POSEIDON WATER

Appendix I
Brine Dilution Salinity Tolerance

Renewal of NPDES CA0109223
Carlsbad Desalination Project

Nautilus Environmental

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 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.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

Results verified by: Adrienne Cibar

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

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

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 salinity-induced 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.

Table 1. Exposure Scenarios and Test Dates for the Pump/Brine Dilution Study

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

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-μm 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 ± 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. Red Abalone Development Test Specifications

Test Organism; age:	Red Abalone (*Haliotis rufescens*); 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-μm 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 ^{o}C
Test Acceptability Criteria:	Mean normal shell development in the lab control must be ≥ 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 3. Purple Urchin Development Test Specifications

Test Organism; age:	Purple sea urchin (*Strongylocentrotus purpuratus*); 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-μm screens
Number of Replicates:	Three replicates per phase, and one water quality surrogate.
Test Temperature:	15 ± 1 ^{o}C
Test Acceptability Criteria:	Mean normal development in the lab control must be ≥ 80 percent; must achieve a MSD of <25%
Concurrent Reference Toxicant Test:	Copper chloride

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™ (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.

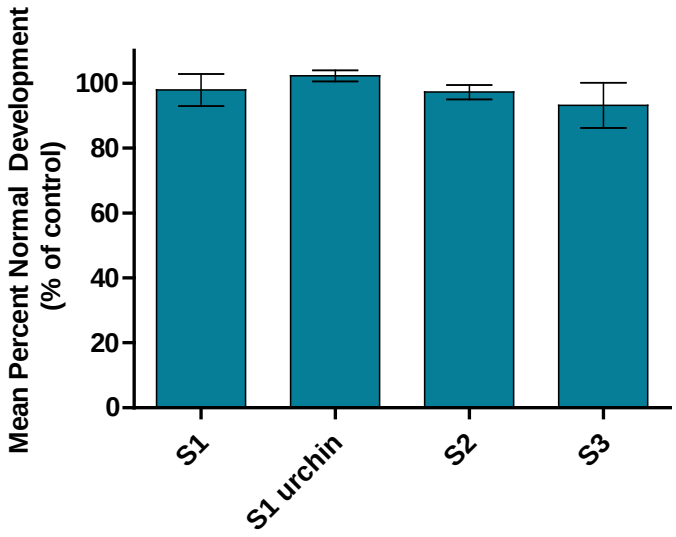


Figure 1. Mean Normal Development after Completion of Phase 3 for all four Scenarios (S1-S3). Scenario #1: P1 44 ppt for 2.8 min, P2: 39 min, P3: 30 min; Scenario #2: P1 42 ppt for 2.2 min, P2: 36 min, P3: 30 min; Scenario #3: P1 40 ppt for 1.7 min, P2: 34 min, P3: 30 min.

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 effects observed in Phase 2 or 3 of this exposure compared to the controls. Therefore, 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 Development			
				Sample	Phase 1	Phase 2	Phase 3
1	P1: 44 ppt for 2.8 minutes; P2: 39 min.; P3: 30 min.	2/6/15	Abalone Development	Control	83.8	77.7	80.5
				Brine Exposure	76.7*	79.1	78.8
1	P1: 44 ppt for 2.8 min.; P2: 39 min.; P3: 30 min.	2/17/15	Urchin Development	Control	93.7	92.0	89.3
				Brine Exposure	91.3	90.3	91.3
2	P1: 42 ppt for 2.2 min.; P2: 36 min.; P3: 30 min.	1/30/15	Abalone Development	Control	94.0	93.7	94.3
				Brine Exposure	95.7	92.7	91.7
3	P1: 40 ppt for 1.7 min.; P2: 34 min.; P3: 30 min.	1/22/15	Abalone Development[a]	Control[a]	66.0	61.0	67.3
				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.

REFERENCES

Alden Research Laboratory, Inc. Carlsbad Desalination Plant Contact Time in Discharge Pond during Stand-alone Operations. August 27, 2015.

California State Water Resources Control Board (SWRCB) 2014. Draft Staff Report Including the Draft Substitute Environmental Documentation Amendment to the Water Quality Control Plan For Ocean Waters of California Addressing Desalination Facility Intakes , Brine Discharges, and the Incorporation of other Non-substantive Changes . Sacramento, CA, July 2014.

GraphPad Software, Inc. 1992-2004. GraphPad Prism, Version 4.02.

Jenkins, S.A., and Wasyl, J. Hydrodynamic Dilution Analysis for the Carlsbad Desalination Project Operating at Sixty Million Gallons per Day Production Rate. August 2015.

Philips, B. et al. University of California, Davis, Department of Environmental Toxicology. Hyper-Salinity Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Prepared for California State Water Resources Control Board, Agreement Number 11-133-250. July 2012.

Tidepool Scientific Software. 2000-2012. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20.

USEPA. 1995. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. United States Environmental Protection Agency Office of Research and Development, Cincinnati, OH. EPA/600/R-95-136.

USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Zar, 1984. Biostatistical Analysis, Second Edition. Prentice-Hall Inc., New Jersey. 718pp.

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

Sample ID[a]	Mean Percent Normal Development		
	Phase 1	Phase 2	Phase 3
	Ambient to 44 ppt in 2.8 minutes[b]	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

[a] 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.

[b] 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 = ~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)	A	--	--	--	--	--
Control (Phase 1)	B	68	80	85.0	83.8	2.4
	C	76	89	85.4		
	D	77	95	81.1		
Control (Phase 2)	E	68	81	84.0	77.7	10
	F	56	85	65.9		
	G	69	83	83.1		
Control (Phase 3)	H	68	84	81.0	80.5	1.5
	I	67	82	81.7		
	J	67	85	78.8		
Brine Exposure (WQ surrogate)	A	--	--	--	--	--
Brine Exposure (Phase 1)	B	58	77	75.3	76.7	3.1
	C	44	59	74.6		
	D	53	66	80.3		
Brine Exposure (Phase 2)	E	74	93	79.6	79.1	1.4
	F	62	80	77.5		
	G	57	71	80.3		
Brine Exposure (Phase 3)	H	46	57	80.7	78.8	4.0
	I	66	89	74.2		
	J	57	70	81.4		
Brine Control[a]	A	53	70	75.7	79.9	4.2
	B	58	69	84.1		
	C	79	99	79.8		
	D	63	85	74.1		
	E	63	76	82.9		

[a] 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 — **Test Species:** *H. rufescens*

Project ID: Pump brine dilution study — **Start Date/Time:** 2/6/2015 1333

End Date/Time: 2/8/2015 1300

Sample	Rep	Number Counted	Number Normal	Technician Initials
Lab Control	A	Surrogate	--	
	B	80	68	CH 2/9/15
	C	89	76	CH
	D	95	77	CH
	E	81	68	CH
	F	85	56	CH
	G	83	69	CH
	H	84	68	CH
	I	82	67	CH
	J	85	67	CH
Brine dilution	A	Surrogate	--	
	B	77	58	CH
	C	59	44	CH
	D	66	53	CH
	E	93	74	CH
	F	80	62	CH
	G	71	57	CH
	H	57	46	CH
	I	89	66	CH
	J	70	57	CH
Brine Control	A	70	53	
	B	69	58	
	C	99	79	
	D	85	63	
	E	76	63	

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:** ~~KFP~~ 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	0.0178
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	One-tailed
t, df	t=3.119 df=4
How big is the difference?	
Mean ± SEM of column A	1.157 ± 0.01875 N=3
Mean ± SEM of column B	1.068 ± 0.02166 N=3
Difference between means	0.08933 ± 0.02864
95% confidence interval	0.009819 to 0.1688
R squared	0.7086
F test to compare variances	
F,DFn, Dfd	1.334, 2, 2
P value	0.8567
P value summary	ns
Are variances significantly different?	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	1.114 ± 0.01073 N=3
Mean ± SEM of column B	1.093 ± 0.02762 N=3
Difference between means	0.02067 ± 0.02963
95% confidence interval	-0.06159 to 0.1029
R squared	0.1084
F test to compare variances	
F,DFn, Dfd	6.628, 2, 2
P value	0.2622
P value summary	ns
Are variances significantly different?	No

EC-AC 7/24/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study
Sample ID: Lab Control
Test No.: 1502-S029

Test Species: *H. rufescens*
Start Date/Time: 2/6/2015 ~~1533~~ 1333
End Date/Time: 2/8/2015 1300

Total Treatment Duration	NA	2.8 minutes		39 minutes				30 minutes		
Brine/ seawater addition	ambient	ambient seawater addition		ambient seawater addition				ambient seawater addition		
Time Between Readings	T0	+1 min.	+ 1 min. 48 sec.	+ 5 min.	+ 10 min.	+ 10 min.	+ 14 min.	+ 5 min.	+ 10 min.	+ 15 min.
Actual Time	~~1348~~ 1347	~~1349~~ 1348	13:49:48	1354	1404	1414	1424	1429	1439	~~1444~~ 1454
Salinity (ppt)	33.5	33.5	33.5	33.5	33.4	33.5	33.5	33.5	33.5	33.5
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: KFP
Dilutions made by: ~~AC~~ PA

Comments:

0 hrs: ambient seawater salinity 33.5 ppt @ KFP 2/6/15
24 hrs:
48 hrs:

QC Check: AC 2/9/15

Final Review: 2/6/15/15

Marine Chronic Bioassay

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 @~~1533~~ 1333
End Date/Time: 2/8/2015 1300

Total Treatment Duration	NA	2.8 minutes		39 minutes				30 minutes		
Brine/ seawater addition	ambient	67 ppt addition to final salinity 44 ppt		seawater addition to final salinity 35.5 ppt				Seawater ~~33.5 ppt~~ addition to final salinity ~~35.5~~ 33.5 ppt (AC 2/10 QA)		
Time Between Readings	T0	+1 min.	+ 1 min. 48 sec.	+ 5 min.	+ 10 min.	+ 10 min.	+ 14 min.	+ 5 min.	+ 10 min.	+ 15 min.
Actual Time	1339	1340	13:41:48	1346	1356	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	14.8

Technician Initials:

WQ Readings: KFP
Dilutions made by: ~~AC/PA~~

Comments:
0 hrs: @KFP QA 2/6/15 ; 42.8 ppt @ 1344 ;
24 hrs:
48 hrs:

QC Check: AC 2/9/15

Final Review: KFP 2/10/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon
Sample ID: Pump Brine Dilution Study
Sample Log No.: --
Test No.: 1502-S029

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

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	33.4			15.4			8.3			8.08		
67 ppt	67.2			15.2			7.9			7.79		
Lab Control Surrogate	33.0	33.1	33.0	15.0	15.7	15.6	8.3	8.3	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	33.7	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:	VCR/AD	AB	BK
Dilutions made by:	AC		

Comments:
0 hrs: Day 0 surrogate readings recorded after final mixing
24 hrs:
48 hrs:

QC Check: AC 2/9/15

Final Review: KFP 2/10/15

Marine Chronic Bioassay **Abalone Embryo-Larval Development**

Client: Poseidon/brine pump/dilution study **Test Species:** *Haliotis rufescens*

Sample ID: frozen brine (Nautilus) **Start Date/Time:** 2/6/15 1333

Test No.: 1502-S029 **End Date/Time:** 2/8/15 1300

Animal Source/Date Received: American Abalone 2/3/15

Number of abalone and condition upon receipt/holding:

Males: 4 good

Females: 4 good

	Males:	Females:
Tris & peroxide addition time	1005	0925
Spawn time	1200	1225
Number of spawners	4	4
Condition of spawn (light, moderate, heavy)	heavy	heavy
Fertilization time	12:52	

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

Time of test Initiation: 1333 **48 hr. QC** 64/94 = 89%

Technician Initials: AC/PH

Comments:

QC Check: AC 2/9/15 **Final Review:** KFP 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

Sample ID[a]	Mean Percent Normal Development		
	Phase 1	Phase 2	Phase 3
	Ambient to 44 ppt in 2.8 minutes[b]	44 to 35.5 ppt in 39[c] 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

[a] 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.

