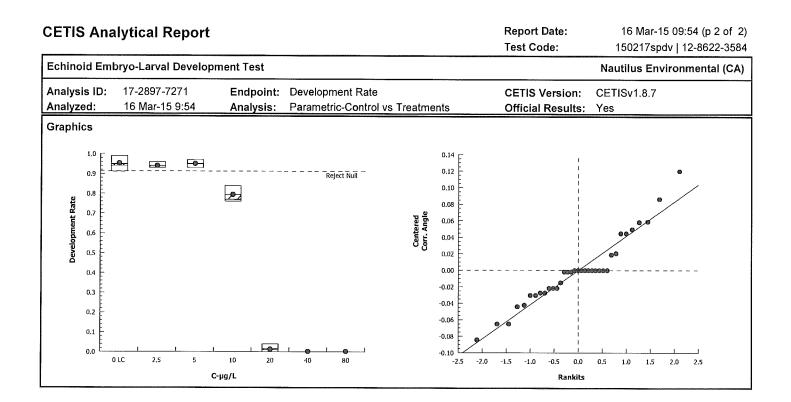
### **CETIS Analytical Report**

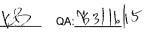
16 Mar-15 09:54 (p 1 of 2) 150217spdv | 12-8622-3584

Report Date:

CE HŞ AN					Test Code: 150217spdv   12-8622-3				2-8622-358		
Echinoid En	nbryo-Larval Dev	elopment	Test				1630			· · · · · · · · · · · · · · · · · · ·	nental (CA)
Analysis ID: Analyzed:		E	ndpoint: Dev	/elopment R ametric-Cor		tments		IS Version: cial Results:	CETISv1.		
Data Transfo		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	τυ
Angular (Cori	rected)	NA	C > T	NA	NA		3.74%	5	10	7.071	
Dunnett Mul	tiple Compariso	n Test								**************************************	
Control	vs C-µg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(	a:5%)		
Lab Control	2.5		0.7431	2.305	0.080 8	0.4940	CDF		icant Effect	*****	
	5		0.09927	2.305	0.080 8	0.7662	CDF	Non-Signif	icant Effect		
	10*		7.187	2.305	0.080 8	<0.0001	CDF	Significant			
	20*		35.58	2.305	0.080 8	<0.0001	CDF	Significant	Effect		
ANOVA Tabl	e										
Source	Sum Squ	lares	Mean Squ	lare	DF	F Stat	P-Value	Decision(	a:5%)		
Between	5.653374		1.413343		4	468.9	<0.0001	Significant	Effect		
Error	0.060277	0.06027799		I	20	_					
Total	5.713652				24						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett E	Equality of	Variance	4.692	13.28	0.3204	Equal Var	iances			
Distribution	Shapiro-	Wilk W No	ormality	0.9573	0.8877	0.3626	Normal Distribution				
Developmen	t Rate Summary										
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL						
0			Mean			Median	Min	Max	Std Err	CV%	%Effect
-	Lab Control	5	0.948	0.9114	0.9846	0.94	Min 0.91	<u>Max</u> 0.99	Std Err 0.01319	<b>CV%</b> 3.11%	%Effect 0.0%
	Lab Control	5 5		0.9114 0.9224							
2.5 5	Lab Control		0.948		0.9846	0.94	0.91	0.99	0.01319	3.11%	0.0%
2.5 5	Lab Control	5	0.948 0.94	0.9224	0.9846 0.9576	0.94 0.93	0.91 0.93	0.99 0.96	0.01319 0.006324	3.11% 1.5%	0.0% 0.84%
2.5	Lab Control	5 5	0.948 0.94 0.95	0.9224 0.9324	0.9846 0.9576 0.9676	0.94 0.93 0.95	0.91 0.93 0.93	0.99 0.96 0.97	0.01319 0.006324 0.006324	3.11% 1.5% 1.49%	0.0% 0.84% -0.21%
2.5 5 10	Lab Control	5 5 5	0.948 0.94 0.95 0.794	0.9224 0.9324 0.747	0.9846 0.9576 0.9676 0.841	0.94 0.93 0.95 0.77	0.91 0.93 0.93 0.76	0.99 0.96 0.97 0.84	0.01319 0.006324 0.006324 0.01691	3.11% 1.5% 1.49% 4.76%	0.0% 0.84% -0.21% 16.24%
