Technical Guidance Document

for Conducting the

Florida Department of Environmental Protection's

Protocols for Determining Major-Seawater-Ion Imbalance Toxicity (MSIIT)

in

Membrane-Technology Water-Treatment Concentrate

Florida Department of Environmental Protection
Bureau of Laboratories
May 4, 2004

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Glossary of terms

Anti-scalant — chemical added to the concentrate at the plant to reduce the build-up of minerals (scaling) in the plant's plumbing prior to being discharged.

Concentrate — facility concentrate ('reject water'). Water containing concentrated amounts of dissolved constituents and resulting from the membrane water-purification process. Concentrate is generally discharged as a waste. For the purposes of these protocols, the concentrate is defined as that which is discharged following any post-treatment through an outfall to waters of the State and is collected at the wastewater permit compliance point.

Controlling ion — the ion to which all other ions are balanced. The controlling ion is typically the ion most out of proportion with natural seawater ion concentrations, with the exception of the carbonate ion. Carbonate does not appear to play a significant role in seawater-ion-imbalance toxicity, so even though it may be the most out of proportion, the next out of proportion ion would be the controlling ion.

Hyper-saline brine — high salinity natural seawater produced by freezing natural seawater in a - 40°C freezer for 24 hours and pouring off the unfrozen concentrated saltwater.

Ion-adjusted concentrate — concentrate adjusted with ACS-grade salts so that the proportions of major seawater ions (Table 1) match that of seawater as closely as possible without individual ions exceeding full seawater (35 part per thousand) concentrations. With some concentrates, the ion-adjusted concentrate may consist of concentrate with the major ions adjusted to match seawater proportions and then diluted to full-seawater concentrations. In other tests with these concentrates, some of the major seawater ions may remain in excess of the full seawater concentrations.

Major seawater ions—those ions making up greater than 1% of the total molar weight of the ionic constituents of standard seawater (*mer de normal*). These ions are listed in Table 1. Bromide is included in the list even though it constitutes less than 1% because its presence is required for survival of the test organism, the mysid shrimp, (*Americamysis bahia*, formerly *Mysidopsis bahia*).

Major-seawater-ion toxicity — toxicity resulting solely from a surplus or deficit of one or more of the major seawater ions listed in Table 1, or from the relative proportions of those ions.

Mock concentrate — synthesized concentrate created using ACS-grade salts but containing only the major seawater ions (Table 1) in the same concentrations as the facility concentrate.

Mock ion-adjusted concentrate — synthesized concentrate created using ACS-grade salts but containing only the major seawater ions (Table 1) in the same concentrations as the ion-adjusted concentrate. Created by adding to mock concentrate the same salts used to create the ion-adjusted concentrate.

Mock-sea-salt slurry — salt slurry (i.e., enough de-ionized water added to permit good mixing of component salts) created using ACS-grade salts such that, once fully dissolved, the slurry will provide the major seawater ions (Table 1) in the same proportions as seawater.

Mock seawater — synthesized seawater created using ACS-grade salts and de-ionized water but containing only the major seawater ions (Table 1) in the same concentrations as seawater.

Natural seawater (NSW) — high-quality, filtered seawater from an uncontaminated source.

Raw water — the untreated source water used in the membrane water-purification process.

Introduction

This document expands on the 1995 FDEP guidance document entitled, "Protocols for Determining Major-Seawater-Ion Toxicity in Membrane-Technology Water-Treatment Concentrate" (FDEP, 1995). The purpose of that paper was to document the development of a set of protocols for determining major-seawater-ion toxicity in membrane-technology water-treatment concentrate, or reject water. This document contains several revisions to the 1995 protocols and is meant to provide more explicit and up to date technical guidance on how to perform the major-seawater-ion toxicity testing protocols. These protocols are used to determine if the toxicity observed in a concentrate is due solely to the imbalance of the major-seawater ions, due to a combination of major-seawater-ion imbalance and some other constituent(s) of the concentrate, or due solely to some other constituent(s) of the concentrate. The protocol is not designed to identify the "other" constituent(s), only to determine if there is a source of toxicity other than an imbalance in the major-seawater ions. It is important to note that the protocols were originally developed under the guidance of a Technical Advisory Committee (TAC) composed of representatives from the membrane-technology industry, the State of Florida, and federal government.

Freshwater vs. Saltwater Test Organisms

These protocols are for determining major-seawater-ion imbalance to saltwater organisms. There may be facilities with concentrates that have elevated conductivity for which it would be more appropriate to test with freshwater test organisms. The Gas Research Institute funded the development of the Salinity Toxicity Relationship (STR) Model that is capable of accurately predicting the toxicity of mixtures of common seawater ions to freshwater organisms (GRI-FW SRT^{TM}, Version 1.02). One should be aware that the STR model does not predict the toxic effects of ions that are present in deficit; it only predicts the toxicity of ions that are present in surplus.

More recently, a second model has been developed that predicts the toxicity of mixtures of common seawater ions to saltwater organisms (GRI-MSTR^{TM}, Version 1.0). These models can be used as screening tools to help the discharger determine if major-seawater-ion imbalance is a potential cause of toxicity in the facility's effluent.

For the purposes of these protocols, major seawater ions to be considered are those that constitute greater than one percent of the total molar weight of the ionic constituents of standard seawater (oceanographic *mer de normal*). In addition, carbonate is included because of its strong effect on pH and the overall chemistry of the solution. The trace seawater ion bromide is also to be included because it is required, in combination with the listed major seawater ions, to form the minimal 'artificial seawater' in which the test organisms could live. These major seawater ions are presented in Table 1. Fluoride was not included as a major-seawater ion since it makes up less than one percent of the total molar weight of the ionic constituents of standard seawater, but it is frequently elevated in groundwater and drinking water facility concentrates. The State of Florida has a

surface water quality criterion for fluoride, so it is treated as an "other" toxicant in the interpretation of these protocols.

Table 1. Major Constituents of 35 part per thousand (ppt) Seawater.

	percent	g/kg	millimoles/kg	millimolar
Na^+	30.68	10.78	469.0	480.0
K^+	1.14	0.399	10.21	10.45
Mg^{++}	3.65	1.284	52.82	54.05
Ca^{++}	1.19	0.4185	10.44	10.68
Cl^-	55.07	19.353	545.87	558.62
$SO_4^=$	7.72	2.712	28.24	28.90
$*HCO_3^-$	0.36	0.126	2.07	2.12
$*Br^-$	0.19	0.0673	0.842	0.862
Total	100.00	35.1398	1,119.492	1,145.682

- The inclusion of bromide, although forming <1% of the ions, is essential for the survival of the mysid shrimp used in the tests. Carbonate is included because of it's strong effect on pH and the overall chemistry of the resulting solutions.

Minimum and Maximum Test Salinities

In order to test "low salinity" concentrates, the salinity of the concentrate must be raised to some minimum salinity. That minimum salinity is based upon the salinity tolerance of the tests species, the mysid shrimp, *Americamysis bahia*, and the ability of the investigators to obtain reliable and reproducible results. Testing conducted by the FDEP Bureau of Laboratories (FDEP, 1997) determined that our laboratory was unable to repeatedly produce acceptable survival at salinities below 6.0 parts per thousand (ppt) (See Table 3, Appendix 1).

The FDEP Bureau of Laboratories conducted a second study in 2002-2003 to determine if the lower test salinity was affecting the sensitivity of the mysids to other stressors (Report in draft). Standard reference toxicant tests with sodium dodecyl sulfate (SDS) or pentachlorophenol (PCP) and mysid shrimp determined that mysids acclimated to, and tested at, a salinity of 7.0 ppt were not more sensitive to SDS or PCP than were mysids tested at the culture salinity of 20.0 ppt (See Tables 4 and 5, Appendix 1). The reference toxicants SDS and PCP were selected because their modes of action (SDS occludes the respiratory membranes, while PCP is a non-specific stressor, affecting enzyme function and cellular respiration) were not believed by FDEP researchers to be strongly influenced by salinity. Other common reference toxicants, especially heavy metal salts (e.g., $CdCl_2$), are more strongly influence by salinity (Roast, 2001). Using a reference toxicant whose toxicity is salinity dependant would have confounded the interpretation of the stress caused by acclimating the mysids to a lower test salinity.

The maximum salinity at which the mysids are tested in these protocols is 35 ppt. A study that examined the effects of salinity on saltwater test organisms that had not been acclimated to the test salinity from their culture salinity prior to testing, reported a high salinity 48-hour LC_{50} for *Americamysis bahia* of approximately 45 ppt (Pillard, 1999). Price noted in his taxonomic key to shallow water *Mysidacea* of the Texas coast, *A. bahia* (formerly *Mysidopsis bahia)* abundance was greatest at 30 ppt (Price, 1982). The FDEP laboratory observed no control mortality problems in more than 36 tests conducted with mysids at 35 ppt during the 1995 MSIIT protocol development study.

Methods

The protocols consist of seven toxicity tests with supporting analytical chemistry. In some instances, only five of the seven tests will need to be performed. This will be discussed in more detail during the individual test descriptions.

Table 2. Major-Seawater-Ion-Imbalance Toxicity Tests

Test Number	Test Name	Test Description
1	Initial Toxicity Test	Unaltered or salinity-adjusted concentrate. Natural seawater (NSW) or artificial seawater (ASW) diluted to the same salinity as the concentrate used as the diluent and control water.
2	Baseline Toxicity Test	Same as Test 1, but performed once the analytical chemistry for the concentrate is available.
3	Mock Concentrate Test	Test solution made using reagent-grade chemicals to match the ionic composition of the facility concentrate. Includes anti-scalants or other chemicals added to concentrate at the facility. NSW or ASW diluted to the same salinity as the concentrate used as the diluent and control water.
4	Ion-adjusted Concentrate All ions balanced.	Concentrate balanced to seawater proportions using reagent-grade chemicals. NSW or ASW diluted to the ion-adjusted concentrate salinity used as the used as diluent and control water.
4a	Ion-adjusted Concentrate (ions in excess of 35 ppt NSW proportions left out of balance).	Concentrate balanced except for ions in excess of 35 ppt NSW proportions. 35 ppt NSW or ASW diluent and control water.

Table 2 cont.

Test Number	Test Name	Test Description
4b	Ion-adjusted Concentrate (all ions balanced to NSW proportions, then diluted with de-ionized water back down to 35 ppt).	Test solution is facility concentrate balanced to NSW proportions using reagent-grade chemicals. Ion-adjusted concentrate then diluted with de-ionized water back down to 35 ppt. 35 ppt NSW or ASW used as the test diluent.
5	Ion-adjusted Concentrate	Same as Test 4, but diluent is ion-adjusted mock concentrate rather than NSW or ASW.
5a	Ion-adjusted Concentrate (ions in excess of 35 ppt NSW proportions left out of balance).	Same as Test 4a, but diluent is mock concentrate ion adjusted the same as the concentrate.
5b	Ion-adjusted Concentrate (all ions balanced to NSW proportions, then diluted with de-ionized water back down to 35 ppt).	Same as Test 4b, but diluent is mock concentrate ion adjusted the same as the concentrate.

It is relatively easy to determine if the toxicity in a concentrate is due, in part, to an ionic imbalance of the major-seawater ions. It is much more difficult to exclude other, possibly unknown, contaminants as potential sources of toxicity. It is the need to determine if the concentrate contains sources of toxicity other than an imbalance in the seawater-ion concentrations that make this protocol so lengthy. **Attempts to omit any of the toxicity tests or chemical analyses outline in this document beyond what is recommended in Tests 4 and 5 would severely compromise the ability of the researcher to make a conclusive statement about seawater-ion imbalance being the sole source of toxicity in the sample.**

Advanced Test Preparation

Prior to the sample arriving in the laboratory, several preparatory steps must be made in advance. SOP #6 outlines the steps to prepare a "mock-sea-salt slurry" (All referenced SOP's can be found in Appendix 2 or on the web at: http://www.floridadep.org/labs/cgi-bin/sop/biosop.asp). The mock-sea-salt slurry is used to adjust the salinity of a concentrate that has an apparent salinity (based on conductivity and temperature) of greater than 1.0 ppt but less than 7.0 ppt, up to the minimum test salinity. If the concentrate being tested has an apparent salinity greater than or equal to 7.0 ppt, a mock-sea-salt slurry is not needed.

SOP #8 outlines the salinity acclimation steps that must be taken if any of the test solution salinities will be less than the 20 ppt mysid culture salinity. A study performed by the FDEP Biology Section noted that mysids that were not salinity acclimated to the low test salinities prior to test initiation would experience unacceptable levels of mortality due to salinity shock (FDEP, 1997). The United States Environmental Protection Agency (U.S. EPA) recommends salinity adjustments not exceed 3.0 ppt per 12-hour period (EPA/600/4-90/027), but this recommendation appears to be extremely conservative. The 1997 FDEP study found that acceptable survival (less than 10% mortality over the 96-hr period following salinity adjustment) was possible with a more rapid acclimation period that allowed mysids to be brought down to as low as 6.0 ppt within an 8-hour period. The mysids were first adjusted from 20 ppt to 15 ppt using de-ionized water. After two hours, the salinity was adjusted to 12 ppt. The salinity was then adjusted down 2 ppt with de-ionized water every two hours until the desired test salinity was reached.

It has been the FDEP Biology Section's observation that salinity adjusting mysids up to 35 ppt does not appear to stress the mysids in a similar fashion to lowering the salinity below 20 ppt, and salinity acclimation periods can be much shorter for acclimating mysids to test concentrations between 20 and 35 ppt.

Several of the test protocols (Tests 4 & 5) could potentially result in test salinities as high as 35 ppt. Hyper-saline brine made from natural seawater is also made in advance by freezing natural seawater in a -40°C freezer for 24 hours and pouring off the unfrozen concentrated seawater. The hyper-saline brine is then used to salinity adjust the natural seawater up to 35 ppt if it is not already at that salinity.

The FDEP Biology Section also found it useful to pre-label the test vessels and measurement vessels prior to Day 1 to reduce the amount of work that would need to be performed on the actual test days. Day 0 is the day the sample arrives and Test 1 is performed. Day 1 is the day the analytical chemistry results are available and Tests 2 though Test 5 are performed.

Test 1—Initial test on unaltered concentrate (SOP #1)

This test is a normal bio-monitoring test, meeting the 36-hr holding time required by EPA methods (U.S. EPA, 2002); however, natural seawater (NSW) diluted to the concentrate's apparent salinity (based upon conductivity and temperature) is used as the control water and diluent. For concentrates with salinities greater than 1.0 ppt but less than 7.0 ppt, the test sample should be salinity adjusted up to 7.0 ppt using the mock-sea-salt slurry. The diluent and control should be 7.0 ppt NSW. If salinity adjusting of the concentrate is necessary, a second control should be added to the test design that consists of NSW diluted to the original concentrate salinity and brought up to 7 ppt using the mock-sea-salt slurry to parallel the sample salinity adjustment. All tests (Test 1 through Test 5) are performed using the mysid shrimp, *Americamysis bahia*. Testing conducted during the 1995 study found that the vertebrate test species, the inland silverside, *Menidia beryllina,* was less sensitive to seawater-ion imbalance than the mysid shrimp for all of the concentrates tested. For this reason, the *Menidia* were dropped from the protocol.