[b] Rise from 33.5 to 44 in one minute, held for an additional 1.8 minutes.

[c] 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 = ~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

Sample ID	Rep	# Normal	# Counted	Percent Normal	Mean Percent Normal	Standard Deviation
Control (WQ surrogate)	A	--	--	--	--	--
Control (Phase 1)	B	94	100	94.0	93.7	2.5
	C	96	100	96.0		
	D	91	100	91.0		
Control (Phase 2)	E	96	100	96.0	92.0	4.6
	F	93	100	93.0		
	G	87	100	87.0		
Control (Phase 3)	H	89	100	89.0	89.3	2.5
	I	92	100	92.0		
	J	87	100	87.0		
Brine Exposure (WQ surrogate)	A	--	--	--	--	--
Brine Exposure (Phase 1)	B	88	100	88.0	91.3	3.5
	C	91	100	91.0		
	D	95	100	95.0		
Brine Exposure (Phase 2)	E	86	100	86.0	90.3	3.8
	F	93	100	93.0		
	G	92	100	92.0		
Brine Exposure (Phase 3)	H	93	100	93.0	91.3	1.5
	I	90	100	90.0		
	J	91	100	91.0		
Brine Control[a]	A	96	100	96.0	94.8	1.6
	B	94	100	94.0		
	C	93	100	93.0		
	D	97	100	97.0		
	E	94	100	94.0		

[a] 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

72
AC 2/23/15
48-hour Development

Client: Poseidon

Test Species: *S. purpuratus*

Project ID: Pump brine dilution study

Start Date/Time: 2/17/2015 1512

End Date/Time: 2/20/2015 1530

Sample	Rep	Number Counted	Number Normal	Technician Initials
Lab Control	A	Surrogate	--	
	B	100	94	
	C	↓	96	↓
	D		91	
	E		96	
	F		93	
	G		87	
	H		89	
	I		92	
	J	↓	87	↓
Brine dilution	A	Surrogate	--	
	B	100	88	
	C	↓	91	↓
	D		95	
	E		86	
	F		93	
	G		92	
	H		93	
	I		90	
	J	↓	91	↓
Brine Control	A	100	96	
	B	↓	94	↓
	C		93	
	D		97	
	E	↓	94	↓

KTP 0/8
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)

QC Check: AC 2/24/15 **Final Review:** KTP 2/24/15

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

RC = AC 7/24/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study
Sample ID: Lab Control
Test No.: 1502 - 5053

Test Species: *S. purpuratus*
Start Date/Time: 2/17/2015 ~~1457~~ Ⓐ 1512
End Date/Time: 2/20/15 1530

Total Treatment Duration	NA	2.8 minutes		38 ~~39~~ minutes AC Q18 2/24/15				30 minutes		
Brine/ seawater addition	ambient	ambient seawater addition		ambient seawater addition				ambient seawater addition		
Time Between Readings	T0	+1 min.	+ 1 min. 48 sec.	+ ~~5~~ 4 Ⓑ min.	+ 10 min.	+ 10 min.	+ 14 min.	+ 5 min.	+ 10 min.	+ 15 min.
Actual Time	15:26:00	15:27:00	15:28:48	1533	1543	1553	1607	1612	1622	1637
Salinity (ppt)	33.6	33.6	33.6	33.6	33.6	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: ambient seawater salinity 33.5 ppt Ⓐ ~~KFP~~ Q18 2/17/15 – Hach Sension5 Salinity meter used for all Salinity measurements
24 hrs:
48 hrs:

Ⓑ ~~KFP~~ 2/24/15

QC Check: AC 2/24/15

Final Review: ~~KFP~~ 2/24/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study
Sample ID: Brine Mixture
Test No.: 1502-S053

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

Total Treatment Duration	NA	2.8 minutes		38 ~~39~~ minutes				30 minutes		
Brine/ seawater addition	ambient	67 ppt addition to final salinity 44 ppt		AC QA 2/23/15 seawater addition to final salinity 35.5 ppt				(A) seawater ~~33.5~~ ppt addition to final salinity ~~35.5~~ 33.5 ppt		
Time Between Readings	T0	+1 min.	+ 1 min. 48 sec.	+ ~~5~~ (B) 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	33.5
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: KFP
Dilutions made by: AC

Comments:

0 hrs: (A) KFP QA 2/17/15 (B) AC QA 2/17/15 – Hach Sension 5 meters used for all salinity measurements
24 hrs:
48 hrs:

QC Check: ~~AC 2/17/15~~ AC QA 2/23/15 AC 2/24/15

Final Review: KFP 2/24/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon

Sample ID: Pump Brine Dilution Study

Sample Log No.: --

Test No.: 1502-S053

Test Species: *S. purpuratus*

Start Date/Time: 2/17/2015 1512

End Date/Time: 2/20/15 1530

Concentration (ppt)	Salinity (ppt)				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	~~33.9~~ Q18 VCR 2/17/15 33.4				15.0				8.6				8.13			
67 ppt	67.2				16.0				8.1				7.63			
Lab Control Surrogate	33.5	33.4	33.4	33.4	14.5	15.4	14.9	15.1	9.4	7.4	7.6	7.9	8.02	7.93	8.04	8.02
Brine Dilution Surrogate	33.5	33.5	33.5	33.5	14.6	15.4	14.6	14.8	8.4	7.4	7.6	8.2	7.97	7.96	8.04	8.07
Brine Control	~~34.2~~	33.5	33.5	33.6	15.8	15.6	15.6	14.7	8.3	7.6	7.7	8.4	8.00	7.91	8.02	8.07
	33.6 Q18 VCR 2/17/15															

	0	24	48	72
WQ Readings:	~~VCR~~	AG	EG	EG
Dilutions made by:	AC			

Technician Initials:

Comments:

0 hrs: Day 0 surrogate readings recorded after final mixing – Salinity meter Hach Sension 5

24 hrs: meter used for all salinity measurements

48 hrs:

72 hrs:

QC Check: AC 2/24/15

Final Review: KTP 2/24/15

Marine Chronic Bioassay **Echinoderm Larval Development Worksheet**

Client: Poseidon-Pump/dilution Study
Sample ID: brine (Nautilus frozen seawater)
Test No.: 1502-5053

Start Date/Time: 2/17/15 / 1512
End Date/Time: 2/20/15 / 1530
Species: S. purpuratus
Date Collected: 2/4/15

Tech initials: AC
Injection Time: 1430

Sperm Absorbance at 400 nm: 1.3 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 25, 19, 18, 22, 23

Mean: 21.4 X 50 = 1070 eggs/ml

(target counts of 20 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)

Initial density: 1070 eggs/ml = 1.07 dilution factor
Final density: 1000 eggs/ml -1.0 part egg stock
/ parts seawater

egg stock 200 ml
seawater — ml

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%):

	Time	No. Fert.	No. Unfert.	%
5 minutes (1st fert. check)	1447	96	4	96
10 minutes (2nd fert. If needed)	—	—	—	—

Fertilization time: 1445

Test Initiation Time: (B) ~~1500~~ 1512 Embryo Stock Added: 0.25 ml

Test initiation must be within 1 hour of fertilization time.

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check 1[a]	96	4	96
QC check 2	—	—	—

Comments: [a] If the embryo development does not meet the mean test acceptability criterion of 80% normally 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.

QC Check: AC 2/24/15 Final Review: KFP 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

Sample ID[a]	Mean Percent Normal Development		
	Phase 1	Phase 2	Phase 3
	Ambient to 42 ppt in 2.2 minutes[b]	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

[a] 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 ($p < 0.05$).

[b] 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 = ~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

Sample ID	Rep	# Normal	# Counted	Percent Normal	Mean Percent Normal	Standard Deviation
Control (WQ surrogate)	A	--	--	--	--	--
Control (Phase 1)	B	91	100	91.0	94.0	2.6
	C	95	100	95.0		
	D	96	100	96.0		
Control (Phase 2)	E	95	100	95.0	93.7	2.0
	F	94	100	94.0		
	G	92	100	92.0		
Control (Phase 3)	H	96	100	96.0	94.3	2.1
	I	92	100	92.0		
	J	95	100	95.0		
Brine Exposure (WQ surrogate)	A	--	--	--	--	--
Brine Exposure (Phase 1)	B	96	100	96.0	95.7	2.5
	C	93	100	93.0		
	D	98	100	98.0		
Brine Exposure (Phase 2)	E	89	100	89.0	92.7	0.58
	F	94	100	94.0		
	G	95	100	95.0		
Brine Exposure (Phase 3)	H	94	100	94.0	91.7	2.1
	I	91	100	91.0		
	J	90	100	90.0		
Brine Control[a]	A	91	100	91.0	92.8	2.2
	B	94	100	94.0		
	C	92	100	92.0		
	D	91	100	91.0		
	E	96	100	96.0		

[a] 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

Test Species: *H. rufescens*

Project ID: Pump brine dilution study

Start Date/Time: 1/30/2015 1522

Test No: 1501-S098

End Date/Time: 2/1/2015 1415

Sample	Rep	Number Counted	Number Normal	Technician Initials
Lab Control	**A**	Surrogate	--	
	B	100	91	CH
P1	**C**	100	95	CH
	D	100	96	CH
	E	100	95	CH
P2	**F**	100	94	CH
	G	100	92	CH
	H	100	96	CH
P3	**I**	100	92	CH
	J	100	95	CH
Brine dilution	**A**	Surrogate	--	
	B	100	96	CH
P1	**C**	100	93	CH
	D	100	98	CH
	E	100	89	CH
P2	**F**	100	94	CH
	G	100	95	CH
	H	100	94	CH
P3	**I**	100	91	CH
	J	100	90	CH
Brine Control	A	100	91	CH
	B	100	94	CH
	C	100	92	CH
	D	100	91	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)