2.5 5 10 20	Lab Control	5 5 5 5	0.948 0.94 0.95 0.794 0.016	0.9224 0.9324 0.747 0	0.9846 0.9576 0.9676 0.841 0.03856	0.94 0.93 0.95 0.77 0.01	0.91 0.93 0.93 0.76 0	0.99 0.96 0.97 0.84 0.04	0.01319 0.006324 0.006324 0.01691 0.008124	3.11% 1.5% 1.49% 4.76%	0.0% 0.84% -0.21% 16.24% 98.31%
2.5 5 10 20 40 80	Lab Control	5 5 5 5 5 5 5	0.948 0.94 0.95 0.794 0.016 0 0	0.9224 0.9324 0.747 0 0	0.9846 0.9576 0.9676 0.841 0.03856 0	0.94 0.93 0.95 0.77 0.01 0	0.91 0.93 0.93 0.76 0 0	0.99 0.96 0.97 0.84 0.04 0	0.01319 0.006324 0.006324 0.01691 0.008124 0	3.11% 1.5% 1.49% 4.76%	0.0% 0.84% -0.21% 16.24% 98.31% 100.0%
2.5 5 10 20 40 80	rected) Transfor Control Type	5 5 5 5 5 5 5	0.948 0.94 0.95 0.794 0.016 0 0	0.9224 0.9324 0.747 0 0 0	0.9846 0.9576 0.9676 0.841 0.03856 0	0.94 0.93 0.95 0.77 0.01 0 0	0.91 0.93 0.93 0.76 0 0	0.99 0.96 0.97 0.84 0.04 0	0.01319 0.006324 0.006324 0.01691 0.008124 0	3.11% 1.5% 1.49% 4.76%	0.0% 0.84% -0.21% 16.24% 98.31% 100.0%
2.5 5 10 20 40 80 Angular (Cor C-μg/L	rected) Transfor	5 5 5 5 5 5 <b>5</b>	0.948 0.94 0.95 0.794 0.016 0 0	0.9224 0.9324 0.747 0 0 0	0.9846 0.9576 0.9676 0.841 0.03856 0 0	0.94 0.93 0.95 0.77 0.01 0 0	0.91 0.93 0.93 0.76 0 0 0	0.99 0.96 0.97 0.84 0.04 0 0	0.01319 0.006324 0.006324 0.01691 0.008124 0 0	3.11% 1.5% 1.49% 4.76% 113.5%	0.0% 0.84% -0.21% 16.24% 98.31% 100.0% 100.0%
2.5 5 10 20 40 80 <b>Angular (Cor</b> <b>C-μg/L</b> 0	rected) Transfor Control Type	5 5 5 5 5 <b>med Sum</b> Count	0.948 0.94 0.95 0.794 0.016 0 0 mary Mean	0.9224 0.9324 0.747 0 0 0 95% LCL	0.9846 0.9576 0.9676 0.841 0.03856 0 0 95% UCL	0.94 0.93 0.95 0.77 0.01 0 0 Median	0.91 0.93 0.93 0.76 0 0 0 0	0.99 0.96 0.97 0.84 0.04 0 0 0	0.01319 0.006324 0.006324 0.01691 0.008124 0 0 Std Err	3.11% 1.5% 1.49% 4.76% 113.5%	0.0% 0.84% -0.21% 16.24% 98.31% 100.0% 100.0% %Effect
2.5 5 10 20 40 80 <b>Angular (Cor</b> <b>C-μg/L</b> 0 2.5	rected) Transfor Control Type	5 5 5 5 5 <b>med Sum</b> 5	0.948 0.94 0.95 0.794 0.016 0 0 <b>mary</b> <u>Mean</u> 1.351	0.9224 0.9324 0.747 0 0 0 0 <b>95% LCL</b> 1.256	0.9846 0.9576 0.9676 0.841 0.03856 0 0 95% UCL 1.446	0.94 0.93 0.95 0.77 0.01 0 0 <b>Median</b> 1.323	0.91 0.93 0.93 0.76 0 0 0 0 <b>Min</b> 1.266	0.99 0.96 0.97 0.84 0.04 0 0 0 <b>Max</b> 1.471	0.01319 0.006324 0.006324 0.01691 0.008124 0 0 <b>Std Err</b> 0.0342	3.11% 1.5% 1.49% 4.76% 113.5% <b>CV%</b> 5.66% 2.34%	0.0% 0.84% -0.21% 16.24% 98.31% 100.0% 100.0% <b>%Effect</b> 0.0% 1.91%