If NSW is not available, a high quality artificial sea salt product can be used, but chemical characterization of the product should be performed to confirm that the major-seawater ions are in seawater proportion and that metals, such as aluminum and iron, are not elevated from their typical seawater concentration. Metals can be elevated in some artificial saltwater mixes due to impurities or the use of aluminum or iron salts. The artificial seawater used in the tests should also be aged for a minimum of 24 hours prior to use, as freshly mixed artificial seawater can be toxic due to a higher initial concentration of free metal ions. During the MSIIT protocol development, the FDEP laboratory did not observe any metal toxicity problems associated with the small salinity adjustments to the concentrate using the aged mock-sea-salt slurry.

If the concentrate in Test 1 is not toxic, the rest of the tests in this suite should be aborted. Initial mortality due to an imbalance in seawater ions typically occurs within the first 24 hours. If no mortality was observed by 24 hours, the rest of the tests should be delayed until 48 hours. If at 48 hours there is not greater than 15% mortality in the 100% sample, the rest of the tests in the suite should be cancelled. This might occur if sufficient variability in the concentrate exists that not all samples collected are toxic. If, for example, a facility uses multiple wells for source water at different times and the wells produce different quality waters, the toxicity associated with that facility's concentrate might fluctuate. For this reason, facilities are strongly encouraged to collect concentrate for use in the MSIIT testing under the same conditions that produced the failed bioassays during their routine monitoring.

Test 2—Baseline test on unaltered concentrate (SOP #2)

Test 2 is similar to Test 1, but it is performed at the same time as the tests of ion-manipulated concentrate. If the concentrate salinity is greater than 1.0 ppt but less than 7.0 ppt, the concentrate should be salinity adjusted up to 7.0 ppt using the mock-sea-salt slurry as in Test 1. If salinity adjusting of the concentrate is necessary, a second control should be added to the test design that consists of NSW diluted to the original concentrate salinity and brought up to 7 ppt using the mock-sea-salt slurry to parallel the sample salinity adjustment. Tests 2 through 5 only require four 0.5 dilutions plus the control(s) [100%, 50%, 25%, 12.5%, and control(s)]. Tests conducted during the 1995 study failed to show toxicity in any of the samples at the 12.5% concentration and below, so the lowest test concentration was dropped from the protocol. The diluent is the same as used for Test 1. The time lag between Test 1 and the remaining tests is caused by the necessity of awaiting the results of chemical analyses of the concentrate before performing any ion manipulations and the time required to perform the manipulations themselves. During the 1995 study, the time between Test 1 and the remaining tests was typically 24 hours. To perform the test protocol properly requires excellent coordination and communication between the bioassay and analytical chemistry laboratories. If the analytical chemistry is not performed in-house, special arrangements will probably need to be made to expedite processing the test solution ion chemistries. Rapid turnaround time is essential to rule out the potential loss of toxicity due to the volatilization or breakdown of toxic constituents in the sample.

Comparing the results from Test 1 to Test 2 indicates changes in toxicity with time, and Test 2 provides the measure of toxicity against which the results of the remaining tests are compared. If the concentrate in Test 2 is not toxic, the cause of toxicity in Test 1 was a degradable toxicant that had been rendered non-toxic during the period between setting up Test 1 and setting up Test 2. This result would indicate that the cause of toxicity in Test 1 was not major-seawater-ion toxicity.

Test 3—Mock concentrate test (SOP #3)

Test 3 uses a synthetic concentrate created by mixing ACS-grade chemical salts in de-ionized water. NSW diluted to the concentrate's salinity is used as the control and diluent. If the concentrate salinity is greater than 1.0 ppt but less than 7.0 ppt, the mock concentrate will also need to be salinity adjusted up to 7.0 ppt using mock sea salt-slurry as in Test 1 and Test 2.

It is important that all products added to the concentrate at the facility are included in the mock concentrate at the same concentration (e,g., anti-scalant, acid). Not including these products can make it difficult or impossible to achieve the concentrations of the ions in the mock concentrate that occur in the facility concentrate due to the failure of some of the salts to go into solution or the formation of precipitates. Most of the "new generation" anti-scalants are believed to exhibit fairly low toxicity. If no toxicity data exists for the anti-scalant being used, a stand-alone toxicity test with the anti-scalant and mysid shrimp should be performed prior to the ion-imbalance testing to determine if the concentration of anti-scalant in the concentrate could be contributing to the concentrate toxicity.

The order of the steps in SOP #3 for preparing the mock concentrate is important to produce the best quality mock concentrate. Not following the order could result in the loss of carbonate to the atmosphere as CO_2 or the failure of some ions to go, or stay, in solution. Due to the limited amount of time available to produce the mock concentrate, it is still possible that some compounds will not go into solution completely or that a precipitate could form. The steps outlined in the SOP are designed to minimize the occurrence of either of these potentially confounding factors. A sample of the mock concentrate needs to be submitted for chemical analyses to determine how accurately the mock concentrate matches the facility concentrate. Given the sources of error in both the measurement of the compounds to produce the mock and in the analytical chemistry, the ions in the mock concentrate should match the concentrate ions to within $\pm$ 20%, with the exception of carbonate. Carbonate concentrations in the mocks produced during the protocol development were frequently greater than 20% different from the facility concentrates. Since carbonate does not appear to play an important role in the toxicity of seawater-ion imbalance, this discrepancy is not considered to be critical to the interpretation of the test results.

Test 4—Ion-adjusted concentrate (SOP #4)

Test 4 uses a concentrate sample to which ACS-grade chemical salts have been added to balance the major seawater ions to the proportions present in seawater. This balancing is controlled by the concentrate ion that is in the highest proportion relative to seawater,

otherwise known as the controlling ion. Carbonate will frequently be the ion most out of proportion; however, carbonate is not used as the controlling ion because it does not appear to play an important role in the toxicity of concentrates. If carbonate is the ion most out of proportion, the next most out of proportion ion should be used as the controlling ion. The controlling ion remains unchanged and the remaining ions (with the exception of carbonate) are adjusted to achieve balance. NSW diluted to the ion-adjusted-concentrate salinity is used as the control and diluent.

If the solution in Test 3 was toxic and that in Test 4 was not toxic, one would nominally conclude that the toxicity seen in the concentrate was a result of the major seawater ions being out of proportion. One would verify this by reviewing the analytical chemistry results of the test solutions to assure that the proportions of the ions in the test solutions were on target.

If the solution in Test 3 was not toxic and that in Test 4 was toxic, one would nominally conclude that the toxicity seen in the concentrate was a result of some unidentified toxicant(s), not from the major seawater ions.

If the solution in Test 3 was toxic and that in Test 4 was toxic (but both possibly less toxic than Test 2), one would nominally conclude that the toxicity seen in the concentrate was a result of both the major seawater ions being out of proportion and the presence of some 'other' toxicant(s).

One wouldn't expect to see results where neither Test 3 nor Test 4 was toxic. If these results were found, the implication would be that the toxicity was not caused by major seawater ion imbalance and the toxicant was rendered non-toxic by the steps used in adding the ions needed to balance the seawater ions. This might be through reactions with the added ions themselves or through effects on the toxicants of the sample manipulations during the ion additions.

The problem with this testing is in creating the ion-adjusted concentrate for Test 4. Figure 1 shows two concentrates tested during the protocol development that have relatively low "salinities". The 4 ppt concentrate does not have any ions in excess of what would be found in 35 ppt seawater, while the 3 ppt concentrate has the same amount of sulfate as 35 ppt seawater and more than twice the carbonate and calcium ion as 35 ppt seawater. Figure 2 shows how the ion concentrations in the concentrates compare to 35 ppt seawater. Since the 4 ppt concentrate has no ions in excess of 35 ppt seawater, salts can be added to bring each of the ions up to 35 ppt seawater proportions. The 3 ppt concentrate however, has ions in excess of 35 ppt seawater proportions, so those ions would remain out of proportion even if the other ions were brought up to 35 ppt seawater proportions. Various ion-exchange resins, precipitation techniques, and gasification methods that could be used to remove the excess ions were considered during the 1995 FDEP study but, it was decided to avoid them because each offered the prospect of inadvertently altering any unidentified toxicants in the concentrate. This left adding ACS-grade salts as the means of achieving seawater proportions. This method has two drawbacks of its own.

a) Adding salts to balance the major seawater-ion proportions increases the total dissolved solids ("salinity") of the concentrate.

b) In some concentrates tested, achieving seawater proportions for all major ions resulted in a concentrate salinity of as much as 219 ppt—well above the salinity range which mysid shrimp can tolerate.

This increased salinity also offered the possibility of toxicity changes in any non-major-seawater-ion toxicants present in the concentrate. The toxicity of some toxicants (e.g., ammonia and heavy metals) is altered by changes in salinity. To address these complications, in cases where the ion-adjusted concentrate salinity would be greater than 35 ppt, Test 4 was split into two tests, Test 4a and 4b.

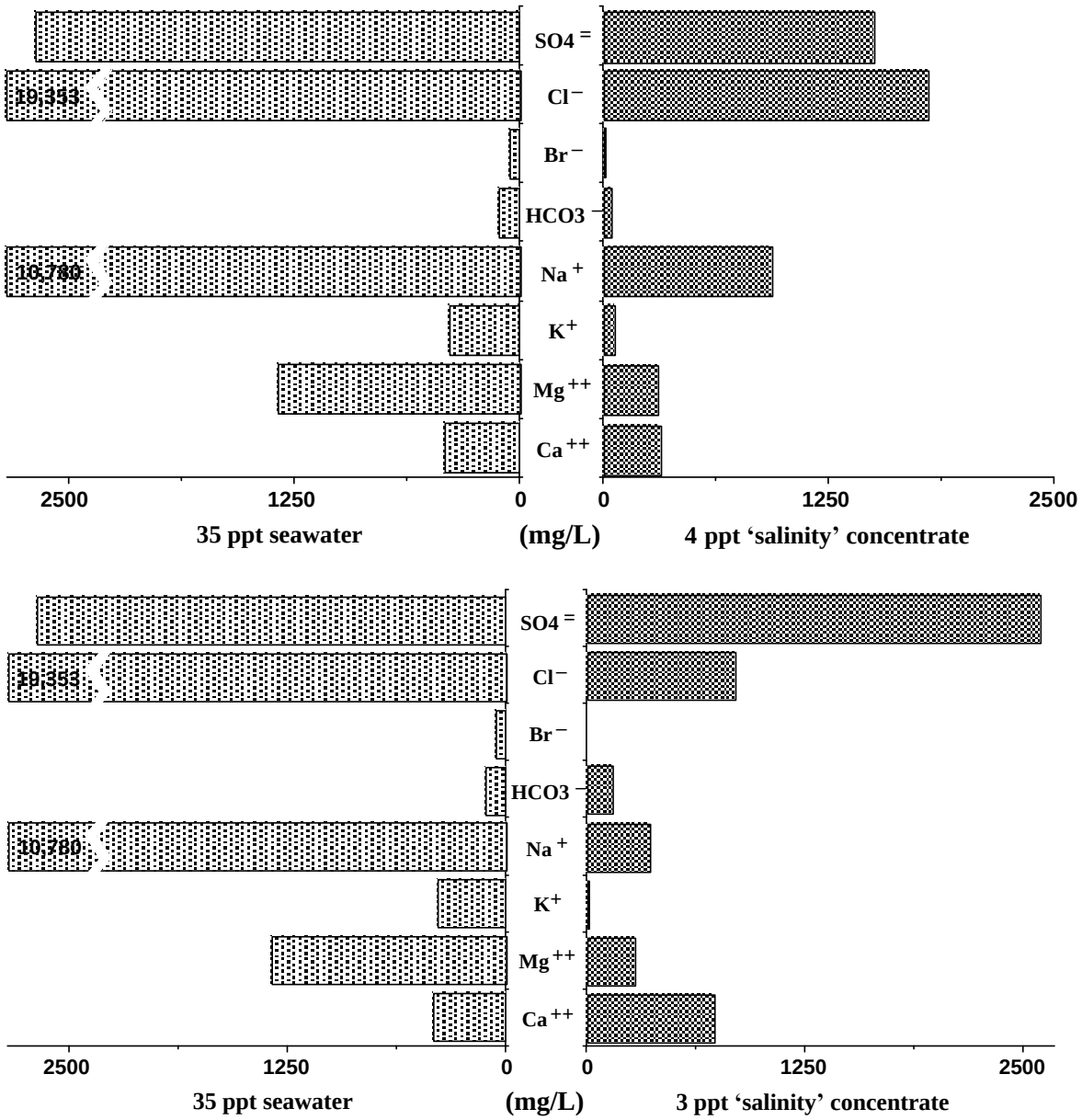


Figure 1. Comparisons of major-ion compositions of seawater and two low-salinity concentrate samples.