QC Check: AC 2/3/15

Final Review: KFP 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	1.317 ± 0.01784 N=3
Mean ± SEM of column B	1.300 ± 0.03426 N=3
Difference between means	0.01700 ± 0.03862
95% confidence interval	-0.09022 to 0.1242
R squared	0.04619
F test to compare variances	
F,DFn, Dfd	3.690, 2, 2
P value	0.4265
P value summary	ns
Are variances significantly different?	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	1.333 ± 0.02530 N=3
Mean ± SEM of column B	1.279 ± 0.02238 N=3
Difference between means	0.05333 ± 0.03378
95% confidence interval	-0.04043 to 0.1471
R squared	0.3840
F test to compare variances	
F,DFn, Dfd	1.278, 2, 2
P value	0.8779
P value summary	ns
Are variances significantly different?	No

QC=AC 7/24/15

Marine Chronic Bioassay

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 1415

Total Treatment Duration	NA	2.2 minutes		36 minutes				30 minutes		
Brine/ seawater addition	ambient	67 ppt addition to final salinity 42 ppt		seawater addition to final salinity 35.5 ppt				AC @ ~~33.5 ppt~~ Seawater addition to final salinity ~~35.5~~ AC 33.5 ppt		
Time Between Readings	T0	+1 min.	+ 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	1643
Salinity (ppt)	33.6	41.5	42.1	39.6	37.2	36.4	35.6	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

KPP QA 1/30/15

Technician Initials:
WQ Readings: ~~KPP~~ /VCR
Dilutions made by: PA/AC

Comments: @Q18 AC 6/15/15
0 hrs: Remove 3 reps after each phase (noted by red box)
24 hrs:
48 hrs:

QC Check: AC 2/3/15

Final Review: KPP 6/15/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study
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 1415

Total Treatment Duration	NA	2.2 minutes		36 minutes				30 minutes		
Brine/ seawater addition	ambient	ambient seawater addition		ambient seawater addition				ambient seawater addition		
Time Between Readings	T0	+1 min.	+ 1 min. 12 sec.	+ 6 min.	+ 10 min.	+ 10 min.	+ 10 min.	+ 5 min.	+ 10 min.	+ 15 min.
Actual Time	1525	15:26:00	15:27:12	1533	1543	1553	1603	1608	1618	1633
Salinity (ppt)	33.6	33.6	33.6	33.6	33.6	33.6	33.6	33.6	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: KFP /NLR
Dilutions made by: PA/AC

Comments:

0 hrs: ambient seawater salinity 33.5 ppt, Remove 3 reps after each phase (noted by red box)
24 hrs:
48 hrs:

QC Check: AC 2/3/15

Final Review: KFP 6/15/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon
Sample ID: Pump Brine Dilution Study
Sample Log No.: --
Test No.: 1501-5098

Test Species: *H. rufescens*
Start Date/Time: 1/30/2015 1522
End Date/Time: 2/1/2015 1415

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	33.6			14.7			8.3			8.03		
67 ppt	67.0			14.7			nm			nm		
Lab Control Surrogate	33.4	33.2	33.2	15.1	15.0	15.2	8.3	7.4	7.7	7.87	7.92	7.95
Brine Dilution Surrogate	33.5	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	7.6	7.5	7.84	7.98	8.03

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

Technician Initials:

Comments:
0 hrs: Day 0 surrogate readings recorded after final mixing nm= not measured; tech error
24 hrs:
48 hrs:

QC Check: AC 2/3/15

Final Review: KPP 6/15/15

Marine Chronic Bioassay **Abalone Embryo-Larval Development**

Client: Poseidon - brine dilutions study (Pump/) **Test Species:** *Haliotis rufescens*

Sample ID: frozen brine (Nautilus) **Start Date/Time:** 1/30/2015 1522

Test No.: 1501-5098 **End Date/Time:** 2/1/2015 1415

Animal Source/Date Received: American Abalone / 1/28/15

Number of abalone and condition upon receipt/holding:

Males: 4 good, small

Females: 4 good

	Males:	Females:
Tris & peroxide addition time	1100	1030
Spawn time	1330	1400
Number of spawners	4	AC ~~2~~ 3
Condition of spawn (light, moderate, heavy)	heavy	moderate
Fertilization time	~~1403~~ Q21 AC 1/30/15 1440	

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

Time of test Initiation: 1522 **48 hr. QC** 97

Technician Initials: RA

Comments: Q21 - used different batch of ~~f~~ eggs (more dense) and new fert. time.

QC Check: AC 2/3/15 **Final Review:** ~~RFF~~ 6/15/15

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

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

[a] 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.

[b] 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.

[c] 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 = ~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)	A	--	--	--	--	--
Control (Phase 1)	B	67	100	67.0	66.0	1.53
	C	65	100	65.0		
Control (Phase 2)[a]	D	64	100	64.0	65.0	3.5
	E	66	100	66.0		
	F	73	100	73.0	71.0	4.7
	G	69	100	69.0		
	H	62	100	62.0	61.0	3.1
	I	60	100	60.0		
Control (Phase 3)	J	62	100	62.0	67.3	5.59
	K	66	100	66.0		
	L	74	100	74.0		
Brine Exposure (WQ surrogate)	A	--	--	--	--	--
Brine Exposure (Phase 1)	B	73	100	73.0	68.5	4.51
	C	64	100	64.0		
Brine Exposure (Phase 2)	D	68	100	68.0	72.0	4.6
	E	76	100	76.0		
	F	68	100	68.0	68.0	1.2
	G	68	100	68.0		
	H	66	100	66.0	67.0	6.2
	I	68	100	68.0		
Brine Exposure (Phase 3)	J	65	100	65.0	60.3	4.90
	K	56	100	56.0		
	L	60	100	60.0		

[a] only reps H & I went through the entire P2, other reps were pulled early.

Embryo Larval Bioassay

48-hour Development

Client: Poseidon

Test Species: *H. rufescens*

Project ID: Pump brine dilution study

Start Date/Time: 1/22/2015 1444

End Date/Time: 1/24/2015 1500

Sample	Rep	Number Counted	Number Normal	Technician Initials
Lab Control	A	Surrogate	--	
P1	B	100	67	AC 1/25/15
	C		65	
10 min	D		64	
	E		66	
P2 20 min	F		73	
	G		69	
34 min	H		62	
	I		60	
	J		62	
P3	K		66	
	L		74	
Brine dilution	A	Surrogate	--	
P1	B	100	73	
	C		AC 1/25 ~~55~~ 64	
10 min	D		68	
	E		76	
20 min P2	F		68	
	G		68	
34 min	H		66	
	I		68	
	J		65	
P3	K		56	
	L		60	

Comments: ~~Reps B, C, and D mixing treament ended after phase 1 (67 to 40 ppt)~~ AC
~~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: AC 1/29/15 **Final Review:** 6/15/15

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

QC = AC 7/24/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study

Sample ID: Lab Control

Test No.: 1501-8097

Test Species: *H. rufescens*

Start Date/Time: 1/22/2015 1444

End Date/Time: 1/24/2015 1500

Total Treatment Duration	NA	1.7 minutes		34 minutes				30 minutes		
Brine/ seawater addition	ambient	33.5 ppt		@ +5min 33.5 ppt addition				33.5 addition		
Time Between Readings	~~ons~~ T0	+1 min.	+42 sec.	+ 5 min.	+ 10 min.	+ 10 min.	+ 9 min.	+ 5 min.	+ 10 min.	+ 15 min.
Actual Time	KFP 1622/15 ~~1447~~ ~~1444~~	1448:00	~~14:48:42~~ ~~14:48:42~~	1454	1504	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	14.7	14.7

Technician Initials:

WQ Readings: KFP/AC

Dilutions made by: PA

Comments:

0 hrs: @ 1459 reps DTE pulled; 15.1°C, 33.6ppt, F+G pulled at 15:~~03~~ 04 BQ 1/8 6/15/15

24 hrs:

48 hrs:

QC Check: AC 1/29/15

Final Review: A 6/15/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon/ Pump Brine Dilution Study
Sample ID: Brine Mixture
Sample Log No.: --
Test No.: 1501-5097

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

Total Treatment Duration	NA	1.7 minutes		34 minutes				30 minutes		
Brine/ seawater addition	ambient	67 ppt addition to final salinity 40 ppt		seawater addition to final salinity 35.5 ppt @ +5				seawater addition to final salinity 33.5		
Time Between Readings	T0	+1 min.	+42 sec.	+ 5 min.	+ 10 min.	+ 10 min.	+ 9 min.	+ 5 min.	+ 10 min.	+ 15 min.
Actual Time	~~KFP~~ ~~1501~~ 1502	1503:00	15:03:42	15:08	1518	1528	1537	1542	1552	1607
Salinity (ppt)	33.5	39.2	40.1	38.8	37.2	35.7	35.5	35.1	34.2	33.6
Temp (◦C)	15.0	14.6	14.8	14.9	14.6	14.5	14.6	14.6	14.6	14.7

Technician Initials:

WQ Readings: KFP/AC
Dilutions made by: PA

Comments:

0 hrs: @1513 reps DHE pulled; 37.9 ppt 14.7°C; ~~PAG~~ pulled at 1518
24 hrs:
48 hrs:

QC Check: AC 1/29/15

Final Review: 6/15/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon
Sample ID: Pump Brine Dilution Study
Sample Log No.: --
Test No.: 1501-5097

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

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			15.9			7.7			7.61		
Lab Control Surrogate	33.6	33.4	33.5	14.8	14.7	14.9	8.7	7.8	8.3	7.84	8.01	8.03
Brine Dilution Surrogate	33.6	33.5	33.5	14.7	14.3	14.8	8.9	7.7	8.4	7.97	8.05	8.01

	0	24	48
WQ Readings:	~~PA~~/AC	NH	~~y~~
Dilutions made by:	PA		

Technician Initials:

Comments:
0 hrs: Day 0 surrogate readings recorded after final mixing
24 hrs:
48 hrs:

QC Check: AC 1/29/15

Final Review: ~~y~~ 6/15/15

Marine Chronic Bioassay | **Abalone Embryo-Larval Development**

Client: Poseidon | **Test Species:** *Haliotis rufescens*

Sample ID: Nautilus frozen brine | **Start Date/Time:** 1/22/2015 1444

Test No.: 1501-SD97 | **End Date/Time:** 1/24/2015 1500

Animal Source/Date Received: American Abalone 1/20/15

Number of abalone and condition upon receipt/holding:

Males: 2 / good

Females: 2 / good

	Males:	Females:
Tris & peroxide addition time	1045	1015
Spawn time	1315	1305
Number of spawners	2	1
Condition of spawn (light, moderate, heavy)	Moderate	light
Fertilization time	1350	

Embryo counts (per 0.5 ml)	
1	180
2	172
3	184
Mean	

Time of test Initiation: 1444 | **48 hr. QC** 83

Technician Initials: PA

Comments:

QC Check: AC 1/29/15 | **Final Review:** 6/15/15

APPENDIX B

Reference Toxicant Test Data

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

CETIS Summary Report

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Batch ID:	11-1621-8697	**Test Type:**	Development	**Analyst:**	
Start Date:	22 Jan-15 14:44	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	24 Jan-15 15:00	**Species:**	Haliotis rufescens	**Brine:**	Not Applicable
Duration:	48h	**Source:**	American Abalone	**Age:**	

