IONIC REQUIREMENTS OF BLUE CRAB, *CALLINECTES SAPIDUS,* IN ENVIRONMENTS CONTAINING LOW CONCENTRATIONS OF TOTAL DISSOLVED SOLIDS

by

Duane A. Friedman, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Aquatic Resources
December 2015

Committee Members:

Joseph R. Tomasso, Chair

Weston H. Nowlin

Benjamin Schwartz

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DEDICATION

This work is dedicated to my father, Joseph Friedman, and to the memory of my loving mother, Ruth Marie Friedman, and my aunt, Hilda Maurer.

ACKNOWLEDGEMENTS

I wish to thank Dr. Joseph R. Tomasso for his guidance and assistance. I would also like to thank Dr. Weston H. Nowlin and Dr. Benjamin Schwartz for their constructive criticisms throughout my graduate career. Furthermore I would like to thank Gabrielle Timmins, the Edwards Aquifer Research and Data Center, whose help was invaluable, and finally, my laboratory colleagues Justin Crow, Shawntel Lopez, Erica Molina, and Megan Cox.

Blue crabs were provided by the Gulf Coast Research Laboratory, in Ocean Springs, Mississippi. Wet lab facilities were provided by Texas State University. Funding was provided by the Mississippi Department of Marine Resources.

I express my sincere appreciation to my parents, Ruth M. and Joseph G. Friedman for their support, encouragement and patience throughout my professional and educational years. Finally, I thank my wife, Lisa, for her consistent help and understanding during my collegiate studies.

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ABSTRACT

The goal of this study was to develop an ionic environment containing 1 g/L total dissolved solids (TDS) that would support the survival and growth of juvenile blue crabs (designated a “mixed-ion solution”). The 1 g/L TDS level was selected in order to minimize the cost of preparing ponds and to potentially reduce the need for effluent permitting in inland areas. The general approach was to provide selected ions in approximate concentrations found in 1 g/L dilute seawater.

In a series of three-week exposures beginning with ~20 mm carapace-width crabs, we found:

1) Growth in artificial sea-salt treatments of 1, 2, 4, and 8 g/L TDS averaged 76% ± 20.2% (mean ± SD) and was not significantly affected by treatments. The number of molts, feed intake and modified feed conversion ratio were not significantly affected by treatments.
2) Growth during exposure to 1 g/L sea-salt, 0.5 g/L sea-salt + 0.5 g/L mixed-ions or 1 g/L mixed-ions averaged 41% ± 0.49% and was not significantly affected by treatment. Although not quantified, some exoskeletons in the mixed-ion treatment appeared soft and off-colored, leading us to investigate the need for environmental strontium in the next experiment.
3) Average survival during the 21-d exposure of 1 g/L mixed-ions with 0, 1 and 2 mg/L strontium was 89% ± 12.7% and did not differ significantly across treatments. Growth across treatments averaged 40% ± 25.3% and

was not significantly affected by treatment. In this experiment and in two failed experiments (not reported), environmental pH was considered lower than optimal for both molting (Perry et al., 2001; Cameron and Wood, 1985; Cameron, 1985) and nitrification by the bio-filters (Spotte, 1979). Consequently, sodium bicarbonate was substituted in the next experiment for approximately 17.2% of the sodium chloride in order to affectively buffer against pH changes.

4) All crabs in the 1 g/L mixed-ion environment (with 1 mg/L strontium) survived the 21-d exposure to temperatures of 26, 29, and 32^{o}C. Growth in all treatments averaged 71% ± 12.1% and was not significantly affected by treatments. Due to two previous, failed experiments, 133 mg/L of sodium bicarbonate was substituted for 133 mg/L of sodium chloride to maintain pH levels above 7.5.

These results indicate that blue crabs can survive and grow in 1 g/L mixed-ion solution. This work was supported with funding by the Mississippi Department of Marine Resources.

1. INTRODUCTION

The blue crab (*Callinectes sapidus*) supports a $138 million fishery along the Mid-Atlantic and Gulf of Mexico states (VanderKooy, 2015; McGaw, 2006) and plays a major role in the local food webs as both predator and prey (Zohar et al., 2008; Johnson et al., 2011). In 1993, 114.7 thousand metric tons in commercial landings (NOAA, 2015) were reported. Landings of blue crabs have trended downward to 60.5 thousand metric tons in 2013, (NOAA, 2015) along both the Atlantic and Gulf coasts (VanderKooy, 2015). With the decrease in wild stock, interest has increased in the possibility of pond culture of blue crabs. If pond aquaculture could be conducted inland, the cost of production would be less than that in expensive and environmentally sensitive coastal sites.