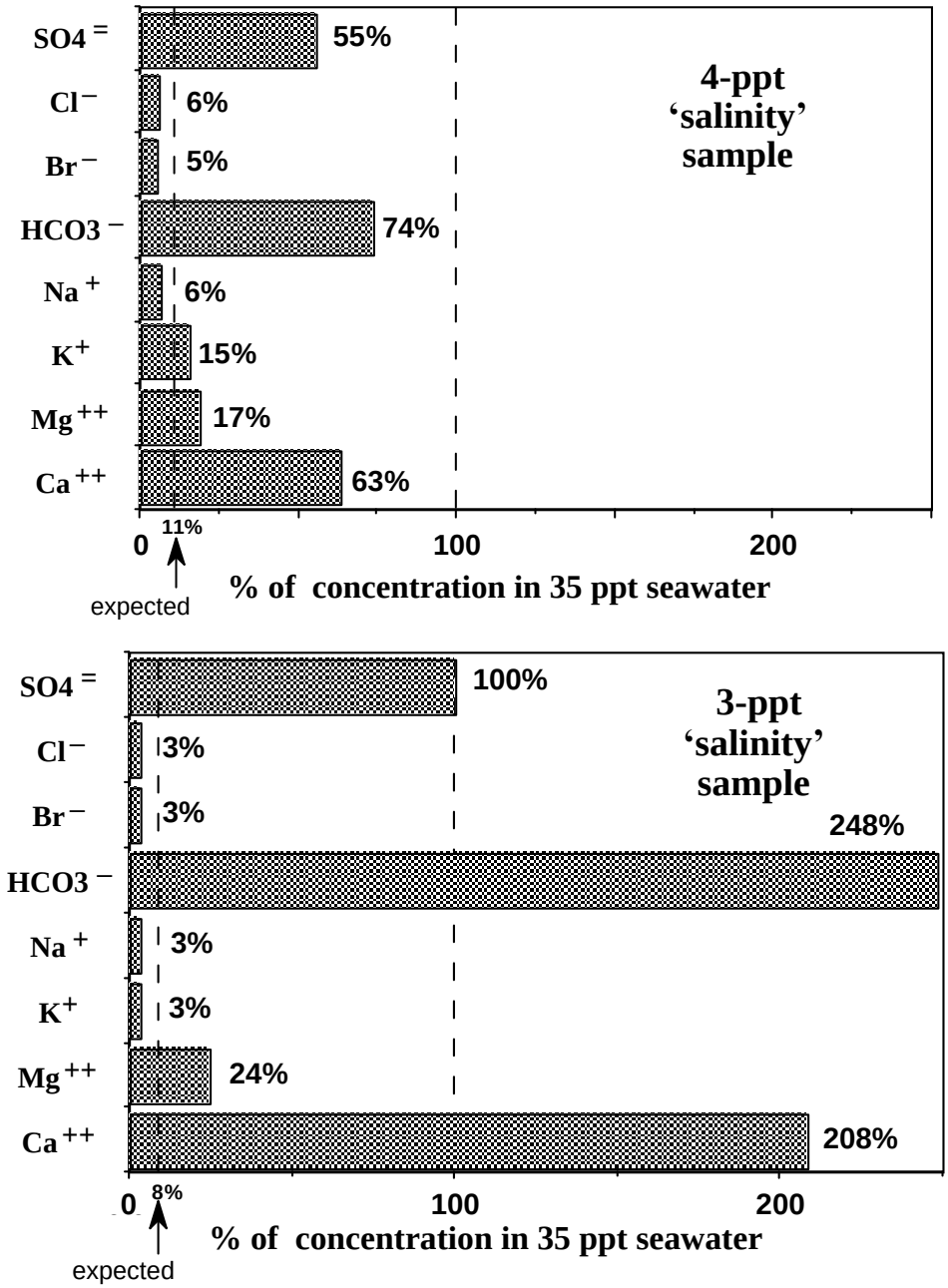


Figure 2. Comparison of proportions of the major-ion compositions of seawater and two low-salinity concentrate samples.

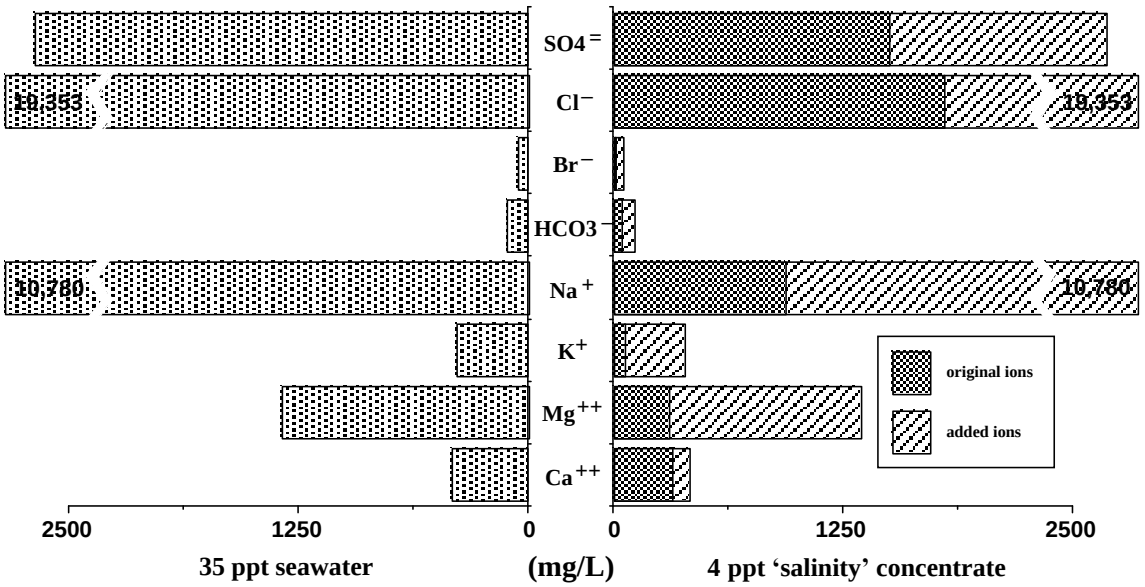


Figure 3. Major-seawater-ion correction of a 'low-salinity' concentrate sample.

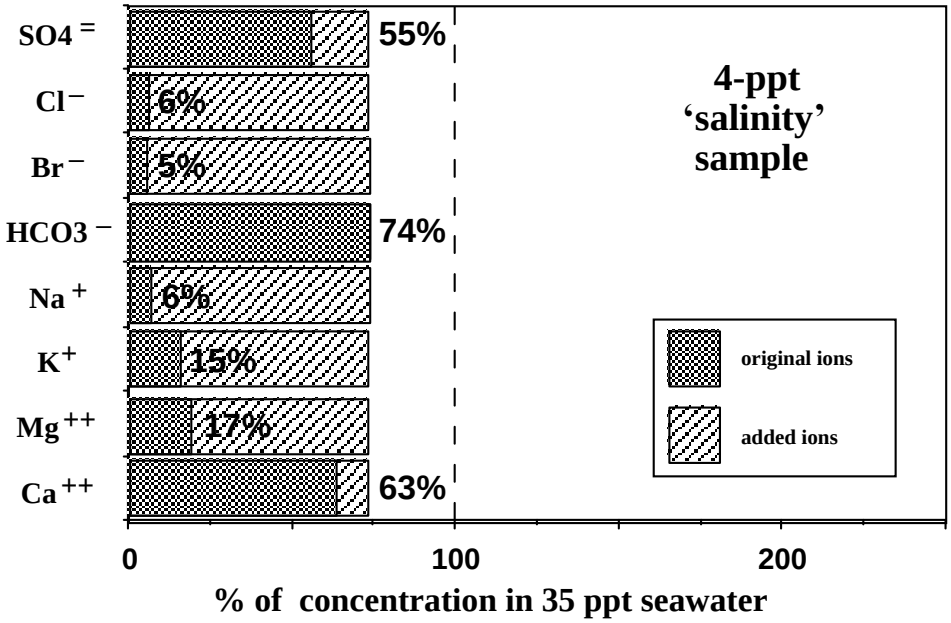


Figure 4. Relative proportions of major-seawater-ion correction of a 'low-salinity' concentrate sample.

Figures 3 and 4 illustrate ion-adjusting the 4-ppt sample shown in Figures 1 and 2 in an attempt to "correct" the ion concentrations to match those of seawater.

We assume Tests 1 and 2 showed toxicity or this suite of tests would not have been completed.

Test 4a—Ion-adjusted concentrate, major seawater ions incompletely adjusted.

Test 4a uses a concentrate sample in which the major seawater ions present in concentrations below that of 35 ppt seawater are adjusted up to the 35 ppt-seawater concentrations. Any of the major seawater ions present in concentrations greater than that of 35 ppt seawater are left unaltered. NSW at 35 ppt is used as the control and diluent. If the NSW collected for the test is less than 35 ppt, use hyper-saline brine (prepared per SOP #7) to raise the salinity of the NSW to 35 ppt.

Figures 5 and 6 show the ion distribution for a 'moderate-salinity' concentrate having calcium and carbonate concentrations greater than those of 35-ppt seawater. Salts were added to bring any concentrate ions that were below 35-ppt seawater concentrations up to that level. This produced a solution with the major seawater ions equivalent to 35-ppt seawater except for the higher concentrations of calcium and carbonate.

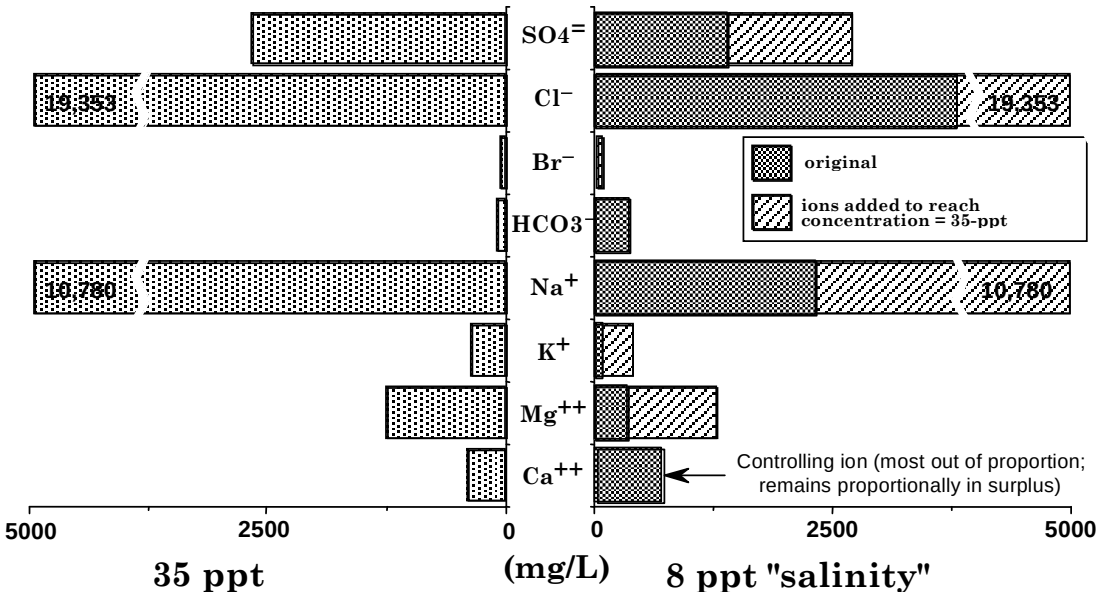


Figure 5. Example of ion correcting a moderate-'salinity' concentrate sample having one ion present at a concentration greater than that in 35-ppt seawater. This ion remains proportionally in surplus, with all other ions made proportional at 35-ppt seawater concentrations (per Tests 4A and 5A).

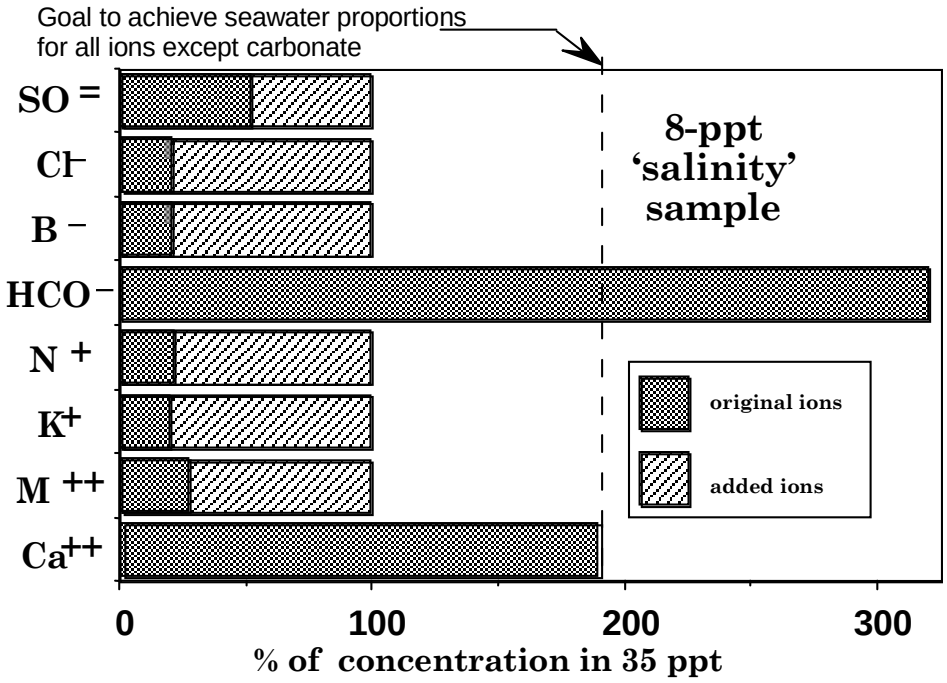


Figure 6. Proportion example of ion correction of a moderate-salinity concentrate sample, leaving one ion proportionally in surplus, with all other ions made proportional at 35-ppt seawater concentrations (per Tests 4A and 5A).

Test 4b—Ion-adjusted concentrate, major seawater ions completely adjusted then diluted to 35 ppt

Test 4b uses a concentrate sample in which ACS-grade chemical salts have been added to balance the major seawater ions to the proportions present in seawater. This balancing is controlled by the controlling ion that is in the highest proportion relative to seawater, excluding carbonate. The resulting sample is then diluted with de-ionized water to 35 ppt salinity. NSW at 35 ppt is used as the control and diluent. In actual practice, the sample manipulations are conducted such that any dilution is performed first then the necessary salts added. This helps minimize problems with salts reaching saturation and precipitating out of solution.

Figures 7 and 8 show the ion distribution for a 'moderate-salinity' concentrate having calcium and carbonate concentrations greater than those of 35-ppt seawater. Salts were added to bring all ions, except carbonate, to the same proportions present in 35-ppt seawater. As a result, the ion concentrations are much greater than those in seawater and dilution with de-ionized water is necessary to reduce the salinity to that of full seawater. Performing these two tests keeps the salinity of the test solutions in the range

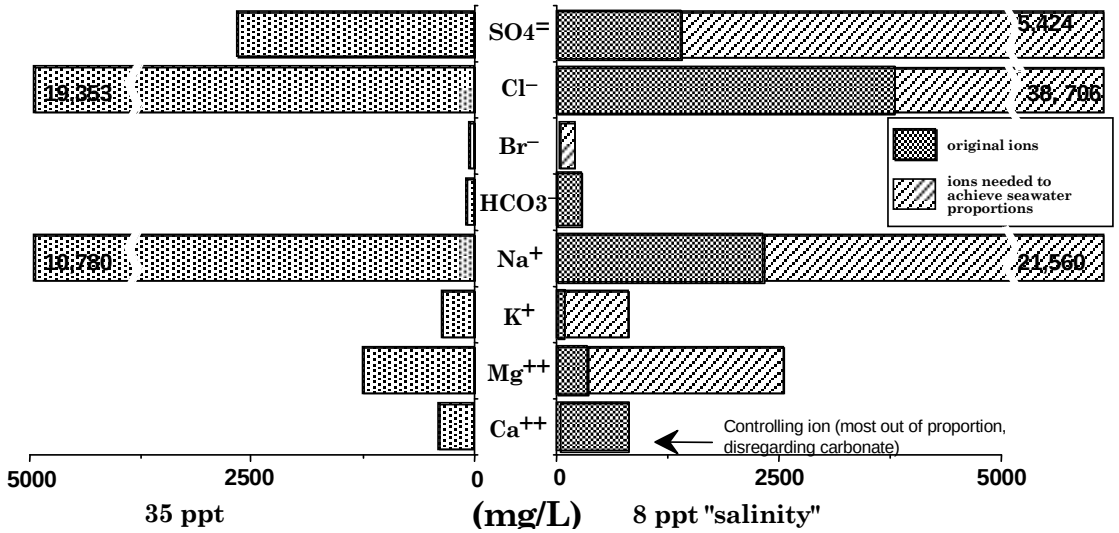


Figure 7. Ion correction of a moderate-salinity concentrate sample, requiring dilution (per Test 4B and 5B).