2.5 5 10 20 40 80 Angular (Cor C-μg/L 0 2.5 5	rected) Transfor Control Type	5 5 5 5 5 <b>med Sum</b> 5 5 5 5	0.948 0.94 0.95 0.794 0.016 0 0 mary <u>Mean</u> 1.351 1.325	0.9224 0.9324 0.747 0 0 0 0 <b>95% LCL</b> 1.256 1.286	0.9846 0.9576 0.9676 0.841 0.03856 0 0 95% UCL 1.446 1.363	0.94 0.93 0.95 0.77 0.01 0 0 <b>Median</b> 1.323 1.303	0.91 0.93 0.93 0.76 0 0 0 0 <b>Min</b> 1.266 1.303	0.99 0.96 0.97 0.84 0.04 0 0 <b>Max</b> 1.471 1.369	0.01319 0.006324 0.006324 0.01691 0.008124 0 0 <b>Std Err</b> 0.0342 0.01384	3.11% 1.5% 1.49% 4.76% 113.5% <b>CV%</b> 5.66%	0.0% 0.84% -0.21% 16.24% 98.31% 100.0% 100.0% 100.0% 1.91% 0.26%
2.5 5 10 20 40 80 <b>Angular (Cor</b> <b>C-μg/L</b> 0 2.5 5 10	rected) Transfor Control Type	5 5 5 5 5 <b>med Sum</b> 5 5 5 5 5 5	0.948 0.94 0.95 0.794 0.016 0 0 mary <u>Mean</u> 1.351 1.325 1.347	0.9224 0.9324 0.747 0 0 0 0 95% LCL 1.256 1.286 1.306	0.9846 0.9576 0.9676 0.841 0.03856 0 0 95% UCL 1.446 1.363 1.388	0.94 0.93 0.95 0.77 0.01 0 0 <b>Median</b> 1.323 1.303 1.345	0.91 0.93 0.93 0.76 0 0 0 0 <b>Min</b> 1.266 1.303 1.303	0.99 0.96 0.97 0.84 0.04 0 0 <b>Max</b> 1.471 1.369 1.397	0.01319 0.006324 0.006324 0.01691 0.008124 0 0 <b>Std Err</b> 0.0342 0.01384 0.01485	3.11% 1.5% 1.49% 4.76% 113.5% <b>CV%</b> 5.66% 2.34% 2.47% 4.32%	0.0% 0.84% -0.21% 16.24% 98.31% 100.0% 100.0% <b>%Effect</b> 0.0% 1.91% 0.26% 18.48%
2.5 5 10 20 40 80 Angular (Cor	rected) Transfor Control Type	5 5 5 5 <b>med Sum</b> 5 5 5 5 5 5 5 5	0.948 0.94 0.95 0.794 0.016 0 0 mary <u>Mean</u> 1.351 1.325 1.347 1.101	0.9224 0.9324 0.747 0 0 0 0 95% LCL 1.256 1.286 1.306 1.042	0.9846 0.9576 0.9676 0.841 0.03856 0 0 95% UCL 1.446 1.363 1.388 1.16	0.94 0.93 0.95 0.77 0.01 0 0 <b>Median</b> 1.323 1.303 1.345 1.071	0.91 0.93 0.93 0.76 0 0 0 0 <b>Min</b> 1.266 1.303 1.303 1.303	0.99 0.96 0.97 0.84 0.04 0 0 <b>Max</b> 1.471 1.369 1.397 1.159	0.01319 0.006324 0.006324 0.01691 0.008124 0 0 <b>Std Err</b> 0.0342 0.01384 0.01485 0.02125	3.11% 1.5% 1.49% 4.76% 113.5% <b>CV%</b> 5.66% 2.34% 2.47%	0.0% 0.84% -0.21% 16.24% 98.31% 100.0% 100.0% 100.0% 1.91% 0.26%