The blue crab is euryhaline and starts life in the waters near the mouth of a bay or estuary where the salinities are relatively high, 23 to 30 g/L (Cameron, 1985; Millikin and Williams, 1984; Turner et al., 2003), and subsequently moving into estuaries of less than 5 g/L (Zohar et al., 2008; Perry et al.,1982). Newly hatched eggs develop into zoea, which resembles a shrimp. In approximately six weeks, depending on salinity and temperature, the zoea undergoes seven molts, and then a metamorphic transition into the magalops stage. A recognizable miniature crab appears approximately two weeks later. Under favorable conditions, the time period to this stage is two months. A healthy crab lives approximately three years in the wild, completing about 20 molts during its lifespan (Cameron, 1985).

The goal of this study was to develop an ionic environment containing 1 g/L total dissolved solids (TDS) that would support the survival and growth of juvenile blue crabs (designated a “mixed-ion solution”). The 1 g/L TDS level was selected in order to minimize the cost of preparing ponds and to potentially reduce the need for effluent permitting in inland areas. The general approach was to provide selected ions in approximate concentrations found in 1 g/L dilute seawater. Specific objectives were:

1) To compare survival and growth of blue crabs in 1 g/L, 2 g/L, 4 g/L and 8 g/L artificial sea salt solutions,
2) To compare survival and growth of blue crabs in 1 g/L artificial sea salt, 1 g/L mixed-ion solution, and 0.5 g/L artificial sea salt + 0.5 g/L mixed ion-solution,
3) To determine the need for strontium in mixed-ion solutions to support survival and growth of blue crabs,
4) To determine the effect of temperature on survival and growth of blue crabs in mixed-ion solutions,
5) To determine the sensitivity of blue crabs to environmental nitrite in mixed-ion solutions.

2. METHODS AND MATERIALS

Blue crabs (~20 mm in carapace width) were produced in a hatchery at the Gulf Coast Research Laboratory (Ocean Springs, Mississippi, USA) and shipped by overnight courier service to Texas State University (San Marcos, Texas, USA). Crabs were held individually in 1-L plastic beakers. The beakers were perforated and suspended in a 1,500 liter recirculating system. Crabs were fed shrimp feed (Ziegler Hiper-Intensivo 35 shrimp feed, Ziegler Bros., Gardeners, Pennsylvania; 35% protein, 7% fat, 2% fiber, 10% moisture and 13% ash) to satiation once daily. Temperature (mean ± SD, 23 ± 0.6 C), dissolved oxygen (7.7 ± 0.34 mg/L), and total dissolved solids (12 ± 0.3 g/L) in the holding system were measured every three days. Total ammonia-N concentrations (0.2 ± 0.30 mg/L), nitrite-N concentrations (0.01 ± 0.007 mg/L), and pH (8.3 ± 0.15) were measured bi-weekly. Before being transferred to experimental aquaria, crabs were transferred and acclimated to 4-5 g/L salinity for 24-48 h.

Individual glass aquaria were placed in a water bath to maintain appropriate temperatures; crabs were randomly assigned. Each contained 30 L of aerated, deionized water with the treatment ions added. All aquaria were equipped with a bio-filter. Temperature and dissolved oxygen (DO), and TDS in the individual aquaria were measured daily. Ammonia, pH, and nitrite were monitored every three days. Concentrations of sodium, potassium, magnesium, calcium, sulfate, chloride, and strontium were determined on samples collected on day 14 of each experiment. Water quality for each experiment is presented in Tables 1-4.

Temperature, DO, and TDS were measured using a YSI 2030 (YSI, Inc., Yellow Springs, Ohio). Ammonia, pH, and nitrite were determined by Nesslerization (APHA, 1989), an Accumet 15 pH meter (Fisher Scientific, Pittsburg, Pennsylvania), and the USEPA (1979) diazotization method, respectively. Analysis of dissolved ions was performed with an ICS 1600 ion chromatograph (Dionex, Inc., Sunnyvale, California).