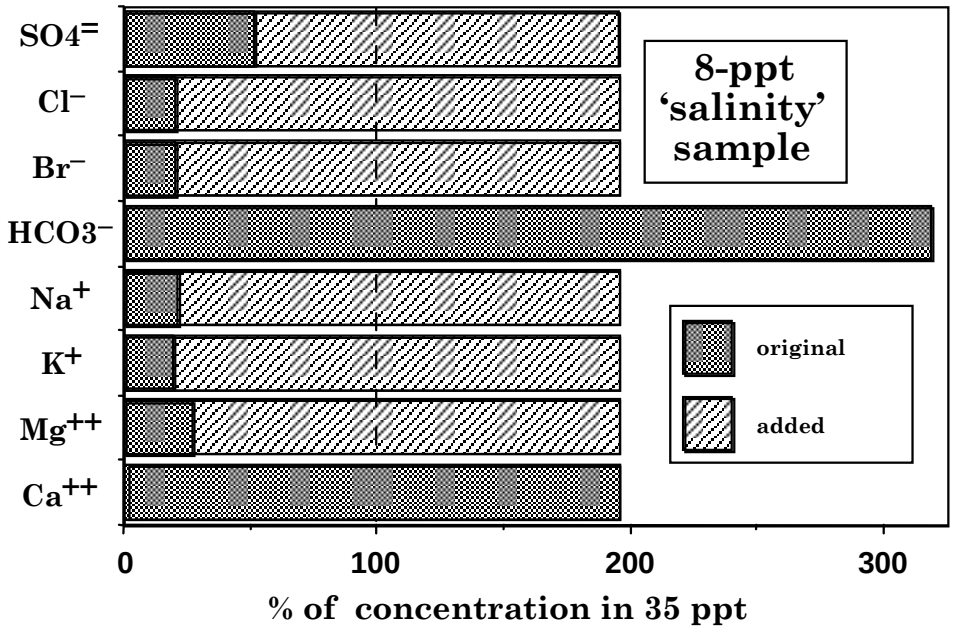


Figure 8. Proportion example of ion correction of a moderate-salinity concentrate sample, requiring dilution (per Test 4B and 5B).

appropriate for the mysids, but Test 4a could incorrectly indicate toxicity from the presence of unidentified toxicants when it actually results from the seawater ions that remain out-of-proportion. Test 4b could incorrectly indicate that the ion adjusting had removed major-seawater-ion toxicity when the loss of toxicity was actually a result of diluting unidentified toxicants to non-toxic concentrations as a side effect of diluting the salinity to 35 ppt.

To address these concerns, an additional test was added to determine the result when the concentration of major seawater ions remains constant while the concentration of any unidentified toxicants is diluted across the test concentration series.

Test 5—Ion-adjusted concentrate (SOP #5)

Test 5 uses a concentrate sample to which ACS-grade chemical salts have been added to balance the major seawater ions to the proportions present in seawater. This balancing is controlled by the controlling ion that is in the highest proportion relative to seawater, excluding carbonate. This is the same as in Test 4. However, the control and diluent for the toxicity test is composed of mock ion-adjusted concentrate.

The mock ion-adjusted concentrate is composed of an aliquot of the mock concentrate prepared for Test 3 that has been ion adjusted using the same amounts of the same salts used to ion adjust the concentrate in Test 4 and 5. By virtue of the dilution water having the same amount of the major seawater ions as are present in the ion-adjusted concentrate, this test results in a concentration series in which the major seawater ions are the same in all test concentrations, but where any unidentified toxicants are diluted along that series. Thus any differences in toxicity along the test concentration gradient should indicate toxicity from causes other than major seawater ions.

Of course, this test suffers from the same problem as Test 4 in that the composition of some concentrates can result in salinities that are too high for mysid survival. Therefore, this test is also split into two tests for this type concentrate.

Test 5a—Ion-adjusted concentrate, major seawater ions incompletely adjusted, using mock ion-adjusted concentrate for diluent.

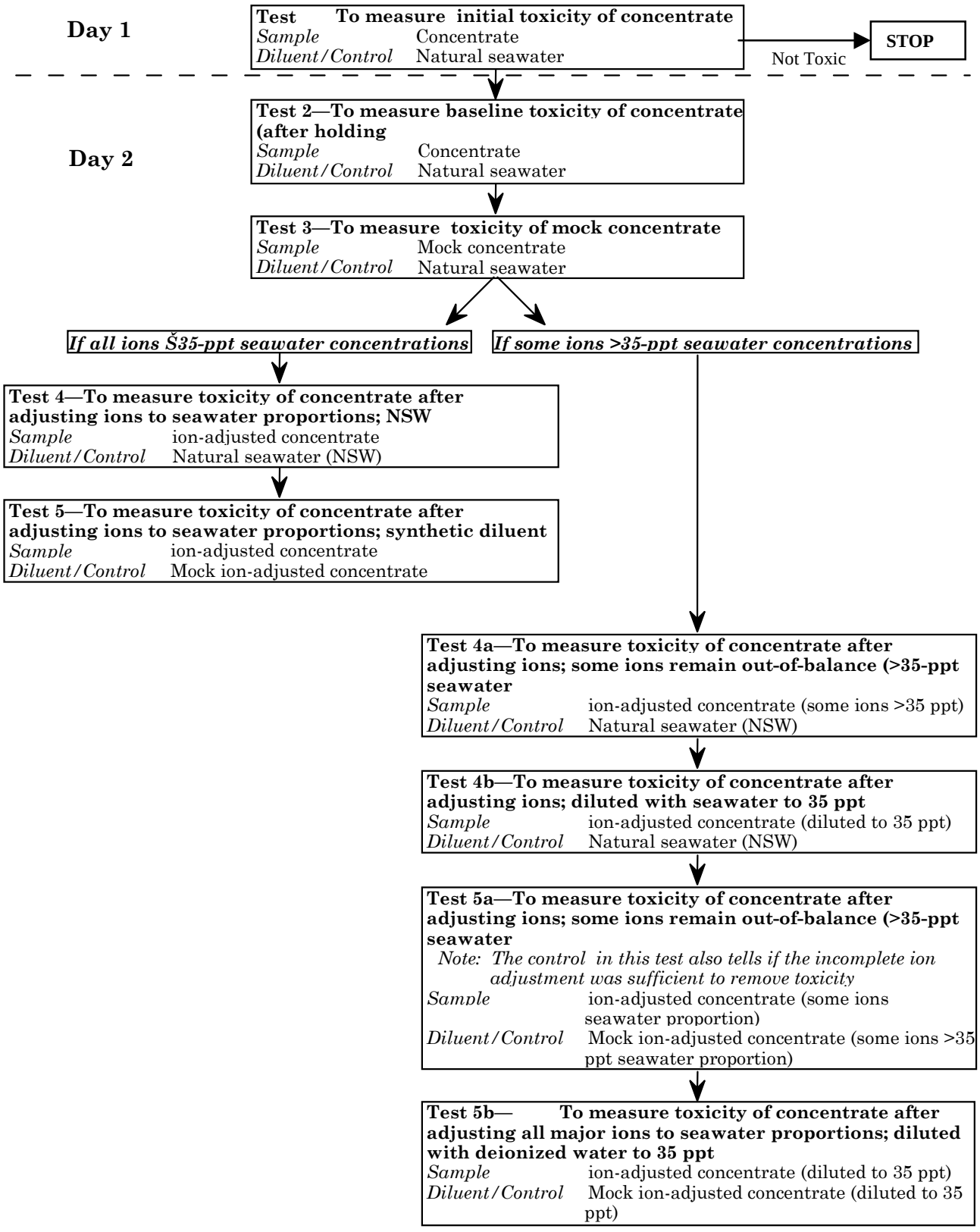
Test 5a uses a concentrate sample in which the major seawater ions present in concentrations below that of 35 ppt seawater are adjusted up to the 35 ppt-seawater concentrations. Any of the major seawater ions present in concentrations greater than that of 35 ppt seawater are left unaltered. This is the same as Test 4a. However, the control and diluent for the toxicity test is composed of a mock ion-adjusted concentrate made to match the major-seawater-ion concentrations of the ion-adjusted concentrate used in this test.

Test 5b— Ion-adjusted concentrate, major seawater ions completely adjusted then diluted to 35 ppt, using mock ion-adjusted concentrate for diluent.

Test 5b uses a concentrate sample in which ACS-grade chemical salts have been added to balance the major seawater ions to the proportions present in seawater. This balancing is controlled by the controlling ion that is in the highest proportion relative to seawater, excluding carbonate. The resulting sample is then diluted with de-ionized water to 35 ppt salinity. This is the same as Test 4b. However, the control and diluent for the toxicity test is composed of a mock ion-adjusted concentrate made to match the major-seawater-ion concentrations of the ion-adjusted concentrate used in this test after dilution to 35 ppt.

NOTE: The two mock ion-adjusted concentrates used as diluent in 5a and 5b are ***not*** the same. In each test, the mock concentrate is ion-adjusted in the same manner as the concentrate in that test is adjusted. As a result, the mock ion-adjusted concentrate in Test 5a has the major seawater ions incompletely adjusted while that created for Test 5b has the major seawater ions completely adjusted to 35 ppt proportions, with the possible exception of carbonate. Also as a result, the control for Test 5a tells us whether the toxicity seen in the ion-adjusted concentrate in Test 4a and Test 5a results from some of the major seawater ions remaining out of proportion, or from the presence of 'other' toxicants.

Figure 9. Summary flowchart of the test methods.



Interpreting Test-Suite Results

After running a concentrate sample through this test suite, the results are interpreted as follows:

Toxicity of the sample due solely to an "imbalance" of the major seawater-ion proportions:

Test 1, Test 2, Test 3 } All three tests show similar results, with toxicity reducing with dilution.

Test 4 & Test 5 } Neither Test 4 or Test 5 should show any toxicity.

Test 4a, Test 4b, & Test 5a, Test 5b } None of the tests should show toxicity, with the possible exception of Test 5a. If Test 5a shows toxicity, all test concentrations, including the the control, should show similar toxicity.

Part of the toxicity due to major seawater-ion "imbalance" and the rest from other toxicants:

Test 1, Test 2, Test 3, Test 4 (a, b), Test 5 (a, b) } All tests should show some toxicity, but Test 3, Test 4 (a, b) and Test 5 (a, b) should show less toxicity than Test 1 and Test 2.

All of the toxicity caused by toxicants other than the major seawater ions:

Test 3 - Test 3 should show no toxicity.

Test 1, Test 2, & Test 4 (a, b), Test 5 (a, b) } All of these tests should show toxicity. Tests 4, 4a, 4b, 5, 5a, and 5b may show less toxicity than Test 1 and Test 2, due to the effects of increased salinity or dilution on the unknown toxicant(s). Test 2, Test 4 (a, b) and Test 5 (a, b) may show less toxicity than Test 1 if the unknown toxicant(s) has been affected by the holding time.

Figure 10. Interpreting Test-Suite Results.

Some areas of uncertainty arose in interpreting the test-suite results during the 1995 study. The situation has been identified in those concentrates where correcting the major seawater ions results in ion-adjusted concentrates with some major seawater ions present in much greater concentrations than seawater and where very high dilutions are needed to create 35 ppt concentrate samples for Tests 4b and Test 5b. In this situation, it is possible to have a set of data where one cannot determine whether a concentrate has unidentified causes of toxicity only or has both major seawater ion plus unidentified causes of toxicity. By excluding carbonate as a controlling ion, fewer extremely high salinity, balanced concentrates will be produced.

The 1995 study encountered no situations where there was difficulty in distinguishing concentrates toxic solely because of major-seawater-ion proportions from concentrates possessing unidentified toxicants, whether solo or in conjunction with major-seawater-ion toxicity.

Note: this suite of tests is not designed to identify the actual toxicant(s). It is designed to determine whether the observed toxicity results from the proportions of major-seawater ions or if it results from other unidentified toxicants. If other ions that can be manipulated in a manner similar to the major-seawater ions are strongly suspected, then this protocol could be readily modified to confirm or deny the toxicity associated with the suspected ion (e.g., fluoride). If desired, Toxicity Identification Evaluation (TIE) protocols (U.S. EPA 1988, 1989a, 1989b) could be used to attempt to identify the specific cause(s) of any toxicity not caused by major-seawater-ion imbalances.

Conclusions

By following these protocols, a facility should be able to determine if the toxicity exhibited by its concentrate is; due solely to major-seawater-ion imbalance, due to a combination of major-seawater-ion imbalance and some other toxicant(s), or due solely to some toxicant(s) other than an imbalance in the major-seawater-ion concentrations.

A comprehensive quality assurance plan and high-quality analytical chemistry support are critical to the successful completion of these protocols. Ion adjustments to the test solutions should be within 20% of the targeted value for all ions except carbonate. Deviations in excess of 20% could potentially make the interpretation of the toxicity test results unreliable.

Attempts to short-cut or eliminate any of the toxicity tests or analytical chemistry analyses in these protocols would severely compromise the ability of the researcher to make a conclusive statement about seawater-ion imbalance being the sole source of toxicity in the sample. Any additional tests or deviations in the protocols should be discussed with, and approved by, qualified FDEP personnel before initiating the study.