Analyst: 15 QA:163 16 [5





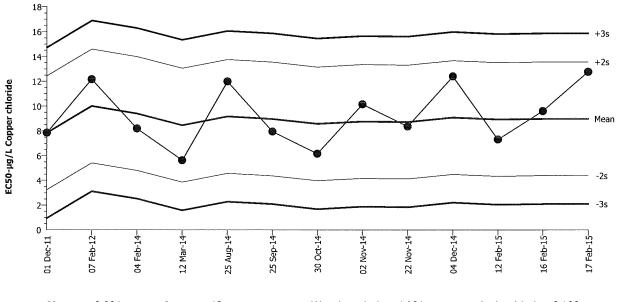
	Analytical Re	eport					-	ort Date: Code:			9:54 (p 1 of 1 12-8622-358
Echinoid	Embryo-Larval D	evelopment 1	est						Nautilu	s Enviror	nmental (CA
Analysis Analyzed			-	Development F Trimmed Spea		er		IS Version: al Results:	CETISv1 Yes	.8.7	
Trimmed	Spearman-Kärbe	er Estimates									
Threshold Option Threshold			Trim	Mu	Sigma		EC50	95% LCL	95% UCL		
Control Threshold 0.052			0.32%	1.106	0.005297	7	12.77	12.46	13.08		
Developn	nent Rate Summa	arv			Calc	ulated Variat	e(A/B)				
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	А	в
0	Lab Control	5	0.948	0.91	0.99	0.01319	0.0295	3.11%	0.0%	474	500
2.5		5	0.94	0.93	0.96	0.006324	0.01414	1.5%	0.84%	470	500
5		5	0.95	0.93	0.97	0.006324	0.01414	1.49%	-0.21%	475	500
10		5	0.794	0.76	0.84	0.01691	0.03782	4.76%	16.24%	397	500
20		5	0.016	0	0.04	0.008124	0.01817	113.5%	98.31%	8	500
40		5	0	0	0	0	0		100.0%	0	500
80		5	0	0	0	0	0		100.0%	0	500
Development Rate											
0	0 10 20	зо 40 С-µg/L	50 6	0 70 80							

Analyst: KB QA: 453/16/15

### CETIS QC Plot

Echinoid Embry	o-Larval Development Test				Nautilus Environmental (CA)
Test Type: Deve	elopment	Organism:	Strongylocentrotus purpuratus (Purpl	Material:	Copper chloride
Protocol: EPA	/600/R-95/136 (1995)	Endpoint:	Development Rate	Source:	Reference Toxicant-REF

Echinoid Embryo-Larval Development Test



Mean:	8.981	Count:	12	-2s Warning Limit:	4.391	-3s Action Limit:	2.096
Sigma:	2.295	CV:	25.60%	+2s Warning Limit:	13.57	+3s Action Limit:	15.87

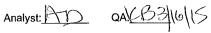
**Quality Control Data** 

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Dec	1	17:40	7.852	-1.129	-0.4918			07-8281-3338	17-7265-1210
2	2012	Feb	7	15:45	12.17	3.189	1.39			12-7990-2055	07-7286-1647
3	2014		4	14:15	8.195	-0.7861	-0.3425			14-5835-0600	07-6515-7453
Ļ		Mar	12	18:20	5.64	-3.341	-1.456			16-4219-4884	02-2584-6206
<b>i</b>		Aug	25	19:10	11.99	3.014	1.313			11-2159-2788	17-1870-3217
6		Sep	25	17:30	7.95	-1.031	-0.4493			09-4928-6784	08-7961-1534
,		Oct	30	15:00	6.16	-2.821	-1.229			16-2563-7748	00-5600-3113
		Νον	2	17:00	10.14	1.162	0.5065			05-9121-9644	01-7691-0405
			22	16:45	8,358	-0.6232	-0.2715			06-8410-0954	18-0830-4230
0		Dec	4	14:10	12.4	3.419	1.49			15-8916-5237	11-1209-5739
1	2015	Feb	12	14:25	7.308	-1.673	-0.7291			21-1011-3319	03-9708-7225
2			16	18:25	9.603	0.6217	0.2709			12-8378-8021	11-4706-6613
3			17	15:12	12.77	3.784	1.649			12-8622-3584	03-8986-1540