Sample ID:	14-4511-7987	**Code:**	150122hrdv	**Client:**	Internal
Sample Date:	22 Jan-15	**Material:**	Zinc sulfate	**Project:**	
Receive Date:	22 Jan-15	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Zinc sulfate		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
19-6293-3678	Development Rate	32	56	42.33	11.6%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
21-0118-1264	Development Rate	EC50	50.62	49.17	52.11		Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
19-6293-3678	Development Rate	Control Resp	0.658	0.8 - NL	Yes	Below Acceptability Criteria Q15
21-0118-1264	Development Rate	Control Resp	0.658	0.8 - NL	Yes	Below Acceptability Criteria
19-6293-3678	Development Rate	NOEL	32	NL - 56	No	Passes Acceptability Criteria
19-6293-3678	Development Rate	PMSD	0.1162	NL - 0.2	No	Passes Acceptability Criteria

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.658	0.6138	0.7022	0.63	0.72	0.01594	0.03564	5.42%	0.0%
10		5	0.678	0.6596	0.6964	0.66	0.7	0.006633	0.01483	2.19%	-3.04%
18		5	0.646	0.5702	0.7218	0.55	0.71	0.02731	0.06107	9.45%	1.82%
32		5	0.632	0.5632	0.7008	0.54	0.68	0.02478	0.05541	8.77%	3.95%
56		5	0.268	0.1841	0.3519	0.2	0.36	0.03023	0.0676	25.22%	59.27%
100		5	0	0	0	0	0	0	0		100.0%

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.63	0.64	0.65	0.65	0.72
10		0.68	0.68	0.66	0.67	0.7
18		0.63	0.71	0.68	0.66	0.55
32		0.64	0.68	0.54	0.63	0.67
56		0.2	0.31	0.26	0.21	0.36
100		0	0	0	0	0

Q15 - coded as unofficial

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID: 19-6293-3678 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 04 Feb-15 12:01 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	11.6%	32	56	42.33	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10	-0.6075	2.305	0.079	8	0.9382	CDF	Non-Significant Effect
		18	0.3524	2.305	0.079	8	0.6675	CDF	Non-Significant Effect
		32	0.7844	2.305	0.079	8	0.4754	CDF	Non-Significant Effect
		56*	11.77	2.305	0.079	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.647029	0.1617573	4	54.72	<0.0001	Significant Effect
Error	0.05912194	0.002956097	20			
Total	0.706151		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.59	13.28	0.1078	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9646	0.8877	0.5135	Normal Distribution

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.658	0.6138	0.7022	0.65	0.63	0.72	0.01594	5.42%	0.0%
10		5	0.678	0.6596	0.6964	0.68	0.66	0.7	0.006633	2.19%	-3.04%
18		5	0.646	0.5702	0.7218	0.66	0.55	0.71	0.02731	9.45%	1.82%
32		5	0.632	0.5632	0.7008	0.64	0.54	0.68	0.02478	8.77%	3.95%
56		5	0.268	0.1841	0.3519	0.26	0.2	0.36	0.03023	25.22%	59.27%
100		5	0	0	0	0	0	0	0		100.0%

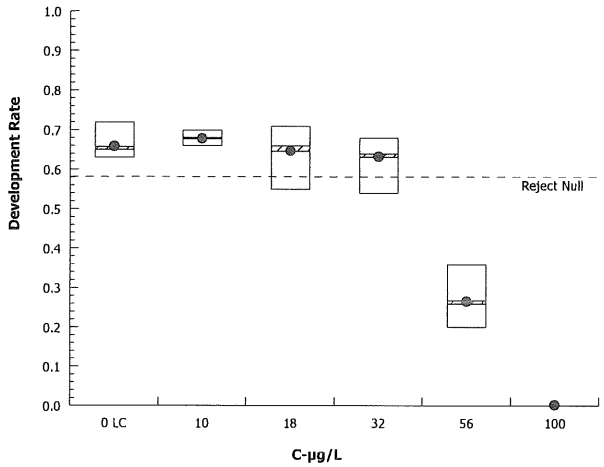
Angular (Corrected) Transformed Summary

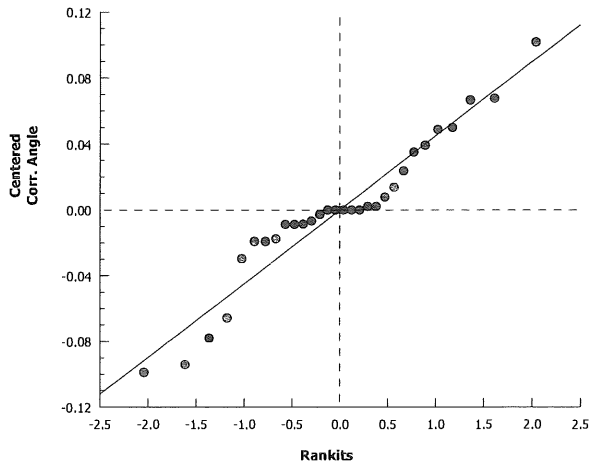
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9466	0.8991	0.994	0.9377	0.9169	1.013	0.0171	4.04%	0.0%
10		5	0.9675	0.9477	0.9872	0.9695	0.9483	0.9912	0.007116	1.65%	-2.21%
18		5	0.9345	0.8557	1.013	0.9483	0.8355	1.002	0.02838	6.79%	1.28%
32		5	0.9196	0.8489	0.9903	0.9273	0.8254	0.9695	0.02546	6.19%	2.85%
56		5	0.5418	0.4472	0.6363	0.5351	0.4636	0.6435	0.03405	14.06%	42.77%
100		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	94.72%

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	19-6293-3678	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	04 Feb-15 12:01	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 150122hrdv | 16-1164-6227

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	21-0118-1264	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	04 Feb-15 12:01	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

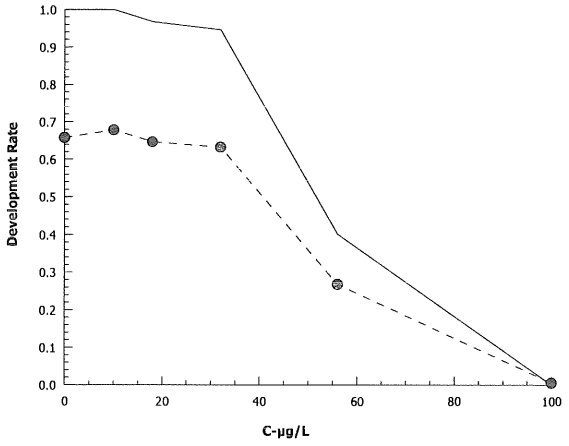
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.342	0.00%	1.704	0.006299	50.62	49.17	52.11

Development Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
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

Graphics



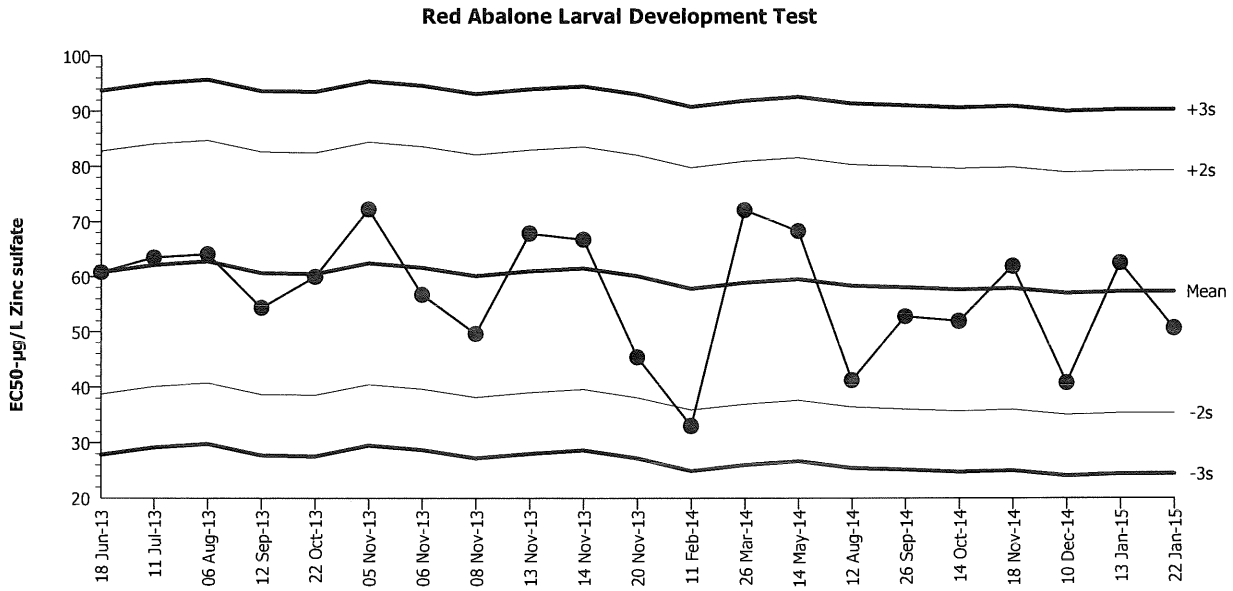
 Analyst: KB QA: AC 2/4/15

CETIS QC Plot

Report Date: 04 Feb-15 12:02 (1 of 1)

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

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			20-4825-5447	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

CETIS Test Data Worksheet

Report Date: 20 Jan-15 13:51 (p 1 of 1)
Test Code: 16-1164-6227/150122hrdv

Red Abalone Larval Development Test			Nautilus Environmental (CA)
Start Date: 22 Jan-15	**Species:** Haliotis rufescens	**Sample Code:** 150122hrdv	
End Date: 24 Jan-15	**Protocol:** EPA/600/R-95/136 (1995)	**Sample Source:** Reference Toxicant	
Sample Date: 22 Jan-15	**Material:** Zinc sulfate	**Sample Station:** Zinc sulfate	

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	68	
			2	100	67	
			3	100	26	
			4	100	0	
			5	100	64	
			6	100	0	
			7	100	63	
			8	100	66	
			9	100	20	
			10	100	0	
			11	100	67	
			12	100	0	
			13	100	63	
			14	100	66	
			15	100	65	
			16	100	63	
			17	100	31	
			18	100	71	
			19	100	0	
			20	100	68	
			21	100	68	
			22	100	64	
			23	100	68	
			24	100	65	
			25	100	70	
			26	100	54	
			27	100	55	
			28	100	36	
			29	100	21	
			30	100	72	

CETIS Test Data Worksheet

Report Date: 20 Jan-15 13:51 (p 1 of 1)
Test Code: 16-1164-6227/150122hrdv

Red Abalone Larval Development Test				Nautilus Environmental (CA)
Start Date:	22 Jan-15	**Species:**	Haliotis rufescens	**Sample Code:** 150122hrdv
End Date:	24 Jan-15	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:** Reference Toxicant
Sample Date:	22 Jan-15	**Material:**	Zinc sulfate	**Sample Station:** Zinc sulfate