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Appendix 1—Tables

Table 3. 1997 salinity tolerance study results for mysid shrimp, *Americamysis bahia.*

Date	Acclimation Rate	Duration of Exposure	Salinity ppt	Natural Seawater Percent Survival	Artificial Seawater Percent Survival
6/10/1997	No acclimation	48 hour	4	0	0
	No acclimation	48 hour	5	0	0
	No acclimation	48 hour	6	10	5
	No acclimation	48 hour	7	0	25
	No acclimation	48 hour	8	55	25
	No acclimation	48 hour	20	100	100
7/21/1997	Slow to 10 ppt	96 hour	4	80	
	Slow to 10 ppt	96 hour	5	60	
	Slow to 10 ppt	96 hour	6	80	
	Slow to 10 ppt	96 hour	7	100	
	Slow to 10 ppt	96 hour	8	95	
	No acclimation	96 hour	20	100	
7/28/1997	Slow to 10 ppt	96 hour	4	20	55
	Slow to 10 ppt	96 hour	5	50	80
	Slow to 10 ppt	96 hour	6	60	95
	Slow to 10 ppt	96 hour	7	85	100
	Slow to 10 ppt	96 hour	8	100	100
	No acclimation	96 hour	20	100	100
8/1/1997	Slow to 8 ppt	96 hour	4		80
	Slow to 8 ppt	96 hour	5		85
	Slow to 8 ppt	96 hour	6		95
	Slow to 8 ppt	96 hour	7		95
	Slow to 8 ppt	96 hour	8		95
	No acclimation	96 hour	20		100
8/1/1997	Rapid to 8 ppt	96 hour	4		40
	Rapid to 8 ppt	96 hour	5		60
	Rapid to 8 ppt	96 hour	6		95
	Rapid to 8 ppt	96 hour	7		100
	Rapid to 8 ppt	96 hour	8		100
	No acclimation	96 hour	20		100
9/2/1997	Slow to 8 ppt	48 hour	4		90
	Slow to 8 ppt	48 hour	5		95
	Slow to 8 ppt	48 hour	6		100
	Slow to 8 ppt	48 hour	7		95
	Slow to 8 ppt	48 hour	8		100
	No acclimation	48 hour	20		100
9/2/1997	Rapid to 8 ppt	48 hour	4		90
	Rapid to 8 ppt	48 hour	5		95
	Rapid to 8 ppt	48 hour	6		100
	Rapid to 8 ppt	48 hour	7		100
	Rapid to 8 ppt	48 hour	8		95
	No acclimation	48 hour	20		100

Slow acclimation - Not more than 5 ppt/24-hour period

Rapid acclimation - not more than 5 ppt/2-hour period

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Appendix 2—Standard Operating Procedures (SOP's)

SOP #1: Test 1—Initial Test

(FDEP Biology Section Internal SOP TA-10.02)

STEPS	COMMENTS
Materials	Refer to SOP #TA-10.01.
Documentation	Refer to SOP #TA-10.01.
Advance Preparation	Refer to SOP #TA-10.01.
Methods	
Test Initiation	
1. Pour 50 mL of concentrate into a clean plastic cup and check the salinity.	This is done to prevent contaminating concentrate in sample bottle.
	Check salinity per SOP #TA-06.06.
If necessary, adjust the salinity of the test concentrate.	If the salinity is <7ppt, then add mock-sea-salt slurry as needed to bring 2.5L of concentrate up to 7ppt.
	Allow at least one-half hour to dissolve salts completely.
2. Measure the pH of the cup of concentrate used in Step 2. If salinity adjustment was necessary, replace it with salinity-adjusted concentrate before measuring pH.	Check pH per SOP #TA-060.3.
	Measuring the pH is done after any salinity adjustment because it may raise the pH.
If necessary, adjust the test-concentrate pH to 7.5–9.0.	The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project.
	Refer to SOP #TA-10.10 and #TA-10.11 for preparation of HCl and NaOH stock solutions.
3. Save 500 mL of the adjusted concentrate in two 250-mL plastic bottles for chemical analysis. Label the bottles with the sample name, test number, date, and your initials.	Send these bottles to the chemistry section to be analyzed for Cl^-, SO_4, Alkalinity, Br^-, and Metals.
4. Put 250 mL of NSW adjusted to the concentrate salinity into each of the four replicate control jars.	If the concentrate was salinity adjusted, a second salinity-adjusted control is added to the test series. The salinity-adjusted control is prepared by diluting NSW to the concentrate salinity and salinity adjusting it up to 7ppt using the mock-sea-salt slurry.

5. The test concentrations are 6.25%, 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of concentrate using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.

Use four 250-mL replicates for each test concentration.

Concentration	Diluent	Sample
Control	1,000 mL	0 mL
Salt Control	1,000 mL	0 mL
6.25%	937.5 mL	62.5 mL
12.5%	875 mL	125 mL
25%	750 mL	250 mL
50%	500 mL	500 mL
100%	0 mL	1,000 mL

6. For each concentration**,** seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.

7. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.

Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.

8. Record any comments pertaining to the condition or appearance of the sample.

Record all readings in appropriate columns on *RO Project Bench Sheet.*

9. Load test organisms.

Per SOP #TA-10.07.

SOP #2: Test 2—Baseline Test

(FDEP Biology Section Internal SOP TA-10.03)

STEPS	COMMENTS
Materials	Refer to SOP #TA-10.01.
Documentation	Refer to SOP #TA-10.01.
Advance Preparation	Refer to SOP #TA-10.01.
Methods	
Test Initiation	
1. Pour 50 mL of concentrate into a clean plastic cup and check the salinity.	This is done to prevent contaminating concentrate in sample bottle. Check salinity per SOP #TA-06.06.
If the salinity is <7 ppt, then add mock-sea-salt slurry as needed to bring 2.5L of concentrate up to 7 ppt.	Allow at least one-half hour to dissolve salts completely. To make mock-sea-salt slurry refer to SOP #TA-10.08.
2. Measure the pH of the cup of concentrate used in Step 2. If salinity adjustment was necessary, replace it with salinity-adjusted concentrate before measuring pH.	Check pH per SOP #TA-06.03. Measuring the pH is done after any salinity adjustment because it may raise the pH.
If necessary, adjust the test-concentrate pH to 7.5–9.0.	The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project. Refer to SOP #TA-10.10 and #TA-10.11 for preparation of HCl and NaOH stock solutions.
3. Save 500 mL of the adjusted concentrate in two 250-mL plastic bottles for chemical analysis. Label the bottles with the sample name, test number, date, and your initials.	Send these bottles to the chemistry section to be analyzed for Cl^-, SO_4, Alkalinity, Br^-, and Metals.
4. Put 250 mL of NSW adjusted to the concentrate salinity into each of the four replicate control jars.	If the concentrate was salinity adjusted, a second salinity adjusted control is added to the test series. The salinity-adjusted control is prepared by diluting NSW to the concentrate salinity and salinity adjusting it up to 7ppt using the mock-sea-salt slurry.

5. The test concentrations are 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of concentrate using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.

Use four 250-mL replicates for each test concentration.

Concentration	Diluent	Sample
Control	1,000 mL	0 mL
Salt Control	1,000 mL	0 mL
12.5%	875 mL	125 mL
25%	750 mL	250 mL
50%	500 mL	500 mL
100%	0 mL	1,000 mL

6. For each concentration**,** seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.

7. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.

Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.

8. Record any comments pertaining to the condition or appearance of the sample.

Record all readings in appropriate columns on *RO Project Bench Sheet*.

9. Load test organisms.

Per SOP #TA-10.07.

SOP #3: Test 3—Mock Concentrate

(FDEP Biology Section Internal SOP TA-10.04)

STEPS	COMMENTS
Materials	Refer to SOP #TA-10.01.
Documentation	Refer to SOP #TA-10.01.
Advance Preparation	Refer to SOP #TA-10.01.
Methods	
Test Initiation	
1. Gather the salts prepared for the mock concentrate.	See SOP #TA-10.01
2. Fill a 20L carboy with 16 liters of DI water.	This carboy will be used to make 18 liters of mock concentrate. Part of this will be used in Tests 4 and 5.
3. If the facility uses an anti-scalant, organic acid, or other product to reduce scaling in the facility's pipes, the same product must be added to the mock concentrate at this time. The amount added should be equivalent to the concentration in the concentrate.	It may not be possible to match the ion concentrations in the concentrate without adding the product, because the product allows the ions to go into and remain in solution at concentrations that exceed the normal saturation concentration in de-ionized water. A toxicity test on the straight product may be necessary if no mysid toxicity data is already available.
3. If $CaSO_4$ is needed, it must be dissolved at a low pH. Bring 16L of DI water down to pH 2 using HCl. After dissolving the $CaSO_4$, bring the pH up to 5 using NaOH. The $CaSO_4$ should be the first salt dissolved.	Refer to SOP #TA-10.10 and #TA-10.11 for preparation of HCl and NaOH stock solutions. **It is very important to adjust the pH above 5 before adding additional salts.** Any carbonates will be lost to the atmosphere as CO_2 below pH 5. Any acid or base used in this step must be recorded. The amount of NaCl needed to create the mock concentrate is likely to change and must be recalculated.
4. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve each remaining salt that is hard to dissolve (such as $MgSO_4$, $CaCO_3$, Na_2SO_4, and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar. Portions of the 16L from Step 3, now at pH 5, can be used to pre-dissolve the other salts except NaCl.	$MgSO_4$ tends to solidify into large flakes without adequate agitation. Adding individual salts using a stir plate gets difficult-to-dissolve compounds more readily into solution.

5. Add the pre-dissolved salts and the salts not needing pre-dissolving to the mock concentrate. Bring the total volume to 18L with DI water.

NaCl should always be dissolved separately then added slowly and last to minimize precipitation of any salts that may be near saturation.

6. Pour 50 mL of mock concentrate into a clean plastic cup and check the salinity.

If the salinity is <7 ppt, then first save 500 mL of the mock concentrate in two separate 250-mL bottles for chemical analysis. In a 4-L Erlenmeyer flask, bring 2.5L of mock concentrate up to 7 ppt by adding mock-sea-salt slurry as needed.

This is done to prevent contaminating concentrate in sample bottle.

Check salinity per SOP #TA-06.06.

Allow at least one-half hour to dissolve salts completely.

To make mock-sea-salt slurry refer to SOP #TA-10.08.

7. Measure the pH of the cup of mock concentrate used in Step 2. If salinity adjustment was necessary, replace it with salinity-adjusted mock concentrate before measuring pH.

If necessary, adjust the mock concentrate pH to 7.5–9.0.

Check pH per SOP #TA-06.03.

Measuring the pH is done after any salinity adjustments because they may raise the pH.

The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project.

Refer to SOP #TA-10.10 and #TA-10.11 for preparation of HCl and NaOH stock solutions.

8. Save 500 mL of the salinity-adjusted mock concentrate in two 250-mL plastic bottles for chemical analysis. If salinity adjustment was necessary, there should be a total of four 250-mL bottles. Label the bottles with the sample name, test number, date, and your initials.

Send these bottles to the chemistry section to be analyzed for Cl^-, SO_4, Alkalinity, Br^-, and Metals.

9. Put 250 mL of NSW adjusted to the concentrate salinity into each of the four replicate control jars.

If the mock concentrate was salinity adjusted, a second salinity-adjusted control is added to the test series. The salinity-adjusted control is prepared by diluting NSW to the concentrate salinity and salinity adjusting it up to 7ppt using the mock-sea-salt slurry.

10. The test concentrations are 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of mock concentrate (sample) using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.

Use four 250-mL replicates for each test concentration.

Concentration	Diluent	Sample
Control	1,000 mL	0 mL
Salt Control	1,000 mL	0 mL
12.5%	875 mL	125 mL
25%	750 mL	250 mL
50%	500 mL	500 mL
100%	0 mL	1,000 mL

11. For each concentration**,** seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.

12. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.

Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.

13. Record any comments pertaining to the condition or appearance of the sample.

Record all readings in appropriate columns on *RO Project Bench Sheet*.

14. Load test organisms.

Per SOP #TA-10.07.

SOP #4: Test 4—Ion-adjusted Concentrate with Natural Seawater as Diluent

(FDEP Biology Section Internal SOP TA-10.05)

STEPS	COMMENTS
Materials	Refer to SOP #TA-10.01.
Documentation	Refer to SOP #TA-10.01.
Advance Preparation	Refer to SOP #TA-10.01.
Methods	
If the ion-adjusted concentrate calculations show that seawater proportions can be achieved with a final salinity ≤35‰, then skip the instructions Test 4A and 4B. Otherwise, skip the instructions for Test 4.	Refer to SOP #TA-10.12.
Test 4 ***Create fully ion-adjusted concentrate***	Use Test 4 only if the resulting ion-adjusted concentrate will have a salinity ≤35 ppt. Otherwise go to Test 4A.
1. Gather the salts prepared for the ion-adjusted concentrate.	Refer to SOP # TA-07.09. The salts in ion-adjusted concentrate should result in major seawater ions that are proportional to seawater.
2. Pour 250 mL of the unaltered concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.	Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.
3. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve each remaining hard-to-dissolve salt (such as $MgSO_4$, $CaCO_3$, Na_2SO_4, and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar. It may be possible to dissolve more than one salt in a single flask.	The volume of concentrate needed will vary based upon the concentrate characteristics. About 250 mL will probably be enough to dissolve most salts. $MgSO_4$ tends to solidify into large flakes without adequate agitation. Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily. NaCl should always be dissolved separately in a full liter of concentrate.
4. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask.	It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes. The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.

5.	Bring the total amount of ion-adjusted concentrate up to the goal volume by adding unaltered concentrate.	The goal volume is determined using the spreadsheet *Ion_Calc* per SOP #TA-10.12. Part of the ion-adjusted concentrate will be saved for use in Test 5.

Test 4A

Create ion-adjusted concentrate A: some ions remaining out-of-proportion.

Tests 4A and 4B are for use with concentrate samples in which the ion adjustment will create a salinity >35 ppt.

1.	Gather the salts prepared for the ion- adjusted concentrate A.	Refer to SOP # TA-07.09. The salts in this ion-adjusted concentrate A are not fully balanced. They are balanced as far as possible without the individual ions going over their concentration in 35‰ seawater.
2.	Pour 2L of the unaltered concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.	Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.
3.	In separate 1,000-mL Erlenmeyer flask(s), pre-dissolve each remaining hard-to-dissolve salts (such as $MgSO_4$, $CaCO_3$, Na_2SO_4, and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar. It may be possible to dissolve more than one salt in a single flask.	The volume of concentrate needed will vary based upon the concentrate characteristics. About 250 mL will probably be enough to dissolve most salts. $MgSO_4$ tends to solidify into large flakes without adequate agitation. Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily. NaCl should always be dissolved separately in a full liter of concentrate. With some high-'salinity' concentrates, it may be necessary to dissolve some salts in DI water. Use the minimum volume of DI possible.
4.	After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask.	It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes. The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.
5.	Bring the total amount of ion-adjusted concentrate A up to the goal volume (generally 5L) with additional unaltered concentrate.	The goal volume is determined using the spreadsheet *Ion_Calc* per SOP #TA-10.12. Part of the ion-adjusted concentrate A will be saved for use in Test 5.

Test 4B

Create ion-adjusted concentrate B: ions fully adjusted, but diluted to 35 ppt.

1. Gather the salts prepared for the ion- adjusted concentrate B.	Refer to SOP # TA-10.01. The salts in ion-adjusted concentrate B should result in major seawater ions that are proportional to seawater.
2. Pour about 500 mL less than the needed volume of the unadjusted concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.	The volumes of concentrate and DI water needed are calculated using the spreadsheet *Ion_Calc* per SOP #TA-10.12. Adjust volumes for dissolving the salts such that these total volumes are not exceeded. Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.
3. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve in DI water each remaining hard-to-dissolve salt (such as $MgSO_4$, $CaCO_3$, Na_2SO_4, and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar. It may be possible to dissolve more than one salt in a single flask.	A total of about 250 mL of concentrate and/or DI water will probably be enough to dissolve most salts. $MgSO_4$ tends to solidify into large flakes without adequate agitation. As it is often difficult to get all salts dissolved in this concentrate before diluting, add most of the dilution water before adding the salts. Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily. NaCl should always be dissolved separately in a full liter of concentrate.
4. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask. Any *increase* in the volume of the DI water as a result of adding the salts should be counted as part of the needed concentrate volume. The initial DI water volume should be counted as part of the needed DI water.	It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes. The NaCl solution should always be added slowly and last to minimize precipitation of any salts that may be near saturation.
5. Bring the total amount of ion-adjusted concentrate B up to the goal volume by first adding any remaining concentrate and then adding the necessary DI water.	The goal volume is determined using the spreadsheet *Ion_Calc* per SOP #TA-10.12. The DI used to dissolve the salts is part of the DI volume determined by the spreadsheet. Part of the ion-adjusted concentrate B will be saved for use in Test 5.