The study objectives were addressed sequentially. After experiments two and three, adjustments were made to the mixed-ion solution. During the course of the study, sodium chloride, sodium bicarbonate, sodium sulfate, potassium chloride, calcium chloride, magnesium chloride, and strontium nitrate (Fisher Scientific, Fair Lawn, New Jersey) were added to culture environments to attain desired ion concentrations. These adjustments are reflected in the water quality reported in Tables 1-4.

Carapace width was measured at the beginning and end of the 21-day experimental period. Crabs were fed to satiation twice a day. Feed consumed was recorded. Excess feed and detritus were removed prior to morning and evening feedings. Immediately following the three-week period, bio-filters were turned off and replaced with 1-inch air stones and 2 mg/L nitrite-N (as sodium nitrite) was added. Mortality was monitored for five days.

Statistical analyses of all results except mortality were conducted using ANOVA and Tukey's HSD test, when appropriate. A Fisher's Exact test was used to analyze mortality when less than 100%. All analyses were performed using R 3.01 (R Core Team, 2014). A $p \leq 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

All crabs survived the 21-d exposure to artificial sea salts (Table 1). Growth across all treatments averaged 76% ± 20.2% and was not significantly affected by treatments. The number of molts, feed intake and modified feed conversion ratio were not significantly affected by treatments. Shell hardness appeared normal. Based on these results, the remaining studies were conducted at 1 g/L TDS.

All crabs survived the 21-d exposure to 1 g/L sea salt, a combination of sea salt and mixed ions, or mixed ions (Table 2). Growth across all treatments averaged 41% ± 0.5% and was not significantly affected by treatment. The number of molts and feed intake were not significantly affected by treatment. However, the modified feed conversion ratio was. Post hoc comparison indicated that the 1 g/L sea salt treatment was significantly lower than the 1 g/L mixed ion treatment. However, the 0.5 g/L sea salt + 0.5 g/L mixed-ions treatment did not significantly differ from the other treatments. Although not quantified, some exoskeletons in the mixed-ion treatment appeared soft and off-colored, leading us to investigate the need for environmental strontium in the next experiment. Environmental strontium has been shown to be involved in the molting cycle (Cameron, 1985; Cameron and Wood, 1985; Neufeld and Cameron, 1992).

Average survival during the 21-d exposure to 1 g/L mixed ions with 0, 1, or 2 mg/L strontium was 89% ± 12.7% and did not vary significantly across treatments (Table 3). Growth across all treatments averaged 40% ± 25.3% and was not significantly affected by treatment. The number of molts, feed intake and modified feed conversion ratio were not significantly affected by treatments. Shells were still soft across all treatments. In this experiment and in two failed experiments (not reported), environmental pH was considered lower than optimal for both molting (Perry et al., 2001; Cameron and Wood, 1985; Cameron, 1985) and nitrification by the bio-filters (Spotte, 1979). Consequently, sodium bicarbonate was substituted in the next experiment for approximately 17.2% of the sodium chloride in order to affectively buffer against pH changes.

All crabs survived the 21-d exposure in a mixed-ion solution at 26, 29, and 32^{o}C (Table 4). Growth across all treatments averaged 71% ± 12.1% and was not significantly affected by treatments. The number of molts was not significantly affected by treatments; however, feed intake and modified feed conversion ratio were with crabs in warmer water requiring more feed to grow than crabs in cooler water. Post hoc comparisons indicated that all groups were significantly different from each other for both feed intake and modified feed conversion ratio. Shell hardness appeared normal.

Across the four experiments, survival of crabs exposed to nominal 2 mg/L nitrite-N in 1 g/L artificial sea salt and 1 g/L mixed-ion solution averaged 42% ± 25.8%. Pacific white shrimp (*Litopenaeus vannamei*) demonstrated a similar tolerance when exposed in 2 g/L sea salt (Sowers et al., 2004). The blue crabs also demonstrated an increase in tolerance to nitrite as salinities increased (Table 1), as has been reported for many species (Tomasso, 2012). Survival among experiments was variable. The basis for the variability is not apparent, although it could be related to variability in the actual nitrite concentrations, variability in other water quality characteristics (Tables 1-4), or differences in the timing of the molting cycle. Regardless of the variability, these results indicate that blue crabs are sensitive to environmental nitrite, and it should be a consideration during the culture of blue crabs in water containing low concentrations of dissolved solids.

The results reported here suggest that the culture of blue crabs in water containing a 1 g/L mixed-ion solution (Table 5) is feasible. Field tests are the next step. In particular, stocking densities, size of crabs at stocking, rates of acclimation to culture solutions, and harvesting techniques need to be investigated.