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	7	100	69	AC 1/25/15
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			
32		4	13			
32		5	11			
56		1	9			
56		2	17			
56		3	3			
56		4	29			
56		5	28			
100		1	12			
100		2	4			
100		3	19			
100		4	10			
100		5	6			

QC=AC

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
Sample ID: $ZnSO_4$
Test ID: 150122hrdv

Test Species: *Haliotis rufescens*
Start Date/Time: 1/22/2015 1444
End Date/Time: 1/24/2015 1500

Concentration (µg/L)	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	32.8	32.8	32.9	15.8	15.0	14.9	8.3	8.3	8.4	7.98	8.01	8.05
10	32.8	32.8	32.9	15.9	14.6	14.9	8.2	8.3	8.3	7.99	8.02	8.03
18	32.8	32.8	32.9	15.6	14.6	14.8	8.3	8.3	8.3	8.01	8.03	8.03
32	32.8	32.9	32.8	15.7	14.6	14.9	8.3	8.3	8.3	8.02	8.03	8.02
56	32.8	32.8	32.8	15.5	14.7	15.0	8.3	8.3	8.3	8.04	8.03	8.03
100	32.6	32.6	32.7	15.4	14.6	14.9	8.3	8.3	8.3	8.05	8.04	8.01

Technician Initials:

	0	24	48
WQ Readings:	VCR	NH	Y
Dilutions made by:	AC		

Dilution calcs. (final volume 500 mL):

Conc.	10	18	32	56	100
Vol. Zn stock (mL):	0.5	0.9	1.6	2.8	5.0

Zn Stock Concentration (µg/L): 10,000

Comments:
0 hrs:
24 hrs:
48 hrs:

QC Check: KB 2/2/15

Final Review: AC 2/5/15

Marine Chronic Bioassay **Abalone Embryo-Larval Development**

Client: Internal **Test Species:** *Haliotis rufescens*

Sample ID: ZnSO4 **Start Date/Time:** 1/22/2015 1444

Test No.: 150124 hrdv **End Date/Time:** 1/24/2015 1500

Animal Source/Date Received: American Abalone 1/20/15

Number of abalone and condition upon receipt/holding:

Males: 2 / good

Females: 2 / good

	Males:	Females:
Tris & peroxide addition time	1045	1015
Spawn time	1315	1305
Number of spawners	2	1
Condition of spawn (light, moderate, heavy)	Moderate	light
Fertilization time	1350	

Embryo counts (per 0.5 ml)	
1	180
2	172
3	184
Mean	179

Time of test Initiation: 1444 **48 hr. QC** 83%

Technician Initials: PA

Comments:

QC Check: KB 2/2/15 **Final Review:** AC 2/5/15

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

CETIS Summary Report

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Batch ID:	20-1429-8396	**Test Type:**	Development	**Analyst:**	
Start Date:	30 Jan-15 15:22	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	01 Feb-15 14:15	**Species:**	Haliotis rufescens	**Brine:**	Not Applicable
Duration:	47h	**Source:**	American Abalone	**Age:**	

Sample ID:	03-2767-9688	**Code:**	150130hrdv	**Client:**	Internal
Sample Date:	30 Jan-15	**Material:**	Zinc sulfate	**Project:**	
Receive Date:	30 Jan-15	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Zinc sulfate		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-0806-0312	Development Rate	32	56	42.33	3.9%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
03-0729-5027	Development Rate	EC50	52.83	51.42	54.27		Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-0729-5027	Development Rate	Control Resp	0.906	0.8 - NL	Yes	Passes Acceptability Criteria
16-0806-0312	Development Rate	Control Resp	0.906	0.8 - NL	Yes	Passes Acceptability Criteria
16-0806-0312	Development Rate	NOEL	32	NL - 56	No	Passes Acceptability Criteria
16-0806-0312	Development Rate	PMSD	0.03899	NL - 0.2	No	Passes Acceptability Criteria

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.906	0.8834	0.9286	0.89	0.93	0.008124	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 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.92	0.89	0.89	0.9	0.93
10		0.91	0.92	0.92	0.94	0.93
18		0.89	0.87	0.88	0.94	0.87
32		0.85	0.91	0.93	0.93	0.92
56		0.4	0.36	0.4	0.42	0.36
100		0	0	0	0	0

CETIS Analytical Report

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	16-0806-0312	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Feb-15 11:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.9%	32	56	42.33	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10	-1.277	2.305	0.057	8	0.9885	CDF	Non-Significant Effect
		18	1.001	2.305	0.057	8	0.3804	CDF	Non-Significant Effect
		32	-0.2227	2.305	0.057	8	0.8642	CDF	Non-Significant Effect
		56*	23.62	2.305	0.057	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.405821	0.3514552	4	226.8	<0.0001	Significant Effect
Error	0.03099143	0.001549571	20			
Total	1.436812		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.287	13.28	0.3686	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9559	0.8877	0.3397	Normal Distribution

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.906	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%
56		5	0.388	0.3547	0.4213	0.4	0.36	0.42	0.012	6.92%	57.17%
100		5	0	0	0	0	0	0	0		100.0%

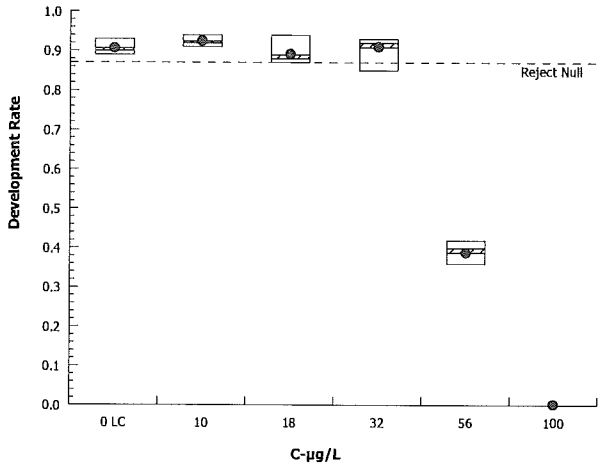
Angular (Corrected) Transformed Summary

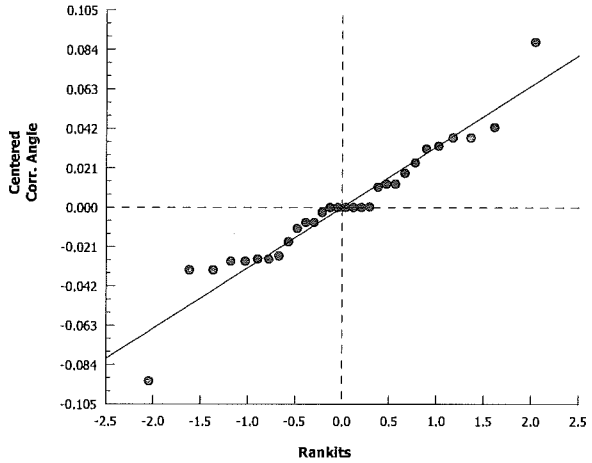
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.26	1.221	1.3	1.249	1.233	1.303	0.01421	2.52%	0.0%
10		5	1.292	1.265	1.319	1.284	1.266	1.323	0.009748	1.69%	-2.52%
18		5	1.235	1.172	1.298	1.217	1.202	1.323	0.02271	4.11%	1.98%
32		5	1.266	1.199	1.333	1.284	1.173	1.303	0.02419	4.27%	-0.44%
56		5	0.6723	0.6381	0.7065	0.6847	0.6435	0.7051	0.01233	4.1%	46.66%
100		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.03%

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	16-0806-0312	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Feb-15 11:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	03-0729-5027	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Feb-15 11:04	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

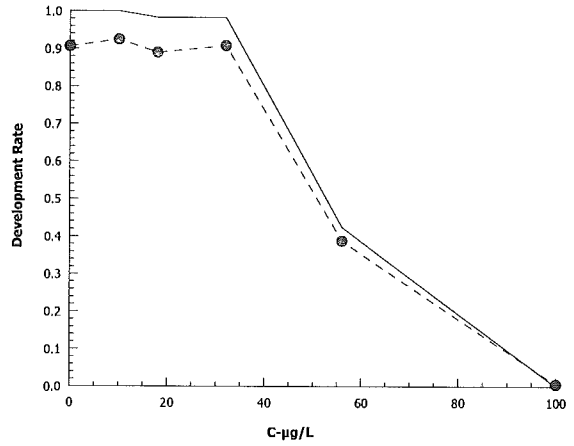
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.094	0.00%	1.723	0.005847	52.83	51.42	54.27

Development Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
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

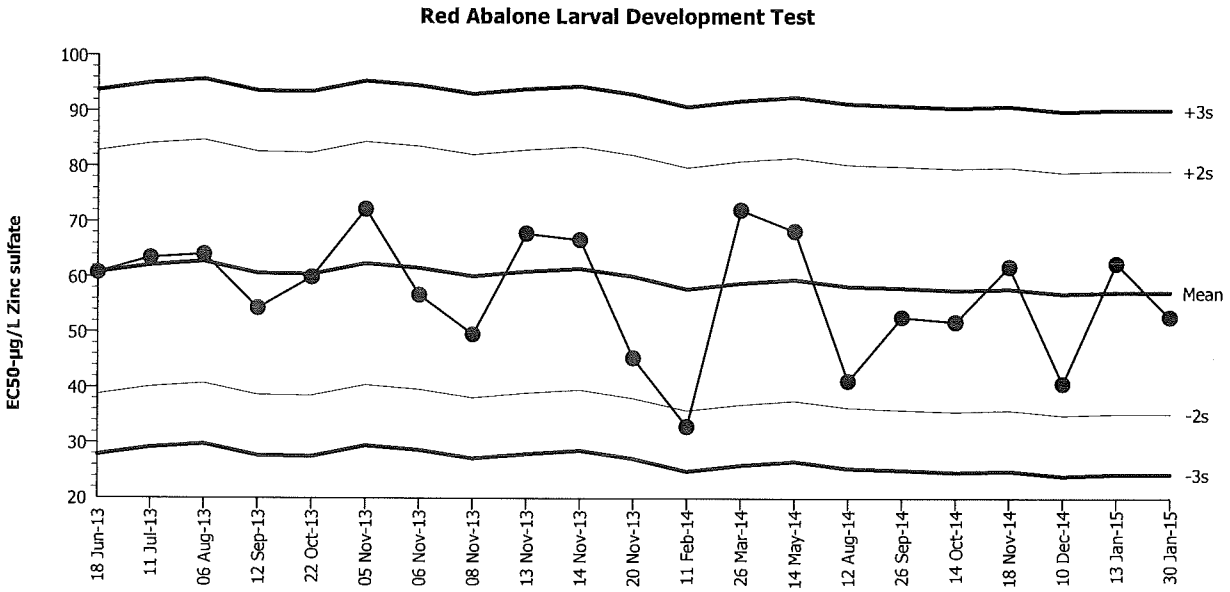
Graphics



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

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			20-4825-5447	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