Test Initiation

If running Tests 4A and 4B, repeat the following instructions using the ion-adjusted concentrates created for each test.

6. Pour 50 mL of ion-adjusted concentrate into a plastic cup and check the salinity to confirm that it is ≤ 35 ppt.

This is done to prevent contaminating concentrate in sample bottle.

Check salinity per SOP #TA-06.06.

7. Measure the pH of the cup of ion-adjusted concentrate used in Step 6.

Check pH per SOP #TA-06.03.

Measure the pH after salt additions because the additions may affect the pH.

If necessary, adjust the ion-adjusted concentrate pH to 7.5–9.0.

The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project.

Refer to SOP #TA-10.10 and #TA-10.11 for preparation of HCl and NaOH stock solutions.

8. Save 500 mL of the ion-adjusted concentrate in two 250-mL plastic bottles for chemical analysis. Label the bottles with the sample name, test number, date, and your initials.

Send these bottles to the Chemistry Section to be analyzed for Cl^-, SO_4, Alkalinity, Br^-, and Metals.

9. Put 250 mL of natural seawater (NSW) adjusted to the ion-adjusted concentrate salinity into each of the four replicate control jars for each test.

10. The test concentrations are 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of sample (ion-adjusted concentrate) using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent (mock ion-adjusted concentrate) to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.

Use four 250-mL replicates for each test concentration.

Concentration	Diluent	Sample
Control	1,000 mL	0 mL
12.5%	875 mL	125 mL
25%	750 mL	250 mL
50%	500 mL	500 mL
100%	0 mL	1,000 mL

11. For each concentration**,** seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.

Save any remaining ion-adjusted concentrate for use in Test 5.

12. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.	Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.
13. Record any comments pertaining to the condition or appearance of the sample.	Record all readings in appropriate columns on *RO Project Bench Sheet.*
14. Load test organisms.	Per SOP #TA-10.07.

SOP #5: Test 5–Ion-adjusted Concentrate with Mock Ion-adjusted Concentrate as Diluent

(FDEP Biology Section Internal SOP TA-10.06)

STEPS	COMMENTS
Materials	Refer to SOP #TA-10.01.
Documentation	Refer to SOP #TA-10.01.
Advance Preparation	Refer to SOP #TA-10.01.
Methods	
If the ion-adjusted concentrate calculations show that seawater proportions can be achieved with a final salinity ≤35‰, then skip the instructions Test 5A and 5B. Otherwise, skip the instructions for Test 5.	Refer to SOP#TA-10.12.
Test 5—fully ion-adjusted concentrate	Use Test 5 only if the resulting ion-adjusted concentrate will have a salinity ≤35 ppt. Otherwise go to Test 5A.
1. Use the remaining ion-adjusted concentrate created in Test 4.	
Create mock ion-adjusted concentrate	
2. Gather the salts prepared for the mock ion-adjusted concentrate.	Refer to SOP # TA-07.09.
3. Pour 2L of the mock concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.	Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.
4. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve each remaining hard-to-dissolve salt (such as $MgSO_4$, $CaCO_3$, Na_2SO_4, and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar. It may be possible to dissolve more than one salt in a single flask.	The volume of mock concentrate needed will vary based upon the concentrate characteristics. About 250 mL will probably be enough to dissolve most salts. $MgSO_4$ tends to solidify into large flakes without adequate agitation. Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily. NaCl should always be dissolved separately in a full liter of mock concentrate.
5. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask.	It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes. The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.

6. Bring the total volume of mock ion-adjusted concentrate up to the goal volume (generally 6L) by adding mock concentrate.	The goal volume is determined using the spreadsheet *Ion_Calc* per SOP #TA-10.12.

Test 5A—ion-adjusted concentrate A: some ions remaining out-of-proportion.

1. Use remaining ion-adjusted concentrate A created in Test 4A as the sample.	The mock ion-adjusted concentrate A that is being prepared will be the diluent.

Create mock ion-adjusted concentrate A

2. Gather the salts prepared for the mock ion-adjusted concentrate A.	Refer to SOP # TA-07.09.
3. Pour 2L of the mock concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.	Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.
4. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve each remaining hard-to-dissolve salt (such as $MgSO_4$, $CaCO_3$, Na_2SO_4, and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar. It may be possible to dissolve more than one salt in a single flask.	The volume of mock concentrate needed will vary based upon the concentrate characteristics. About 250 mL will probably be enough to dissolve most salts. $MgSO_4$ tends to solidify into large flakes without adequate agitation. Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily. NaCl should always be dissolved separately in a full liter of mock concentrate.
5. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask.	It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes. The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.
6. Bring the total volume of mock ion-adjusted concentrate A up to the goal volume (generally 6L) by adding mock concentrate.	The goal volume is determined using the spreadsheet *Ion_Calc* per SOP #TA-10.12.

Test 5B— ion-adjusted concentrate B: ions fully adjusted, but diluted to 35 ppt.

1. Use remaining ion-adjusted concentrate B created in Test 4B as the sample.	The mock ion-adjusted concentrate B that is being prepared will be the diluent.

Create mock ion-adjusted concentrate B

2. Gather the salts prepared for the mock ion-adjusted concentrate B.	Refer to SOP # TA-07.09.

3. Pour about 500 mL less than the needed volume of the mock concentrate into a 6-L Erlenmeyer flask and add any easy-to-dissolve salts.	The volumes of mock concentrate and DI water needed are determined using the spreadsheet *Ion_Calc* per SOP #TA-10.12. Adjust volumes for dissolving the salts such that these total volumes are not exceeded. Salts added in small amounts, such as KBr and KCl, can be added directly to this portion.
4. In separate 1,000-mL Erlenmeyer flasks, pre-dissolve in DI water each remaining hard-to-dissolve salt (such as $MgSO_4$, $CaCO_3$, Na_2SO_4, and K_2SO_4) and NaCl on a magnetic stirrer with a stir bar. It may be possible to dissolve more than one salt in a single flask.	A total of about 250 mL of mock concentrate and/or DI water will probably be enough to dissolve most salts. $MgSO_4$ tends to solidify into large flakes without adequate agitation. Pre-dissolving the salts on a stir plate allows difficult to dissolve compounds to go into solution more readily. NaCl should always be dissolved separately in a full liter of mock concentrate.
5. After salts are dissolved, accurately measure the volume of each pre-dissolved-salt solution and add to the 6-L Erlenmeyer flask. Any *increase* in the volume of the DI water as a result of adding the salts should be counted as part of the needed mock concentrate volume. The initial DI water volume should be counted as part of the needed DI water.	It is necessary to measure the concentrate to ensure accurate volume. Erlenmeyer flasks are not accurately calibrated and the salt additions will slightly increase the volumes. The NaCl solution should always added slowly and last to minimize precipitation of any salts that may be near saturation.
6. Bring the total volume of mock ion-adjusted concentrate B up to the goal volume (generally 6L) by first adding any remaining mock concentrate and then adding the necessary DI water.	The goal volume is determined using the spreadsheet *Ion_Calc* per SOP #TA-10.12. The DI used to dissolve the salts is part of the DI volume determined by the spreadsheet.

Test Initiation

If running Tests 5A and 5B, repeat the following instructions using the ion-adjusted concentrates and mock ion-adjusted concentrates created for each test.

6. Pour 50 mL of mock ion-adjusted concentrate into a plastic cup and check the salinity to confirm that it is $\leq$ 35 ppt.	This is done to prevent contaminating concentrate in sample bottle. Check salinity per SOP #TA-06.06.

7. Measure the pH of the cup of mock ion-adjusted concentrate used in Step 6.

Check pH per SOP #TA-06.03.

Measure the pH after salt additions because the additions may affect the pH.

If necessary, adjust the mock ion-adjusted concentrate pH to 7.5–9.0.

The acceptable range for pH is 7.5–9.0. Adjust as needed by adding a minimal amount of acid or base. Use only those acid or base stock solutions designated for the RO Project.

Refer to SOP #TA-10.10 and #TA-10.11 for preparation of HCl and NaOH stock solutions.

8. Save 500 mL of the mock ion-adjusted concentrate in two 250-mL plastic bottles for chemical analysis. Label the bottles with the sample name, test number, date, and your initials.

Send these bottles to the Chemistry Section to be analyzed for Cl^-, SO_4, Alkalinity, Br^-, and Metals.

9. Put 250 mL of mock ion-adjusted concentrate into each of the four replicate control jars for each test.

10. The test concentrations are 12.5%, 25%, 50%, and 100%. Starting with the lowest concentration, measure the desired volume of sample (ion-adjusted concentrate) using a graduated cylinder of the appropriate size and add it to a 1,000-mL graduated cylinder. Add diluent (mock ion-adjusted concentrate) to bring the volume of the cylinder up near the 1,000 mL mark. Add the final amount of diluent with a 10-mL pipette so as not to overshoot.

Use four 250-mL replicates for each test concentration.

Concentration	Diluent	Sample
Control	1,000 mL	0 mL
12.5%	875 mL	125 mL
25%	750 mL	250 mL
50%	500 mL	500 mL
100%	0 mL	1,000 mL

11. For each concentration**,** seal the cylinder opening with Parafilm and invert 5 or 6 times. Pour 250 mL of the solution into each of the four replicate test jars for that test concentration. Repeat for each test concentration.

12. Measure pH, temperature, DO, and conductivity of the solutions in all jars and record on bench sheets.

Read pH, conductivity, DO, and temperature to one decimal place; however, read low-conductivity measurements (<10 mmhos/cm) to two decimal places. Take measurements proceeding from the lowest to the highest concentration.

13. Record any comments pertaining to the condition or appearance of the sample.

Record all readings in appropriate columns on *RO Project Bench Sheet*.

14. Load test organisms.

Per SOP #TA-10.07.

SOP #6: Preparation of Mock Seasalt Slurry

(FDEP Biology Section Internal SOP TA-10.08)

STEPS	COMMENTS
Materials	
1. Mock slurry salts: **a.** Sodium chloride, ACS grade **b.** Sodium sulfate, ACS grade **c.** Potassium chloride, ACS grade **d.** Potassium bicarbonate, ACS grade **e.** Potassium bromide, ACS grade **g.** Magnesium chloride, ACS grade **h.** Calcium chloride, ACS grade	Salts are kept in a dessicator.
2. Deionized (DI) water	
3. Mettler PC 440 Balance	Calibrate per SOP #TA-06.15
4. Mettler H51AR balance	Calibrate per SOP # BB-18
5. Metal spatula	
6. Magnetic stirrer	
7. Magnetic stir bar, 2.5 cm	
8. Magnetic stir-bar retriever	
9. Small weigh boats	
10. Medium weigh boats	
11. 500-mL Erlenmeyer flask	
12. 100-mL graduated cylinder	
13. Calibrated Epindorf pipette	
14. Parafilm	
Methods	
1. Add 100 mL of DI water to the 500-mL Erlenmeyer flask. Place the flask on the magnetic stirrer, put the stir bar into the flask, and turn the stirrer on.	A 500-mL Erlenmeyer flask is used to allow room for mixing before each use.
2. Measure an additional 100 mL of DI water to use as rinse water when adding salts to the flask.	

3. Use the Mettler PC 440 balance to weigh each of the following salts. Use separate medium sized weigh boats for each and use a small amount of DI water to rinse the clinging salts into the flask. Clean the spatula before and after each salt.

NaCl ±0.001g	24.083
Na_2SO_4 ±0.001g	4.010
$MgCl_2 \cdot 6H_2O$ ±0.001g	10.774
$CaCl_2 \cdot 2H_2O$ ±0.001g	1.534

Weigh per SOP #TA-06.15.

Use DI water previously measured as the rinse water.

4. Use the Mettler H51AR balance to weigh the following salt. Use a small-sized weigh boat and use a small amount of DI water to rinse the salt into the flask. Clean the spatula before and after use.

KCl ±0.00001g	0.54347

Weigh per SOP #BB-18

Use DI water previously measured as rinse water.

5. Use stock salt solutions to add the following salts.

Add 9.996 mL of 0.01 g/mL KBr stock.

Add 2.073 mL of 0.1 g/mL $KHCO_3$ stock.

For stock salt solutions see SOP #TA-10.09.

Use a calibrated Epindorf pipette.

These volumes of stock salts equals:

KBr	0.09996 ±0.00001g
$KHCO_3$	0.20725 ±0.00001g

6. Add any remaining DI water from the graduated cylinder into the Erlenmeyer flask and mix.

The mock slurry is very concentrated and will not dissolve completely.

7. Label the flask with "Mock Slurry for RO Only", the date, and your initials. Store in the walk-in refrigerator.

8. Mix thoroughly before and during each use.

SOP #7: Preparation of Natural Seawater Hyper-saline Brine

(FDEP Biology Section Internal SOP TA-03.09)

STEPS	COMMENTS
Materials	
1. Two 5-gallon buckets	
2. Walk-in freezer (kept at –20°C)	Nor-lake model W00787-4R
3. Natural seawater (NSW)	Collected per SOP #TA-3.10
4. One 2-L Erlenmeyer flask	
5. 0.45µm sterilized Versa flow filter capsule	Gelman Science
6. Parafilm™	
Methods	
1. Fill a 5-gallon bucket with NSW that has been filtered through a sterilized 0.45 µm Versa flow capsule.	
2. Freeze the NSW for 48-96 hours at –20°C. The salinity of the brine will be higher if frozen longer, but the volume yielded will be lower.	There is no advantage to keeping larger volumes of lower-salinity brine. It can always be diluted with DI water if necessary.
3. Remove the bucket of frozen NSW from the freezer. Put an empty 5-gallon bucket into the sink. Invert the bucket with the frozen NSW over the empty bucket in such a way that the block of ice shifts and settles on the lip of the empty bucket.	
4. Collect the concentrated NSW that drips into the empty bucket. When all of the hypersaline brine is collected, transfer it to a 2-L Erlenmeyer flask.	
5. Check the salinity of the hypersaline brine. Label the flask with "Hypersaline brine", the salinity, the date, and your initials. Cover with Parafilm, and store in the walk-in refrigerator.	Check salinity per SOP #TA-06.06.
6. Mix thoroughly before each use.	