Analyst: KB QA: KS 3 [18] [5

Start Date:						ngylocentrotus purpuratus	Nautilus Environmental (CA 150217spdv		
nd Date:	20 F	eb-18	5			/600/R-95/136 (1995)	Sample Code: Sample Source:	Reference Toxicant	
Sample Date	: 17 F	eb-18	5			per chloride		Copper Chloride	
C-µg/L	Code	Rep	Pos	# Count	d #Norma	1	Notes		
			1	180	95				
			2		95				
			3			- 0			
			4	0	12 16 99 12 16 99				
			5	2	G-1				
			6		- C	* ·			
			7		- AI				
			8		0				
			9		82	>			
	-		10		96				
			11		- <u>70</u> 				
			12		84 96				
			13		0				
			14		1				
			15		83				
			16		3				
			17		0				
			18		45				
			19		$\overline{O}$				
			20		95				
<u></u>			21	ļ	4				
			22	- d		94 EG 3/13/15			
			23	678 316	5 1	רוןכווכ ייי ויו			
			24						
			24		0				
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			27		1-9-	<u> </u>			
			28	2	40 -4+	Υ			
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			30		· ·				
No. C. T. T.			31		93				
					0				
			33		94				
	1		34 35		70				

ı,



Report Date:	16 Feb-15 19:04 (p 1 of 1)
Test Code:	12-8622-3584/150217spdv

Echinoid Em	nbryo-	Larva	Deve	lopment Te	st			Nautilus Environmental (CA
Start Date: End Date: Sample Date	20	=eb-14 =eb-14 =eb-14	5	Protoc		ylocentrotus purpuratus 00/R-95/136 (1995) r chloride	Sample Code: Sample Source: Sample Station:	150217spdv Reference Toxicant Copper Chloride
C-µg/L	Code	Rep	Pos	# Counted	# Normal		Notes	
0	LC	1	22	100	96	AC 2/20/15		
0	LC	2	7		عد ا	7(*************************************		
0	LC	3	10					
0	LC	4	33					
0	LC	5	4					
2.5		1	29	100	94			
2.5	1	2	20					
2.5	1	3	31					
2.5		4	15					
2.5		5	12					
5		1	18	100	94			
5		2	28					
5		3	2					
5		4	1					
5		5	5					
10		1	25	100	74			
10		2	34	100				
10		3	11				· · · · · · · · · · · · · · · · · · ·	
10		4	30					
10		5	9					
20		1	16	26	$\frac{1}{2}$	<ul> <li>Phillip Annual and a set of the AMM Annual framework is the analysis of the AMM Annual Sector (AMM).</li> </ul>	· · · · · · · · · · · · · · · · · · ·	
20		2	3					
20		3	23					
20		4	8					
20		5	21					
40		1	32	100	0			
40		2	35	, w				
40		3	13					
40		4	14					
40		5	17					u
80		1	19	100	0			
80		2	26		~			
80		3	27			· · · · · · · · · · · · · · · · · · ·		
80		4	24					
80		5	6					

QCEAC

000-089-170-2

# Water Quality Measurements

Client:	Internal	
Sample ID:	CuCl <sub>2</sub>	
Test No.:	1502175pdJ	

Test Species: <u>S. purpuratus</u> Start Date/Time: 2/17/15 1512 End Date/Time: 2/20/15 1530