Table 1. Results (counts or means ± SD) and water quality for Experiment 1 (three week exposure to 1, 2, 4, or 8 g/L sea salt). Ion values are presented as percent nominal (the percent of the specific ion found in 1 g/L dilute seawater).

	1 ppt	2 ppt	4 ppt	8 ppt	*P*
# animals beginning exp.	9	9	9	9	
% survival	100	100	100	100	
Initial width (mm)	26.3 ± 4.47	27.3 ± 4.61	27.8 ± 4.09	26.3 ± 4.80	0.871
Final width (mm)	48.9 ± 7.41	50.6 ± 6.25	54.3 ± 11.45	45.6 ± 4.50	0.140
% growth	89 ± 33.6	88 ± 26.7	95 ± 30.5	76 ± 20.2	0.531
Number of Molts	2.1 ± 0.60	2.1 ± 0.60	2.3 ± 0.50	1.9 ± 0.33	0.366
Feed intake (mg wet weight)	8,153 ± 2,097.0	9,621 ± 2,588.7	8,334 ± 2,538.7	7,741 ± 2,569.5	0.414
Modified Feed Conversion Ratio (mg feed : mm growth)	391 ±161.3	450 ± 208.1	321 ± 48.0	430 ± 225.9	0.427
Temperature ^{o}C	28.1 ± 0.43	28.2 ± 0.37	28.1 ± 0.40	28.2 ± 0.38	0.902
Total dissolved solids (g/L)	1.0 ± 0.02	2.0 ± 0.03	4.0 ± 0.05	7.9 ± 0.11	
Dissolved oxygen (mg/L)	6.3 ± 0.02	6.2 ± 0.29	6.2 ± 0.28	6.2 ± 0.25	
Total ammonia-N (mg/L)	0.07 ± 0.069	0.05 ± 0.036	0.07 ± 0.053	0.09 ± 0.069	
Nitrite-N (mg/L)	0.07 ± 0.029	0.10 ± 0.024	0.20 ± 0.097	0.19 ± 0.054	
pH	7.7 ± 0.04	7.7 ± 0.06	7.6 ± 0.13	7.7 ± 0.06	
Sodium (% nominal)	116 ± 4.8	109 ± 4.0	101 ± 1.3	100 ± 1.6	
Calcium (% nominal)	86 ± 16.0	107 ± 46.7	145 ± 8.2	137 ± 3.9	
Magnesium (% nominal)	94 ± 3.5	94 ± 3.4	89 ± 1.9	91 ± 1.8	
Potassium (% nominal)	158 ± 8.7	141 ± 7.9	119 ± 1.9	123 ± 4.3	
Chloride (% nominal)	95 ± 2.6	95 ± 1.8	88 ± 2.3	82 ± 1.1	
Sulfate (% nominal)	110 ± 11.4	102 ± 4.7	97 ± 5.3	100 ± 3.6	
5 Day Nitrite Exposure					
% surviving 2 mg/L nitrite-N for 5 days	78	100	100	100	
Nitrite-N (mg/L)	2.3 ± 0.14	2.2 ± 0.17	2.3 ± 0.06	2.4 ± 0.21	0.241
Total ammonia-N (mg/L)	0.4 ± 0.24	0.4 ± 0.19	0.3 ± 0.22	0.3 ± 0.18	0.226
pH	8.0 ± 0.16	7.7 ± 0.22	7.6 ± 0.30	7.8 ± 0.15	0.014

Table 2. Results (counts or means ± SD) and water quality for Experiment 2 (three week exposure to 1 g/L sea salt, 0.5 g/L sea salt + 0.5 g/l mixed ions or 1 g/L mixed ions). Ion values are presented as percent nominal (the percent of the specific ion found in 1 g/L dilute seawater).