SOP #8: Advance Test Preparation

(FDEP Biology Section Internal SOP TA.10.01)

STEPS	COMMENTS
Materials	
1. A minimum of 900 young mysids, *Mysidopsis bahia,* (1–5 days old, 24-hr age range) acclimated to appropriate test salinities.	Obtain Young mysids per SOP #TA-05.04 Acclimate 140 mysids for Test 1 like the *Menidia* above. Acclimate 240 mysids for Tests 2 and 3 to the salinity of the concentrate. Acclimate 260 mysids for Tests 4 and 5 (520 mysids if performing tests 4A & 4B, 5A & 5B) to the salinity of the ion-adjusted concentrates as determined by the *Ion-Adjustment Calculation Spreadsheet*. The salinity of the ion-adjustments will be higher than the concentrates but will not exceed 35 ppt. Records of monthly SRT (Standard Reference Toxicant) tests should be maintained for both test species.
2. Approximately 168 1-L glass bioassay jars. Each test should have jars labeled using a different colored tape to avoid lab errors.	Four replicates per concentration plus 4 replicates for the controls are used for each of the tests, for a total of 24 jars per test. As many as 7 tests may be required.
3. Natural Seawater (NSW)	The NSW is used for diluent and will need to be available at different salinities depending on the test and concentrate. Adjust it by adding deionized water (DI) to lower the salinity or by adding hypersaline brine to raise the salinity. Collect NSW per SOP #TA-03.08.
4. Hypersaline brine.	Prepare per SOP #TA-03.09.
5. Mock-sea-salt slurry	Prepare per SOP #TA-10.08.
6. Stock HCl and NaOH solutions for pH adjustments	Prepare per SOP #TA-10.10 and #TA-10.11.
7. ACS-grade salts: NaCl, Na_2SO_4, $NaHCO_3$, NaBr, KCl, K_2SO_4, $KHCO_3$, KBr, $MgCL_2 \cdot 6H_2O$, $MgSO_4$, $CaCl \cdot 2H_2O$, $CaSO_4 \cdot 2H_2O$, $CaCO_3$.	Store salts in dessicator.
8. Stock salt solutions.	If less than 0.5g of a salt is needed, it should be added as a concentrated stock solution. Prepare per SOP #TA-10.09.
9. 8" Carolina dishes	Transfer mysids from generator larvae cups to Carolina dishes daily. Also, hold the test animals in Carolina dishes during the different salinity adjustments, and when loading bioassay jars.
10. Small hand held screens, 500-µm Nitex®.	Used to transfer mysids from Carolina dish to test jars.
11. Meters to measure pH, temperature, D.O., and conductivity	Calibrate per SOP #TA-06.03 through #TA-06.06.
12. Hach® DR-100 chlorine test kit.	Calibrate per SOP #TA-06.07.

13. Hach® Alkalinity kit.	Calibrate per SOP #TA-06.08. Hardness is typically not measured if conductivity >5,000 µmhos/cm.
14. Orion® 901 specific-ion meter with ammonia probe	Calibrate per SOP #TA-06.10.
15. Temperature controlled testing area	Room should maintain 25±1°C and 16h:8h light:dark.
16. Live *Artemia* nauplii for feeding.	Prepared per SOP #TA-01.03.
17. Two 20-L carboys	
18. Six 6-L Erlenmeyer flasks	For mixing the ion-adjusted concentrates and mock ion- adjusted concentrates.
19. Graduated Cylinders: Two 1,000-mL; One 500-mL; Two 250-mL; One 100-mL	For measuring the dilution volumes.
20. Pipettes: Mechanical pipettor or pipette bulb; Calibrated 1-mL Epindorf pipettor with plastic pipette tips; Calibrated Epindorf adjustable micropipettor (up to 1mL, capable of reading to three decimal places) with plastic pipette tips.	For small volume measurements.
21. Sharpie marking pens	For labeling the jars, flasks, and beakers.
22. Parafilm	Used to cover open containers.
23. Mettler H51AR Balance	Calibrate per SOP #BB-18
24. Mettler PC440 Balance	Calibrate per SOP #TA 06.15
25. Weigh boats, assorted sizes	
26. Lab Con Co. dessicator	See *Comments* in SOP #TA-09.06 for desiccator operation.
27. Spatula**s**	For transferring chemicals during weighing.
28. Assorted stir bars	
29. Magnetic Stir Plate	
30. Magnetic stir-bar retriever	

Documentation

1. Log concentrate receipt, temperature, and condition into *Log-In Book: Bioassay*. In the *RO Logbook* record the above information plus the concentrate salinity, conductivity, and dissolved oxygen.	Per SOP #TA-06.14, including LIMS numbers from Chemistry. Use only black, waterproof pen for all logbook and bench-sheet entries. All entries must be initialed and dated.

2. Fill in information requested at top of the *RO Project Bench Sheets* as completely as possible—including daily calibrations—prior to test initiation.

Include full name of facility, date and time collected, field TRC, LIMS sample and job numbers, and chamber numbers. Some of this information will be available only after the login folder comes down from Chemistry. This step usually can be partially done ahead of time.

3. Corrections to bench sheet entries must be performed by placing a single line through the incorrect entry, writing the corrected entry as near its appropriate space as possible, and initialing and dating the correction.

Write an explanation of the error below the corrected entry; initial and date.

4. As the test progresses, any unusual occurrences or procedures must be recorded in the *RO Logbook* and on the *RO Project Bench Sheets.* The procedures used for doing the salt additions for the ion-adjustments are also recorded in the *RO Logbook.*

Typical entries might be a salt or pH adjustment or a precipitate noted.

Methods—Advance preparation

Before the concentrate arrives

1. Clean and label with color-coded tape all the necessary glassware and organize them on carts

8 possible tests, 5 concentrations +1 control per test, and 4 replicates per test + the possibility of extra controls. Approximately 200 jars need to be prepared.

2. Prepare bench sheets

3. Prepare a 20-L carboy of NSW adjusted to the concentrate salinity (if known).

4. Acclimate the mysids to the expected salinity of the initial concentrate test.

The salinity of the concentrate should be measured at the time of sampling and that information should be communicated to the bioassay laboratory so they can start acclimating some of the mysids to the appropriate salinity.

5. If the salinity of the concentrate is less than 7 ppt, the concentrate will need to be salinity adjusted up to 7 ppt in the bioassay laboratory using the mock seasalt slurry. The mysids used in the initial and baseline and mock concentrate tests must therefore be salinity adjusted down from their culture salinity of 20 ppt to 7 ppt.

Salinity adjust the mysids by placing the appropriate number in a Carolina bowl in their culture water and adding de-ionized water to bring the salinity down from 20 ppt to 15 ppt. The mysids should be held at 15 ppt for a minimum of two hours. Add de-ionized water to bring the salinity down to 12 ppt and hold for a minimum of two hours. Continue to bring the salinity down by 2 ppt with a minimum of two hours between each adjustment until the test salinity is reached.

On arrival of sample (Day 0)

1. Warm 5 L of concentrate up to test temperature (25˚C)	On day 0, approximately 4.5 L of concentrate will be needed for test 1.
2. Do preliminary physical-chemical analyses.	These include chlorine, alkalinity, pH, temperature, D.O., conductivity, and salinity. Record these in the *RO Logbook*. Refer to SOP's #TA 06.03–06.10.
3. Perform any final salinity acclimation of test animals in the Carolina dishes.	See Step 1 of *Materials* for this procedure.
4. Adjust a 20-L carboy of NSW to the salinity of the concentrate.	This is the diluent for Tests 1, 2, and 3.

On receipt of chemical analyses (Day 1)

1. If needed, make final salinity acclimation of mysids.	
2. Warm the remaining concentrate to test temperature (25˚C).	
3. Calculate the required salt additions for the mock concentrate and for the ion- adjustments.	See SOP #TA-10.12 for calculation methods.
4. Weigh out all salts needed for each test in separate weigh boats. If the required weight is less than 0.5g, then use a stock solution and measure a corresponding volume.	Salts are kept in the dessicator. Salts ≥1g are weighed on the Mettler PC440 balance per SOP #TA 06.15. Weights between 0.5g and 1g are weighed on the Mettler H51AR balance per SOP #BB 18. For weights <0.5g, prepare stock solutions per SOP #TA-10.09) For example, if 0.47790g of KBr is needed and the concentration of the stock is 0. 1 g/mL then the amount of stock necessary would be 4.779 mL. Use a calibrated Epindorf pipettes. Accuracy is very important in salt measurements.
5. Group the salts by test.	The chemicals going into the mock concentrate should be in one area. The chemicals going into the ion- adjusted concentrate should be in a separate area and so on. This is to help prevent errors.

Methods

Test 1- NSW at the concentrate salinity is the diluent. Concentrate is the effluent.	Refer to SOP #TA-10.02.
Test 2 - NSW at the concentrate salinity is the diluent. Concentrate is the effluent.	Refer to SOP #TA-10.03.
Test 3 - NSW at the concentrate salinity is the diluent. Mock concentrate is the effluent.	Refer to SOP #TA-10.04.
Test 4 - NSW at the salinity of the ion-adjusted concentrate is the diluent. Ion-adjusted concentrate is the effluent	Refer to SOP #TA-10.05. There is the possibility of 2 tests (4A and 4B).
Test 5 - Mock ion-adjusted concentrate is the diluent. Ion-adjusted concentrate is the effluent.	Refer to SOP #TA-10.06. There is the possibility of 2 tests (5A and 5B).

SOP #9: Test-Organism Loading, Interim Readings, and Test Termination

(FDEP Biology Section Internal SOP TA-10.07)

STEPS	COMMENTS
Materials	Refer to SOP #TA-10.01.
Documentation	Refer to SOP #TA-10.01.
Advance Preparation	Refer to SOP #TA-10.01.
Test Setup	Refer to SOP #TA-10.02 through #TA-10.06.
Test-Organism Loading	
Mysid shrimp loading	
1. Using a 4 cm x 6 cm piece of 100-µm Nitex® screen, lift 5 mysids from the Carolina dish of the appropriate salinity into each test jar. Start at the lowest test concentration and rinse the screen in DI water between transfers. Verify loading counts over a light table. Record counts in appropriate columns on acute bioassay bench sheets in laboratory notebook	"Appropriate salinity" refers to test organisms that have been acclimated to salinity within ±2‰ of the test salinity. See SOP #TA-10.01 for test-organism acclimation. (5 mysids per replicate) x (4 replicates per test concentration) = 20 mysids per concentration tested. Record counts and physical-chemical readings in appropriate columns in *R/O Project Bench Sheets.*
2. Add one to two drops of *Artemia* concentrate daily to each test jar (approximately 50 *Artemia* nauplii per mysid).	Mysids are fed daily throughout test. Prepare *Artemia* nauplii by light separation (SOP #TA-01.03) then pouring onto a 60-µm mesh screen and rinsing with DI water to remove the saltwater. Rinse nauplii off the screen with a small volume of DI water to obtain a concentrated *Artemia* solution.
3. Cover test jars with a sheet of clear acrylic, allowing a 1/4'' gap between the lips of the test jars and the acrylic for air circulation.	Covers prevent accidental contamination and minimize evaporation.
Interim Readings	
1. At 24, 48, and 72 hrs into the test, record the number of surviving mysids in all jars and the pH, temperature, DO, and conductivity from jars that have 100% mortality. After recording parameters, feed the mysids and cover. Record in small superscript next to the number alive the status of any animals that are dead or missing	Counting is done over a light table. Mysids are recorded as alive if they move when lightly prodded or appendage movement is seen. Record counts and physical-chemical readings in appropriate columns in *R/O Project Bench Sheet.* D = Dead animals, body seen. M = Missing animals, no body found.

2. As the test progresses, any unusual observations or procedures must be recorded in the *RO Logbook* and on the *RO Project Benchsheets.*

An example of an entry might be a precipitate noted or unusual behavior or appearance of the test organisms. Any observations, whether seemingly important or not, may prove invaluable for later test interpretation.

Test Termination

1. On termination day, t = 96 hr, count the number of surviving mysids. Afterward, measure parameters of the solutions in all test jars. Record all counts and readings. Record any comments pertaining to the appearance or condition of the mysids. Record test termination date and time.

Counting is done over a light table. Physical-chemical readings (pH, temperature, D.O., and conductivity) are taken in all test jars. If, at t = 0, the total ammonia was greater than 1 mg/L, then a final ammonia reading must be taken.

Record counts, readings, date, and time in appropriate columns in *RO Project Bench Sheet.*

2. Pour contents of all jars through a fine mesh sieve into sink and flush drain with water. Remove labels and take glassware to be washed.

3. Kill mysids collected in mesh sieve by placing them in iced DI water.

4. Enter test results into statistical program to obtain the LC_{50} if 50% or greater mortality occurs in any test concentration.

Per SOP #TA-07.07.

5. Enter test results into *Sample Log*.

SOP #10: Preparation of Stock Salt Solutions

(FDEP Biology Section Internal SOP TA-10.09)

STEPS	COMMENTS
Materials	
1. ACS-grade salts, as desired	Salts are kept in a dessicator.
2. Deionized (DI) water	
3. Mettler PC 440 Balance	Calibrate per SOP #TA-06.15
4. Metal spatula	
5. Magnetic stirrer	
6. Magnetic stir bar, 1.0 cm	
7. Magnetic stir-bar retriever	
8. Medium weigh boats	
9. 100-mL volumetric flasks	
10. Calibrated Epindorf pipette	
11. Parafilm™	
Method to create 0.1g/mL salt solutions	
1. Use the Mettler PC440 balance to weigh 10g of a salt into a weigh boat.	Weigh per SOP #TA-06.15 Multiples of 10 are easy numbers to work with.
2. Fill a 100-mL volumetric flask half way with DI water.	
3. Add the 10g of salt to the 100-mL volumetric flask using a little DI water to rinse the salt into the flask.	
4. Add a stir bar and place the flask on the magnetic stirrer. Allow the salt to dissolve.	It may be difficult to dissolve 10g of some salts. These salts may be made up in 0.01g/mL solutions.
5. When the salt is dissolved, remove the stir bar and add DI water to bring the flask up to the 100-mL mark.	
6. Label the flask with "0.1g/mL [salt] stock", batch number, the date, and your initials. Store in the walk-in refrigerator.	The batch number is obtained by adding one to the last batch number recorded in the *Media Preparation* book.
7. Record batch information in the *Media Preparation* book.	
8. Mix thoroughly before each use.	
Method to create 0.01g/mL salt solutions from 0.1 g/mL stock salt solution	

1. If you were able to make a 0.1g/mL stock solution of the desired salt and you wish to make a 0.01g/mL stock solution**,** remove 10 mL of the 0.1g/mL salt stock solution and put it into a 100-mL volumetric flask.

   Use a calibrated Epindorf pipette.

2. Fill the flask with DI water to the 100-mL mark. Shake vigorously.

3. Label the flask with "0.01g/mL (salt) stock", batch number, the date, and your initials. Store in the walk-in refrigerator.

   The batch number is obtained by adding one to the last batch number recorded in the *Media Preparation* book.