Concentration (µg/L)			inity pt)		Temperature (°C)				Dissolved Oxygen (mg/L)				pH (pH units)			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
Lab Control	33.1	33.6	33.0	33.6	15.0	15.3	14,6	14.5	8.9	8.0	7.7	8.3	8.09	8.00	8-69	80.3
2.5	33.5	33,2	33.2	33.4	14.8	12:0	14.4	14.3	8.5	8.1	7.9	8.4	8.07	7.94	8.06	Ere
5	33.5	33 <sub>.</sub> 2	33,2	33.3	14.8	15.0	14.4	14,3	8.4	8.2	7,9	8.4	8.10	7.96	8,05	8,10
10	33.5	33, \	33.1	33. <b>B</b>	14.8	5.0	14.4	17.3	8.4	8.2	79	8.4	8.11	7.96	8.06	8.10
20	33.5	33.1	33, 1	33.2	15.0	15,1	14.4	14.3	8.3	8.1	7.9	8.4	8.12	7.97	8,06	8,10
40	33.4	33.0	33,6	33.1	15.0	15.1	14,4	14,3	8.4	8.1	7.9	8.5	8.10	7.97	8.06	8.16
80	33.3	32.9	32-9	33, ů	14.8	15.1	14.4	14,3	8.4	8.1	7,9	8,5	8.12	7.97	8,06	8.6

Technician Initials:	WQ Readings: Dilutions made by:	 24 AG	<b>48</b> E9	<b>72</b> EG	High conc. made ( $\mu$ g/L): 80 Vol. Cu stock added (mL): 4.6 Final Volume (mL): $5^{\circ}o$ Cu stock concentration ( $\mu$ g/L): $\mathcal{E}_{j}$ 635
Comments:	0 hrs:				
	24 hrs:				· ·
	48 hrs:				
	72 hrs:				
QC Check:	AC 2/24/15	_			Final Review: KB 3/16/15

#### **Echinoderm Larval Development Worksheet**

Client: Sample ID: Test No.:	Internal Cucto- 1502-175pdv	Start Date/Time: $\frac{2/17/15}{152}$ End Date/Time: $\frac{2/20/15}{1530}$ Species: $\frac{5}{5}$ funguratus Date Collected: $\frac{2}{9}/\frac{4}{15}$				
Tech initials: Injection Time:	<u>AC</u> <u>1430</u>					
Sperm Absorbance at 400 nm: $1.3$ (target range of 0.8 - 1.0 for density of 4x10 <sup>6</sup> sperm/ml)						
Free Oriented	25 Mean: 21.4 X 5					
Eggs Counted:	<u></u> Mean <u>: Ст. Т</u> X 5	0 = <u>() / 0</u> eggs/ml				
	slide for a final density of 10	r vertical pass on Sedgwick-Rafter 00 eggs/ml)				
	$\frac{\gamma z}{z_3}$					
Initial density: Final density:	1000 eggs/ml part e	on factor egg stock <u>ZUU</u> ml egg stock seawater ml seawater				

Prepare the egg 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).

Add 100 µL sperm stock per 100mL of egg stock. For example, if you have 60mL of egg stock, add 60µL sperm stock.

Embryo Stock Fertilization Checks (Initiate test only when fertilization is ≥90%):

5 minutes (1st fert.) 10 minutes (2nd fert. If	needed)	<u>Time</u> <u>14."イソ</u>	No	<u>t. Ur</u>	No. hfert. 4	96	
fernization	time:	1445	~ 				
Test Initiation Time:	(B) +500	31512	Em	bryo Stock /	Added:	0.25 ml	
Test initiation must be within 1 hour of fertilization time.							
Test Termination:							
	No.	No.	%				
72-hour QC check 1 <sup>a</sup> QC check 2	<u>Normal</u> <u><u> </u></u>	Abnormal	Normal 94				
Comments: <u>a If the embryo development does not meet the mean test acceptability criterion of 80% normally</u>							
developed, continue the test to 96-hrs (ASTM 1999).							
(B)Q21 AC 2/17/15 Did not get to target salinity in 2.8 min. re-started test with new inoculation of embryos from same stock. (Civer test)							
QC Check:	AC 2/2	415	·			Final Review: KB 3 16 15	

APPENDIX C

Lab Qualifier Codes



### **Glossary of Qualifier Codes:**

#### Laboratory Procedures

- 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  $\leq 110\%$

### Data Analysis/Reporting

- 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.

### Error Correction

- Q18 Incorrect Entry
- Q19 Illegible Entry
- Q20 Miscalculation
- Q21 Other (provide reason in comments section)