	1 g/L Sea Salt	0.5 g/L Sea-Salt + 0.5 g/L Mixed Ions	1.0 g/L Mixed Ions	*P*
# animals beginning exp.	10	13	13	
% survival	100	100	100	
Initial width (mm)	23.9 ± 1.45	24.2 ± 2.30	24.1 ± 1.98	0.954
Final width (mm)	35.4 ± 5.76	34.9 ± 5.27	31.5 ± 3.15	0.113
% Growth	49 ± 28.7	44 ± 19.0	32 ± 19.0	0.158
Number of Molts	1.1 ± 0.57	1.4 ± 0.51	1.1 ± 0.49	0.268
Feed intake (mg wet weight)	2,842 ± 278.7	2,581 ± 428.5	2,462 ± 438.5	0.086
Modified Feed Conversion Ratio (mg feed / mm growth)	246 ± 149.9	274 ± 95.5	407 ± 179.1	0.023
Temperature ^{o}C	26.0 ± 0.22	26.0 ± 0.24	26.1 ± 0.17	
Total dissolved solids (g/L)	0.9 ± 0.04	0.9 ± 0.01	0.9 ± 0.01	
Dissolved oxygen (mg/L)	7.0 ± 0.22	7.0 ± 0.16	7.1 ± 0.17	
Total ammonia-N (mg/L)	1.0 ± 0.28	1.2 ± 0.57	1.2 ± 0.48	
Nitrite-N (mg/L)	0.007 ± 0.004	0.007 ± 0.002	0.009 ± 0.004	
pH	6.9 ± 0.18	6.9 ± 0.17	6.7 ± 0.27	
Sodium (% nominal)	88 ± 5.6	84 ± 1.8	81 ± 3.1	
Calcium (% nominal)	116 ± 14.6	128 ± 22.0	148 ± 35.1	
Magnesium (% nominal)	85 ± 3.8	95 ± 2.0	103 ± 1.0	
Potassium (% nominal)	122 ± 9.5	132 ± 5.4	138 ± 8.8	
Chloride (% nominal)	98 ± 6.7	98 ± 1.7	96 ± 1.1	
Sulfate (% nominal)	94 ± 7.0	91 ± 1.8	110 ± 3.1	
5 Day Nitrite Exposure				
% surviving 2 mg/L nitrite-N for 5 days	60	69	85	
Nitrite-N (mg/L)	2.0 ± 0.06	2.0 ± 0.27	2.0 ± 0.18	0.227
Total ammonia-N (mg/L)	2.6 ± 0.28	2.4 ± 0.79	2.6 ± 0.39	0.622
pH	7.2 ± 0.15	7.4 ± 0.84	7.3 ± 0.54	0.868

Table 3. Results (counts or means ± SD) and water quality for Experiment 3 (three week exposure to 1 g/L mixed ions, 1 g/L mixed ions + 1 mg/L strontium, or 1 g/L mixed ions + 2 mg/L strontium). Ion values are presented as percent nominal (the percent of the specific ion found in 1 g/L dilute seawater).

	0.0 mg/L Sr	1.0 mg/L Sr	2.0 mg/L Sr	*P*
# animals beginning exp.	12	11	12	
% survival	100	91	75	
Initial width (mm)	28.7 ± 2.81	29.36 ± 3.14	29.83 ± 4.39	0.718
Final width (mm)	40.1 ± 7.15	41.4 ± 6.31	40.2 ± 5.98	0.872
% growth	41 ± 26.9	43 ± 29.0	36.0 ± 21.7	0.810
Number of Molts	1.3 ± 0.65	1.3 ± 0.79	1.1 ± 0.51	0.628
Feed intake (mg wet weight)	4,440 ± 578.6	4,631 ± 1367.2	4,049 ± 1981.4	0.612
Modified Feed Conversion Ratio (mg feed : mm growth)	456 ± 382.4	312 ± 190.7	388 ± 239.6	0.492
Temperature ^{o}C	26.7 ± 0.11	26.7 ± 0.13	26.7 ± 0.15	
Total dissolved solids (g/L)	0.9 ± 0.11	0.9 ± 0.01	0.9 ± 0.01	
Dissolved oxygen (mg/L)	6.7 ± 0.17	6.7 ± 0.22	6.7 ± 0.33	
Total ammonia N (mg/L)	2.3 ± 0.84	2.3 ± 0.32	2.0 ± 0.55	
Nitrite-N (mg/L)	0.014 ± 0.026	0.014 ± 0.011	0.039 ± 0.062	
pH	7.0 ± 0.12	7.0 ± 0.19	7.1 ± 0.42	
Sodium (% nominal)	88 ± 1.7	84 ± 3.3	84 ± 2.9	
Calcium (% nominal)	123 ± 10.7	92 ± 9.3	100 ± 20.5	
Magnesium (% nominal)	101 ± 2.3	96 ± 3.7	100 ± 8.6	
Potassium (% nominal)	133 ± 9.0	112 ± 9.8	126 ± 25.5	
Chloride (% nominal)	96 ± 1.6	96 ± 1.3	96 ± 1.0	
Sulfate (% nominal)	95 ± 1.6	96 ± 1.8	96 ± 1.3	
	5 Day Nitrite Exposure			
% surviving 2 mg/L nitrite-N for 5 days	33	20	44	
Nitrite-N (mg/L)	2.5 ± 0.83	2.5 ± 0.94	2.2 ± 0.96	0.706
Total ammonia-N (mg/L)	2.5 ± 0.61	2.8 ± 0.61	3.3 ± 1.05	0.062
pH	7.6 ± 0.47	7.5 ± 0.38	7.5 ± 0.47	0.809