Report Date: 29 Jan-15 16:15 (p 1 of 1)
Test Code: 06-2409-9903/150130hrdv

Red Abalone Larval Development Test				Nautilus Environmental (CA)
Start Date:	30 Jan-15	**Species:**	Haliotis rufescens	**Sample Code:** 150130hrdv
End Date:	01 Feb-15	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:** Reference Toxicant
Sample Date:	30 Jan-15	**Material:**	Zinc sulfate	**Sample Station:** 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	0	
			5	100	0	
			6	100	85	
			7	100	89	
			8	100	91	
			9	100	89	
			10	100	88	CH 2/3 2/3/15 Q18 2/2/15
			11	100	92	
			12	100	42	
			13	100	92	
			14	100	36	
			15	100	87	
			16	100	87	
			17	100	93	CH 2/3/15 Q18
			18	100	93 ~~91A3~~	
			19	100	94	
			20	100	91	
			21	100	0	
			22	100	40	
			23	100	92	
			24	100	93	
			25	100	0	
			26	100	40	
			27	100	94	
			28	100	93	
			29	100	36	
			30	100	0	

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

CETIS Test Data Worksheet

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Start Date: 30 Jan-15 | **Species:** Haliotis rufescens | **Sample Code:** 150130hrdv
End Date: 01 Feb-15 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 30 Jan-15 | **Material:** Zinc sulfate | **Sample Station:** Zinc sulfate

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	11	100	88	
0	LC	2	1			
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	100	93	
32		5	13			
56		1	22			
56		2	14			
56		3	26	100	39	
56		4	12			
56		5	29			
100		1	21			
100		2	5			
100		3	25			
100		4	30			
100		5	4	100	0	

QC=AC

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
Sample ID: $ZnSO_4$
Test ID: 150130hrdv

Test Species: *Haliotis rufescens*
Start Date/Time: 1/30/2015 1522
End Date/Time: 2/1/2015 1415

Concentration (µg/L)	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	33.6	33.2	33.1	14.7	14.9	14.9	8.3	7.9	8.0	8.03	7.98	8.01
10	33.5	33.4	33.3	14.4	14.7	14.7	8.3	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	14.6	14.8	8.3	8.1	7.9	8.05	8.01	8.01
56	33.5	33.7	33.6	14.7	14.6	14.8	8.3	8.1	8.0	8.05	8.01	8.01
100	33.3	33.5	33.5	14.8	14.5	14.9	8.3	8.1	8.0	8.05	8.01	8.01

Technician Initials:

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

Dilution calcs. (final volume 500 mL): AC 2/3/15

Conc.	10	18	32	56	100
Vol. Zn stock (mL):	0.25	0.45	0.8	1.4	2.5

Zn Stock Concentration (µg/L): 10,000

Comments:
0 hrs:
24 hrs:
48 hrs:

QC Check: AC 2/3/15

Final Review: KB 2/5/15

Marine Chronic Bioassay | Abalone Embryo-Larval Development

Client: Internal | **Test Species:** *Haliotis rufescens*

Sample ID: ZnSO4 | **Start Date/Time:** 1/30/2015 1522

Test No.: 150130hrdv | **End Date/Time:** 2/1/2015 1415

Animal Source/Date Received: American Abalone / 1/28/15

Number of abalone and condition upon receipt/holding:

Males: 4 good, small

Females: 4 good

	Males:	Females:
Tris & peroxide addition time	1100	1030
Spawn time	1330	1400
Number of spawners	4	~~AC 2~~ 3
Condition of spawn (light, moderate, heavy)	heavy	moderate
Fertilization time	~~1403~~ Q21 AC 1/30/15 1440	

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

Time of test Initiation: 1522

48 hr. QC 97%

Technician Initials: RA

Comments: Q21 - used different batch of eggs (more dense) and new fert. time.

QC Check: AC 2/3/15 **Final Review:** KB 2/5/15

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

CETIS Summary Report

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Batch ID:	16-2450-8328	**Test Type:**	Development	**Analyst:**	
Start Date:	06 Feb-15 13:33	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	08 Feb-15 13:00	**Species:**	Haliotis rufescens	**Brine:**	Not Applicable
Duration:	47h	**Source:**	American Abalone	**Age:**	

Sample ID:	17-5259-9068	**Code:**	150206hrdv	**Client:**	Internal
Sample Date:	06 Feb-15	**Material:**	Zinc sulfate	**Project:**	
Receive Date:	06 Feb-15	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Zinc sulfate		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-5283-0346	Development Rate	32	56	42.33	7.73%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
07-8543-5535	Development Rate	EC50	54.69	53.03	56.4		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-8543-5535	Development Rate	Control Resp	0.8089	0.8 - NL	Yes	Passes Acceptability Criteria
20-5283-0346	Development Rate	Control Resp	0.8089	0.8 - NL	Yes	Passes Acceptability Criteria
20-5283-0346	Development Rate	NOEL	32	NL - 56	No	Passes Acceptability Criteria
20-5283-0346	Development Rate	PMSD	0.07735	NL - 0.2	No	Passes Acceptability Criteria

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.8089	0.7669	0.8508	0.77	0.8602	0.01512	0.03382	4.18%	0.0%
10		5	0.7644	0.7023	0.8264	0.7143	0.8293	0.02236	0.04999	6.54%	5.5%
18		5	0.8006	0.7558	0.8455	0.77	0.84	0.01615	0.03611	4.51%	1.02%
32		5	0.7739	0.7314	0.8164	0.7216	0.81	0.0153	0.03421	4.42%	4.32%
56		5	0.3833	0.3011	0.4655	0.3226	0.49	0.02961	0.06621	17.27%	52.61%
100		5	0	0	0	0	0	0	0		100.0%

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8171	0.79	0.77	0.807	0.8602
10		0.7143	0.72	0.8	0.8293	0.7582
18		0.78	0.84	0.84	0.7732	0.77
32		0.7216	0.81	0.79	0.76	0.7879
56		0.49	0.4022	0.3529	0.3488	0.3226
100		0	0	0	0	0

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID: 20-5283-0346 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 10 Feb-15 13:47 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.73%	32	56	42.33	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10	1.618	2.305	0.077	8	0.1665	CDF	Non-Significant Effect
		18	0.3105	2.305	0.077	8	0.6849	CDF	Non-Significant Effect
		32	1.307	2.305	0.077	8	0.2614	CDF	Non-Significant Effect
		56*	13.61	2.305	0.077	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.7352132	0.1838033	4	66.49	<0.0001	Significant Effect
Error	0.05528483	0.002764241	20			
Total	0.790498		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.433	13.28	0.8384	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9429	0.8877	0.1727	Normal Distribution

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.8089	0.7669	0.8508	0.807	0.77	0.8602	0.01512	4.18%	0.0%
10		5	0.7644	0.7023	0.8264	0.7582	0.7143	0.8293	0.02236	6.54%	5.5%
18		5	0.8006	0.7558	0.8455	0.78	0.77	0.84	0.01615	4.51%	1.02%
32		5	0.7739	0.7314	0.8164	0.7879	0.7216	0.81	0.0153	4.42%	4.32%
56		5	0.3833	0.3011	0.4655	0.3529	0.3226	0.49	0.02961	17.27%	52.61%
100		5	0	0	0	0	0	0	0		100.0%

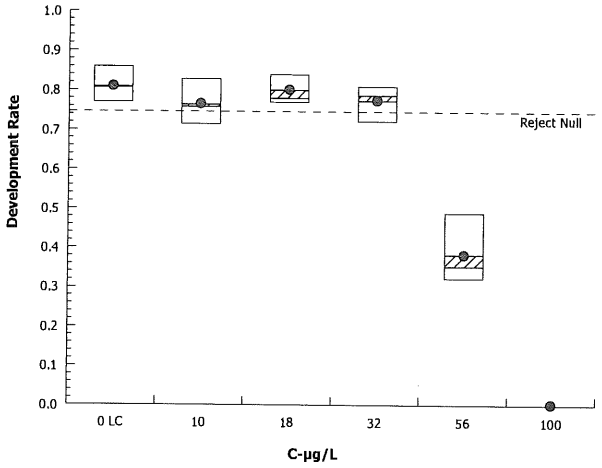
Angular (Corrected) Transformed Summary

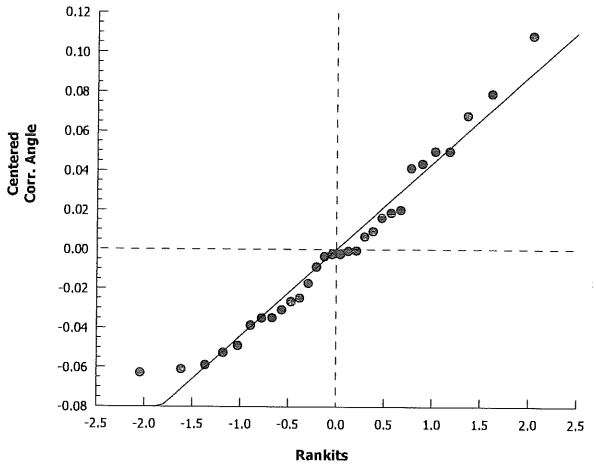
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.12	1.065	1.174	1.116	1.071	1.188	0.01967	3.93%	0.0%
10		5	1.066	0.9916	1.14	1.057	1.007	1.145	0.02672	5.61%	4.81%
18		5	1.109	1.052	1.166	1.083	1.071	1.159	0.02052	4.14%	0.92%
32		5	1.076	1.026	1.126	1.092	1.015	1.12	0.01808	3.76%	3.88%
56		5	0.6669	0.5829	0.7509	0.6361	0.604	0.7754	0.03025	10.14%	40.44%
100		5	0.05249	0.04788	0.05711	0.05159	0.05002	0.05896	0.001662	7.08%	95.31%

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	20-5283-0346	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 Feb-15 13:47	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	07-8543-5535	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 Feb-15 13:47	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

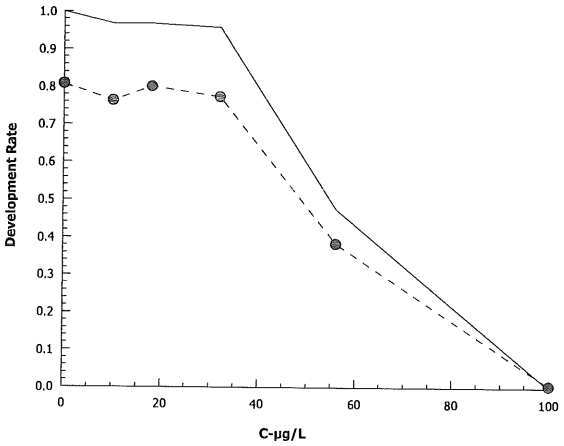
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.1921	3.17%	1.738	0.006685	54.69	53.03	56.4

Development Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
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

Graphics



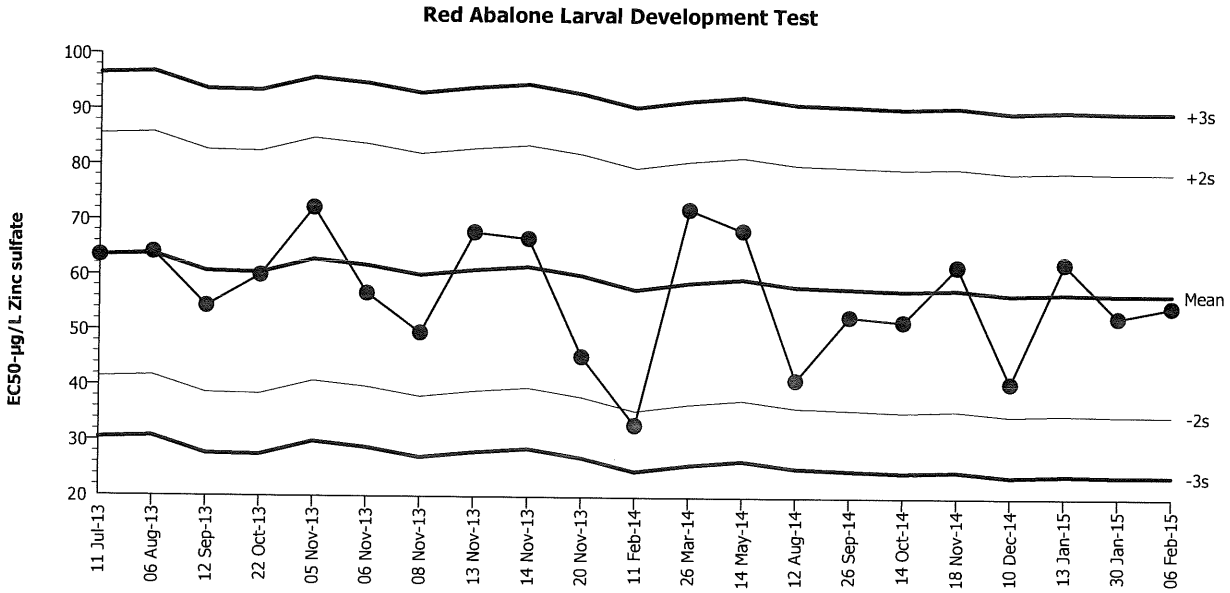
Analyst: _____ QA: AC 2/10/15

CETIS QC Plot

Report Date: 10 Feb-15 13:51 (1 of 1)

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:	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

Quality Control Data

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 Test Data Worksheet

Red Abalone Larval Development Test — **Nautilus Environmental (CA)**

Start Date: 06 Feb-15 13:33 **Species:** Haliotis rufescens **Sample Code:** 150206hrdv
End Date: 08 Feb-15 13:00 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 06 Feb-15 **Material:** Zinc sulfate **Sample Station:** Zinc sulfate

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	92	37	
			2	100	76	
			3	85	30	
			4	93	80	
			5	91	69	
			6	94	0	
			7	57	46	
			8	82	67	
			9	93	30	
			10	82	68	
			11	160	77	
			12	100	77	
			13	100	0	
			14	100	72	
			15	100	81	
			16	100	78	
			17	100	84	
			18	97	75	
			19	97	70	
			20	100	79	
			21	86	30	
			22	100	80	
			23	100	79	
			24	99	78	
			25	100	0	
			26	84	60	
			27	100	49	
			28	100	84	
			29	72	0	
			30	93	0	

CETIS Test Data Worksheet

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

Red Abalone Larval Development Test				Nautilus Environmental (CA)
Start Date: 06 Feb-15	**Species:** Haliotis rufescens		**Sample Code:** 150206hrdv	
End Date: 08 Feb-15	**Protocol:** EPA/600/R-95/136 (1995)		**Sample Source:** Reference Toxicant	
Sample Date: 06 Feb-15	**Material:** Zinc sulfate		**Sample Station:** Zinc sulfate	

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			
32		5	24	100 79 AC 2/18/15	79.	
56		1	27			
56		2	1			
56		3	3			
56		4	21			
56		5	9	100	37.	
100		1	30			
100		2	29			
100		3	25			
100		4	6			
100		5	13	100	0	

QC=VCR

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
Sample ID: $ZnSO_4$
Test ID: 150206hrdv

Test Species: *Haliotis rufescens*
Start Date/Time: 2/6/2015 1333
End Date/Time: 2/8/2015 1300

Concentration (µg/L)	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	32.8	33.1	32.8	16.0	15.3	15.5	8.2	7.9	7.6	7.99	7.94	7.97
10	33.0	33.4	33.2	15.8	15.1	15.4	8.1	7.9	7.9	7.98	7.97	8.01
18	32.9	33.4	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	8.1	8.0	7.9	8.00	7.95	8.00
56	32.8	33.3	33.1	16.0	15.0	15.4	8.1	8.0	7.9	8.00	7.99	8.01
100	32.6	33.2	32.9	16.0	15.0	15.4	8.1	8.0	7.8	7.98	7.98	8.00
									~~7.8~~ BC 2/9/15			

Technician Initials:

	0	24	48
WQ Readings:	VCR	AMB	BK
Dilutions made by:	VCR		

Dilution calcs. (final volume ~~500 mL~~ 250 mL): AC 2/8/15

Conc.	10	18	32	56	100
Vol. Zn stock (mL):	0.25	0.45	0.8	1.4	2.5

Zn Stock Concentration (µg/L): 10,000

Comments:
0 hrs:
24 hrs:
48 hrs:

QC Check: AC 2/9/15

Final Review: 4 2/10/15

Marine Chronic Bioassay | Abalone Embryo-Larval Development

Client: Internal | Test Species: *Haliotis rufescens*

Sample ID: ZnSO4 | Start Date/Time: 2/6/15 1333

Test No.: 150206 hrdv | End Date/Time: 2/8/15 1300

Animal Source/Date Received: American Abalone 2/3/15

Number of abalone and condition upon receipt/holding:

Males: 4 good

Females: 4 good

	Males:	Females:
Tris & peroxide addition time	1005	0925
Spawn time	1200	1225
Number of spawners	4	4
Condition of spawn (light, moderate, heavy)	heavy	heavy
Fertilization time	12:52	

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

Time of test Initiation: 1333 | 48 hr. QC 64/94

Technician Initials: AC/PK

Comments:

QC Check: AC 2/9/15 | Final Review: CA 2/10/15

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

CETIS Summary Report

Echinoid Embryo-Larval Development Test — **Nautilus Environmental (CA)**

Batch ID:	01-1508-0642	**Test Type:**	Development	**Analyst:**	
Start Date:	17 Feb-15 15:12	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	20 Feb-15 15:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	72h	**Source:**	Pt. Loma	**Age:**	

Sample ID:	12-2030-5344	**Code:**	150217spdv	**Client:**	Internal
Sample Date:	17 Feb-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	17 Feb-15	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-2897-7271	Development Rate	5	10	7.071	3.74%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
03-8986-1540	Development Rate	EC50	12.77	12.46	13.08		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-8986-1540	Development Rate	Control Resp	0.948	0.8 - NL	Yes	Passes Acceptability Criteria
17-2897-7271	Development Rate	Control Resp	0.948	0.8 - NL	Yes	Passes Acceptability Criteria
17-2897-7271	Development Rate	PMSD	0.03742	NL - 0.25	No	Passes Acceptability Criteria

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.948	0.9114	0.9846	0.91	0.99	0.01319	0.0295	3.11%	0.0%
2.5		5	0.94	0.9224	0.9576	0.93	0.96	0.006325	0.01414	1.5%	0.84%
5		5	0.95	0.9324	0.9676	0.93	0.97	0.006325	0.01414	1.49%	-0.21%
10		5	0.794	0.747	0.841	0.76	0.84	0.01691	0.03782	4.76%	16.24%
20		5	0.016	0	0.03856	0	0.04	0.008124	0.01817	113.5%	98.31%
40		5	0	0	0	0	0	0	0		100.0%
80		5	0	0	0	0	0	0	0		100.0%

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.94	0.91	0.96	0.94	0.99
2.5		0.93	0.95	0.93	0.93	0.96
5		0.95	0.93	0.95	0.95	0.97
10		0.77	0.76	0.84	0.77	0.83
20		0.03	0	0.01	0	0.04
40		0	0	0	0	0
80		0	0	0	0	0

Echinoid Embryo-Larval Development Test — Nautilus Environmental (CA)

Analysis ID:	17-2897-7271	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Mar-15 9:54	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.74%	5	10	7.071	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	0.7431	2.305	0.080	8	0.4940	CDF	Non-Significant Effect
		5	0.09927	2.305	0.080	8	0.7662	CDF	Non-Significant Effect
		10*	7.187	2.305	0.080	8	<0.0001	CDF	Significant Effect
		20*	35.58	2.305	0.080	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.653374	1.413343	4	468.9	<0.0001	Significant Effect
Error	0.06027799	0.0030139	20			
Total	5.713652		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.692	13.28	0.3204	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9573	0.8877	0.3626	Normal Distribution

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.948	0.9114	0.9846	0.94	0.91	0.99	0.01319	3.11%	0.0%
2.5		5	0.94	0.9224	0.9576	0.93	0.93	0.96	0.006324	1.5%	0.84%
5		5	0.95	0.9324	0.9676	0.95	0.93	0.97	0.006324	1.49%	-0.21%
10		5	0.794	0.747	0.841	0.77	0.76	0.84	0.01691	4.76%	16.24%
20		5	0.016	0	0.03856	0.01	0	0.04	0.008124	113.5%	98.31%
40		5	0	0	0	0	0	0	0		100.0%
80		5	0	0	0	0	0	0	0		100.0%