Table 4. Results (counts or means ± SD) and water quality for Experiment 4 (three week exposure to 1 g/L mixed ions + 1 mg/L strontium at 26, 29 or 32 ^{o}C). Ion values are presented as percent nominal (the percent of the specific ion found in 1 g/L dilute seawater).

	26 Degrees	29 Degrees	32 Degrees	*P*
# animals beginning exp.	12	12	12	
% survival	100	100	100	
Initial width (mm)	29.3 ± 2.05	31.1 ± 2.61	30.3 ± 3.14	0.246
Final width (mm)	51.4 ± 3.36	53.2 ± 4.82	50.3 ± 7.57	0.434
% growth	76 ± 7.7	71 ± 12.3	65 ± 14.0	0.091
Number of Molts	2.0 ± 0.00	2.0 ± 0.00	1.9 ± 0.29	0.379
Feed intake (mg wet weight)	6,821 ± 1,903.9	11,184 ± 1,412.1	14,330 ± 2,557.9	<0.001
Modified Feed Conversion Ratio (mg feed / mm growth)	309 ± 78.2	520 ± 120.6	759 ± 191.3	<0.001
Temperature ^{o}C	26.3 ± 0.10	29.0 ± 0.18	31.9 ± 0.08	
Total dissolved solids (g/L)	0.9 ± 0.02	0.9 ± 0.01	0.9 ± 0.01	
Dissolved oxygen (mg/L)	7.1 ± 0.11	6.2 ± 0.17	5.7 ± 0.14	
Total ammonia-N (mg/L)	0.2 ± 0.04	0.1 ± 0.09	0.1 ± 0.08	
Nitrite-N (mg/L)	0.009 ± 0.003	0.019 ± 0.007	0.032 ± 0.008	
pH	7.9 ± 0.04	7.8 ± 0.05	7.9 ± 0.06	
Sodium (% nominal)	82 ± 2.5	81 ± 1.5	81 ± 2.7	
Calcium (% nominal)	86 ± 14.7	82 ± 11.6	76 ± 13.0	
Magnesium (% nominal)	105 ± 2.8	104 ± 2.1	109 ± 2.5	
Potassium (% nominal)	152 ± 4.6	157 ± 2.8	154 ± 4.5	
Chloride (% nominal)	89 ± 1.5	90 ± 1.8	90 ± 1.9	
Sulfate (% nominal)	111 ± 2.3	115 ± 3.1	110 ± 9.7	
		5 Day Nitrite Exposure		
% surviving 2 mg/L nitrite-N for 5 days	17	8	42	
Nitrite-N (mg/L)	2.2 ± 0.31	2.3 ± 0.16	2.5 ± 0.24	0.009
Total ammonia-N (mg/L)	0.6 ± 0.35	0.8 ± 0.33	1.0 ± 0.51	0.071
pH	8.0 ± 0.12	7.9 ± 0.08	7.9 ± 0.14	0.070

Table 5. Compounds and grams use to create the 1 g/L mixed-ion solution used in experiment 4.

Compound	Formula	Grams
Sodium Chloride	$NaCl$	0.445 g
Sodium Sulfate Anhydrous	Na_2SO_4	0.120 g
Potassium Chloride	KCl	0.022 g
Calcium Chloride Di-hydrate	$CaCl_2 \cdot 2H_2O$	0.045 g
Magnesium Chloride Hexahydrate	$MgCl_2 \cdot 6H_2O$	0.349 g
Sodium Bicarbonate	$NaHCO_3$	0.133 g
Strontium Nitrate Anhydrous	$Sr(NO_3)_3$	0.002 g

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