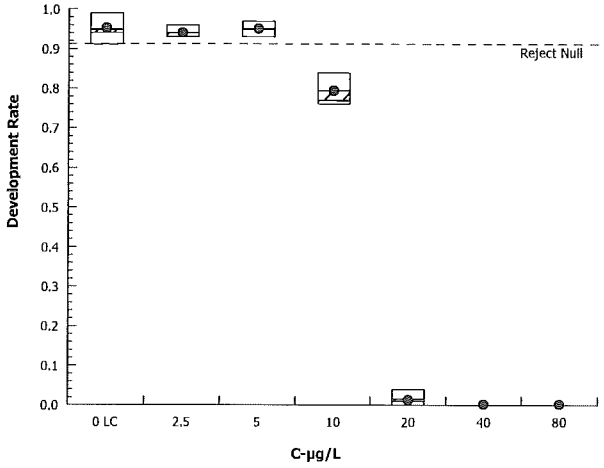
Angular (Corrected) Transformed Summary

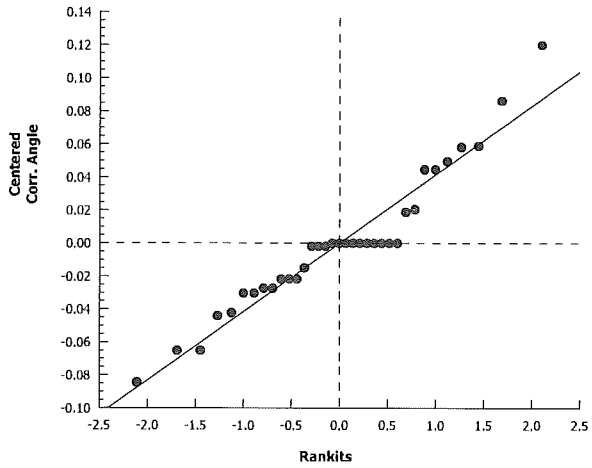
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.351	1.256	1.446	1.323	1.266	1.471	0.0342	5.66%	0.0%
2.5		5	1.325	1.286	1.363	1.303	1.303	1.369	0.01384	2.34%	1.91%
5		5	1.347	1.306	1.388	1.345	1.303	1.397	0.01485	2.47%	0.26%
10		5	1.101	1.042	1.16	1.071	1.059	1.159	0.02125	4.32%	18.48%
20		5	0.1151	0.02818	0.2021	0.1002	0.05002	0.2014	0.03132	60.82%	91.48%
40		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.3%
80		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.3%

Echinoid Embryo-Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	17-2897-7271	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Mar-15 9:54	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Echinoid Embryo-Larval Development Test — **Nautilus Environmental (CA)**

Analysis ID:	03-8986-1540	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Mar-15 9:54	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

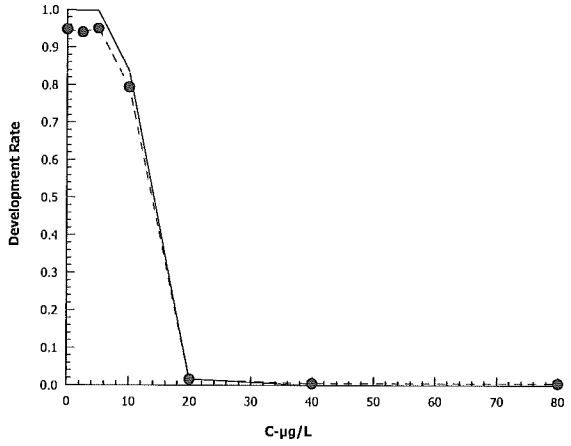
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.052	0.32%	1.106	0.005297	12.77	12.46	13.08

Development Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
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

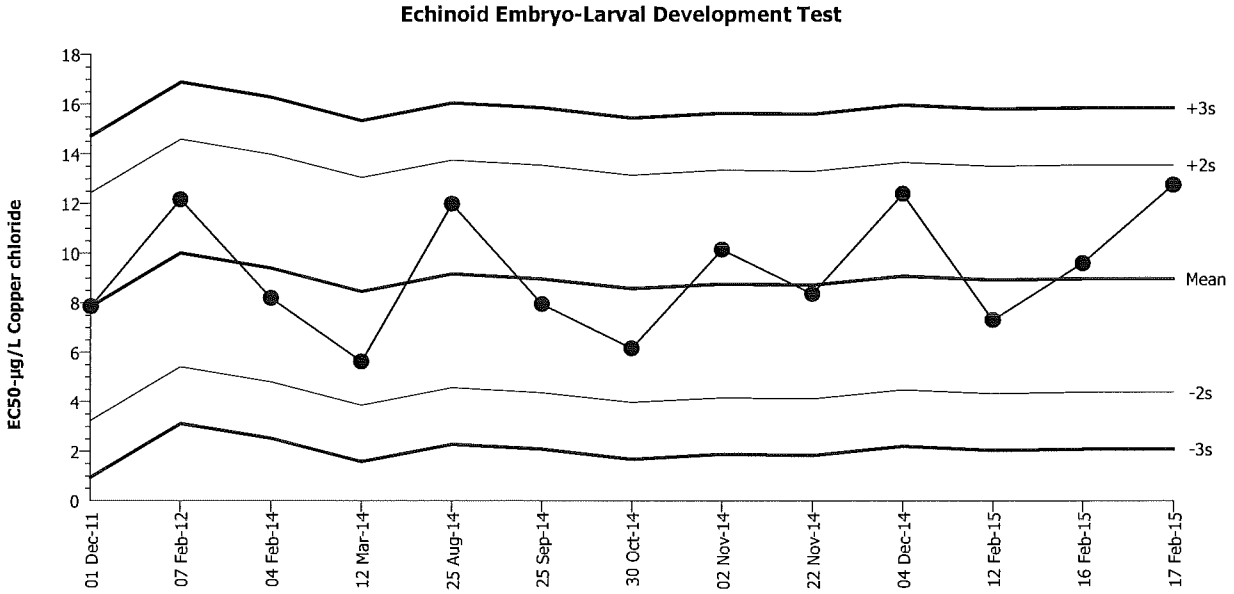
Graphics



CETIS QC Plot

Echinoid Embryo-Larval Development Test — **Nautilus Environmental (CA)**

Test Type:	Development	**Organism:**	Strongylocentrotus purpuratus (Purpl	**Material:**	Copper chloride
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Development Rate	**Source:**	Reference Toxicant-REF



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
4		Mar	12	18:20	5.64	-3.341	-1.456			16-4219-4884	02-2584-6206
5		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
7		Oct	30	15:00	6.16	-2.821	-1.229			16-2563-7748	00-5600-3113
8		Nov	2	17:00	10.14	1.162	0.5065			05-9121-9644	01-7691-0405
9			22	16:45	8.358	-0.6232	-0.2715			06-8410-0954	18-0830-4230
10		Dec	4	14:10	12.4	3.419	1.49			15-8916-5237	11-1209-5739
11	2015	Feb	12	14:25	7.308	-1.673	-0.7291			21-1011-3319	03-9708-7225
12			16	18:25	9.603	0.6217	0.2709			12-8378-8021	11-4706-6613
13			17	15:12	12.77	3.784	1.649			12-8622-3584	03-8986-1540

CETIS Test Data Worksheet

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

Echinoid Embryo-Larval Development Test				Nautilus Environmental (CA)
Start Date:	17 Feb-15	**Species:**	Strongylocentrotus purpuratus	**Sample Code:** 150217spdv
End Date:	20 Feb-15	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:** Reference Toxicant
Sample Date:	17 Feb-15	**Material:**	Copper chloride	**Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	95	
			2		95	
			3	AD	~~99~~ 0	
			4	QI 8 2/24/15	99	
			5		97	
			6		0	
			7		91	
			8		0	
			9		83	
			10		96	
			11		84	
			12		96	
			13		0	
			14		0	
			15		93	
			16		3	
			17		0	
			18		95	
			19		0	
			20		95	
			21		4	
			22	KB	~~3~~	94 EG 3/13/15
			23	3/16/15	1	
			24		0	
			25		77	
			26	AD QI 8 2/25/15	~~97~~ 0	
			27	AD	~~94~~ 0	
			28	QI 8 2/25/15	93	
			29		93	
			30		77	
			31		93	
			32		0	
			33		94	
			34		76	
			35		0	

Analyst: AD QA: KB 3/16/15

CETIS Test Data Worksheet

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

Echinoid Embryo-Larval Development Test				Nautilus Environmental (CA)
Start Date: 17 Feb-15	**Species:** Strongylocentrotus purpuratus		**Sample Code:** 150217spdv	
End Date: 20 Feb-15	**Protocol:** EPA/600/R-95/136 (1995)		**Sample Source:** Reference Toxicant	
Sample Date: 17 Feb-15	**Material:** Copper chloride		**Sample Station:** 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			
0	LC	3	10			
0	LC	4	33			
0	LC	5	4			
2.5		1	29	100	94	
2.5		2	20			
2.5		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			
10		3	11			
10		4	30			
10		5	9			
20		1	16	2 ↔ 100		
20		2	3			
20		3	23			
20		4	8			
20		5	21			
40		1	32	100	0	
40		2	35			
40		3	13			
40		4	14			
40		5	17			
80		1	19	100	0	
80		2	26			
80		3	27			
80		4	24			
80		5	6			

QC=AC

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 150217SpdJ

Test Species: *S. purpuratus*

Start Date/Time: 2/17/15 1512

End Date/Time: 2/20/15 1530

Concentration (___µg/L___)	Salinity (ppt)				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	33.0	33.0	33.0	15.0	15.3	14.6	14.5	8.9	8.0	7.7	8.3	8.09	8.00	8.04	8.08
2.5	33.5	33.2	33.2	33.4	14.8	15.0	14.4	14.3	8.5	8.1	7.9	8.4	8.07	7.94	8.06	8.10
5	33.5	33.2	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	33.1	33.1	33.3	14.8	15.0	14.4	14.3	8.4	8.2	7.9	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	33.0	33.1	15.0	15.1	14.4	14.3	8.4	8.1	7.9	8.5	8.10	7.97	8.06	8.10
80	33.3	32.9	32.9	33.0	14.8	15.1	14.4	14.3	8.4	8.1	7.9	8.5	8.12	7.97	8.06	8.10

Technician Initials:

	0	24	48	72
WQ Readings:	VCR	AG	EG	EG
Dilutions made by:	AC			

High conc. made (µg/L):	80
Vol. Cu stock added (mL):	4.6
Final Volume (mL):	500
Cu stock concentration (µg/L):	8,630

Comments:

0 hrs: _____

24 hrs: _____

48 hrs: _____

72 hrs: _____

QC Check: AC 2/24/15

Final Review: KB 3/16/15

Marine Chronic Bioassay | **Echinoderm Larval Development Worksheet**

Client: Internal
Sample ID: CuCl2
Test No.: 150217SpdV

Start Date/Time: 2/17/15 / 1512
End Date/Time: 2/20/15 / 1530
Species: S. purpuratus
Date Collected: 2/4/15

Tech initials: AC
Injection Time: 1430

Sperm Absorbance at 400 nm: 1.3 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 25, 19, 18, 22, 23

Mean: 21.4 X 50 = 1070 eggs/ml

(target counts of 20 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)

Initial density: 1070 eggs/ml = 1.07 dilution factor
Final density: 1000 eggs/ml
-1.0 part egg stock
parts seawater

egg stock 700 ml
seawater — ml

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%):

	Time	No. Fert.	No. Unfert.	%
5 minutes (1st fert.)	1447	96	4	96
10 minutes (2nd fert. If needed)	—	—	—	—

fertilization time: 1445

Test Initiation Time: (B) ~~1500~~ 1512 Embryo Stock Added: 0.25 ml

Test initiation must be within 1 hour of fertilization time.

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check 1[a]	96	4	96
QC check 2	—	—	—

Comments: [a] If the embryo development does not meet the mean test acceptability criterion of 80% normally 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. (Client test)

QC Check: AC 2/24/15

Final Review: KB 3/16/15

APPENDIX C

Lab Qualifier Codes

Nautilus Environmental

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 ≤ 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 below 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 above 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)