Method to create 0.01g/mL salt solutions from reagent salts

4. If a 0.1g/mL stock solution cannot be made (i.e. 10g would not dissolve in 100 mL of DI water), a 0.01g/mL stock solution can be made. Use the Mettler PC440 balance to weigh 1.0g of a salt.

   It is preferable to make this stock solution as a serial dilution to maximize the accuracy of the resulting concentration; however, in those cases where the salt has low solubility, they can be made using the dry salt.

   Weigh per SOP #TA-06.15

5. Fill a 100-mL volumetric flask half way with DI water.

6. Add the 1.0g of salt to the 100-mL volumetric flask using a little DI water to rinse the salt into the flask.

7. Add a stir bar and place the flask on the magnetic stirrer. Allow the salt to dissolve.

8. When salt is dissolved, remove the stir bar and add DI water to bring the flask up to the 100-mL mark.

9. Label the flask with "0.01g/mL (salt) stock", batch number, the date, and your initials. Store in the walk-in refrigerator.

   The batch number is obtained by adding one to the last batch number recorded in the *Media Preparation* book.

10. Record batch information in the *Media Preparation* book.
11. Mix salt solutions thoroughly before each use.

SOP #11: Preparation of Stock HCl (Acid) Solutions

(FDEP Biology Section Internal SOP TA-10.10)

STEPS	COMMENTS

Materials

1. Technical Grade 12N hydrochloric acid
2. Deionized (DI) water
3. 1-L volumetric flask
4. Fume hood
5. Parafilm

Methods

1. Add 800 mL of DI water to the 1-L volumetric flask.

Caution!

Exothermic Reaction!

HOT!

Wear Goggles!

Wear Gloves

Use the Fume Hood!

2. Under the fume hood, **slowly** add 100 mL of 12N HCl to the flask, making sure to mix the solution well.

Handle the HCl with care! Strong acid.

3. Carefully, bring the total volume of the flask up to 1-L with DI water. Tightly seal with a teflon stopper or acid resistant plastic.

Seal the flask. Any evaporation will result in the HCl concentration increasing.

4. Label the flask with "1.2N HCl Acid Stock Solution", batch number, the date, and your initials.

The batch number is obtained by adding one to the last batch number recorded in the *Media Preparation* book.

5. Record batch information in the *Media Preparation* book.

SOP #12: Preparation of Stock NaOH (Base) Solutions

(FDEP Biology Section Internal SOP TA-10.11)

STEPS	COMMENTS
Materials	
1. Sodium hydroxide, ACS grade	Salts are kept in a dessicator.
2. Deionized (DI) water	
3. 1-L volumetric flask	
4. 250-mL volumetric flask	
4. Mettler PC 440 balance	Calibrate per SOP #TA-06.15
5. Metal spatula	
6. Medium weigh boats	
7. Teflon or plastic stopper	
8. Parafilm™	
Method to make 10N NaOH stock solution	
1. Use the Mettler balance to weigh 100g of NaOH into a weight boat.	Weigh per SOP #TA-06.15.
2. Add 100 mL of DI water to a 250-mL volumetric flask.	
Caution!	
Exothermic Reaction!	
HOT!	
Wear Goggles!	
Wear Gloves	
Use the Fume Hood!	
3. Under the fume hood, **slowly** add 100g of NaOH crystals to the flask, 2–3g at a time. Allow NaOH to dissolve completely before adding more.	*Do not allow flask to get too hot!*
4. Once all the NaOH crystals are added and dissolved, carefully bring the volume up to 250-mL with DI water.	
5. Label the flask with "10N NaOH Base Stock Solution", batch number, the date, and your initials.	The batch number is obtained by adding one to the last batch number recorded in the *Media Preparation* book.
Method to make 1.0N NaOH stock solution	
1. Add 800 mL of DI water to a 1-L volumetric flask.	

Caution!

Exothermic Reaction!

HOT!

Wear Goggles!

Wear Gloves

Use the Fume Hood!

2. Under the fume hood, **slowly** add 100 mL of 10N NaOH stock solution to the flask, mixing the solution well. *Do not allow flask to get too hot!*

3. Carefully, bring the total volume of the flask to 1-L with DI water.

4. Do not use a glass stopper to seal the volumetric flask, as it will stick to the flask. Use a teflon or plastic stopper, or cover with Parafilm. Seal the flask. Any evaporation will result in the NaOH concentration increasing.

5. Label the flask with "1.0N NaOH Base Stock Solution", batch number, the date, and your initials. The batch number is obtained by adding one to the last batch number recorded in the *Media Preparation* book.

6. Record batch information for both stock base solutions in the *Media Preparation* book.

SOP #13: Calculation of Ion Additions

(FDEP Biology Section Internal SOP TA-10.12)

STEPS	COMMENTS
Materials	
1. Microsoft Excel™ 5.0 spreadsheet, "Ion_Calc".	Available from Florida Dept. of Environmental Protection, Biology Section, Tallahassee, FL 32399.
2. Results of major-seawater-ion analyses of concentrate sample.	To include: Na^{+}, Ca^{++}, Mg^{++}, K^{+}, Cl^{-}, Br^{-}, $SO_4^{=}$, and alkalinity
Method	Note: the term ions will be used to indicate the major seawater ions being manipulated by this test protocol.
1. Open spreadsheet file *Ion Calc Template*, which consists of a set of eight work sheets in a 'workbook'.	
2. Click on the tab labeled *Salts 1 (4&5, 4B & 5B)* at the bottom of the workbook if it is not already selected.	If salts with hydration numbers different than those included are needed, unlock the spreadsheet and alter the appropriate value in the lookup tables on worksheets *Salts 1* (headed by cell H41) and *Salts 2* (headed by cell G39).
Worksheet *Salts 1 (4&5, 4B & 5B)*	This worksheet is used with all samples and tests
Enter basic data and determine test protocol	
1. Enter the facility name, sampling date, salinity, and concentrate alkalinity in the appropriate cells at the top of the worksheet.	Determine salinity by measuring conductivity. Spreadsheet cells where data is to be entered are light blue with a bold black border. Other cells are locked. Cells containing important information needed for the calculations are generally light yellow.
2. In column B under *Sample Concentration*, enter the amounts of each ion occurring in the unaltered concentrate sample.	Note that the carbonate concentration is calculated from the alkalinity and is not entered manually.
3. Column I under *Key ion for adjustment* indicates the ion most out of proportion relative to its seawater concentration.	This ion will not be altered in subsequent manipulations and will determine the amount of adjustment necessary to bring the other ions into seawater proportions.

4. Cell E17 shows the 'salinity' of the concentrate that would result from adjusting the ions to seawater proportions based upon the previously determined key ion.

If this salinity is greater than 35 ppt, then the A&B portions of Tests 4 & 5 will be necessary and the worksheet *Salts 2* must also be used.

Note: the following calculations determine the combinations of salts to use for ion-adjusting a sample or to create a mock sample. This can be achieved using many different combinations of the available salts. There is no single "correct" answer; the goal is to minimize the amount of any ions remaining incompletely adjusted.

Determine salts for creating ion-adjusted concentrate (or ion-adjusted concentrate B)

Ion-adjusted concentrate is used in tests 4 & 5 or in tests 4B and 5B.

5. In the black-bordered blue cells headed by cell B40, enter the number of moles of salts needed to ion adjust the concentrate.

Beginning with cell F27, the light-yellow cells within the double red border show the needed moles of each ion needed to create a balanced, ion-adjusted concentrate. All concentrations are in molar units so that charges can be simply balanced.

Typically, entering the moles of KBr (into cell B47) equivalent to the needed moles of Br^- (cell F34) is an appropriate first step. Entering the salts to provide the smallest needed-ion concentrations first generally works best.

Leave the Na^+ and Cl^- ions until last. One should normally be able to reduce all the needed ions to zero with the exception of Na^+ and/or Cl^-. However, these ions are in much greater concentrations and the error should be minor. Cells G27 and G31 will give you information on the percent difference between these two ions.

If the 'salinity' is high or moderate, split any charge imbalance between Na^+ and Cl^-. If it is low, leave either Na^+ or Cl^- in excess and neither in deficit. This is to provide the highest 'salinity' practical.

Determine salts for creating mock concentrate

Mock concentrate is used in tests 3 and in preparing solutions for Tests 4B & 5B.

6. In the black-bordered blue cells headed by cell B75, enter the number of moles of salts needed to create the mock concentrate.

Proceed similarly to step 5.

Beginning with cell D62, the light-yellow cells within the double red border show the needed moles of each ion needed to create a balanced, ion-adjusted concentrate.

Typically, entering the moles of KBr (into cell B82) equivalent to the needed moles of Br^- (cell D69) is an appropriate first step. Entering the salts to provide the smallest needed-ion concentrations first generally works best.

Leave the Na^+ and Cl^- ions until last. One should normally be able to reduce all the needed ions to zero with the exception of Na^+ and/or Cl^-. However, these ions are in much greater concentrations and the error should be minor. Cells E62 and E66 will give you information on the percent difference between these two ions.

If "salinity" is low, don't leave Na^+ or Cl^- in deficit. If "salinity" is high, don't leave Na^+ or Cl^- in excess. This is to minimize very high or low ionic strengths.

7. If it is necessary to add acid or base in the process of dissolving the salts, the acid and base amounts (in mL) should be entered into the appropriate black-bordered blue cell (headed by cell D89) before the NaCl is added. Any resulting corrections to the amount of NaCl needed should be made on the spreadsheet and in the lab.

Be sure that the acid and base amounts are entered into the cell for the correct strength of acid or base.

With some concentrates, all Na^+ and Cl^- may be added using salts other than NaCl. In those cases, the corrections will need to be made for the appropriate Na^+ and Cl^- salts.

8. If the salinity in cell E17 was 35 ppt or less, proceed to *Worksheet Makeup 1* and click on the tab labeled *Makeup 1* at the bottom of the workbook. Otherwise, click on the tab labeled *Salts 2 (4A & 5A)* at the bottom of the workbook and continue with the next step.

Worksheet *Salts 2 (4A & 5A)*

Determine salts for ion-adjusted concentrate A

Ion-adjusted concentrate A is used in tests 4A & 5A.

1. The information at the top of the worksheet duplicates that on worksheet *Salts 1*.

2. In the black-bordered blue cells headed by cell B38, enter the number of moles of salts needed to ion adjust the concentrate.

Proceed similarly to step 5.

Beginning with cell F25, the light-yellow cells within the double red border show the needed moles of each ion needed to create a balanced, ion-adjusted concentrate. Some of the ions will show negative numbers. These ions are the ones being left unbalanced. Ignore them.

Typically, entering the moles of KBr (into cell B45) equivalent to the needed moles of Br^- (cell F32) is an appropriate first step. Entering the salts to provide the smallest needed-ion concentrations first generally works best.

Leave the Na^+ and Cl^- ions until last. One should normally be able to reduce all the needed ions to zero with the exception of Na^+ and/or Cl^-. However, these ions are in much greater concentrations and the error should be minor. Cells G25 and G29 will give you information on the percent difference between these two ions.

If the 'salinity' is high or moderate, split any charge imbalance between Na^+ and Cl^-. If it is low, leave either Na^+ or Cl^- in excess and neither in deficit. This is to provide the highest 'salinity' practical.

3. Click on the tab labeled *Makeup 1* at the bottom of the workbook.

Worksheet *Makeup 1*

This worksheet calculates the amount of each salt needed for the solutions in *Salts 1* based upon the volume of each solution to be made. This volume will depend in part on the volume of the test containers. The default volumes can serve as starting points.

Ion-adjusted concentrate (or ion-adjusted concentrate B)

1. Cell C24 (dark yellow with double red border) will show the total volume of ion-adjusted concentrate (or ion-adjusted concentrate B) that will result. Enter various volumes of concentrate into cell C5 (blue with bold black border) until cell C24 shows the desired total volume.

This is a somewhat indirect method, but avoids the need for an iterative calculation and is quick to perform.

2. The light-yellow cells within the double red border headed by cell C8 show the amount of each salt to weigh out.

Add these amounts of salts to the appropriate amount of unaltered concentrate in order to produce the appropriate ion proportions.

3. If the 'salinity' of the resulting ion-adjusted concentrate would be >35 ppt, cell C23 (light yellow with double red border) shows the amount of DI water to include in producing the final solution.

Salts will normally dissolve more easily if most of the DI water is added before the salts.

Mock ion-adjusted concentrate (or mock ion-adjusted concentrate B)

4. As above, cell H24 (dark yellow with double red border) will show the total volume of mock ion-adjusted concentrate (or mock ion-adjusted concentrate B) that will result. Enter various volumes of mock concentrate into cell H5 (blue with bold black border) until cell H24 shows the desired total volume.

5. The light-yellow cells within the double red border headed by cell H8 show the amount of each salt to weigh out.

Add these amounts of salts to the appropriate amount of mock concentrate in order to produce the appropriate ion proportions.

6. If the 'salinity' of the resulting mock ion-adjusted concentrate would be >35 ppt, cell H23 (light yellow with double red border) shows the amount of DI water to include in producing the final solution.

Salts will normally dissolve more easily if most of the DI water is added before the salts.

Mock concentrate

7. Enter the needed volume of mock concentrate into cell C29 (blue with bold black border).

8. The light-yellow cells within the double red border headed by cell C32 show the amount of each salt to weigh out.

Add these amounts of salts to the appropriate amount of DI water in order to produce the appropriate ion proportions.

There is some saturation information to the right of some of the salts. It may sometimes be necessary to assemble the mock concentrate from a different array of salts to get the needed ions into solution.

9. This sheet is made to be printed out and includes columns to help QA/QC during the test manipulations.

Columns are included for the initials of the staff weighing out each salt, the balance used to weigh the salt, and the initials of the staff adding the salt to the solution. It is strongly recommended that these steps be taken to minimize errors. More than one copy of the sheets may be used.

10. Click on the tab labeled *Makeup 2* at the bottom of the workbook.

Worksheet *Makeup 2*

This worksheet calculates the amount of each salt needed for the solutions in *Salts 2* based upon the volume of each solution to be made. This volume will depend in part on the volume of the test containers. The default volumes can serve as starting points.

Ion-adjusted concentrate A

1. Enter the needed volume of ion-adjusted concentrate A into cell C4 (blue with bold black border).

2. The light-yellow cells within the double red border headed by cell C7 show the amount of each salt to weigh out.

Add these amounts of salts to the appropriate amount of unaltered concentrate in order to produce the appropriate ion proportions.

Mock ion-adjusted concentrate A

3. Enter the needed volume of mock concentrate into cell H4 (blue with bold black border).

4. The light-yellow cells within the double red border headed by cell H7 show the amount of each salt to weigh out.

Add these amounts of salts to the appropriate amount of mock concentrate in order to produce the appropriate ion proportions.

5. This sheet is made to be printed out and includes columns to help QA/QC during the test manipulations.

Columns are included for the initials of the staff weighing out each salt, the balance used to weigh the salt, and the initials of the staff adding the salt to the solution. It is strongly recommended that these steps be taken to minimize errors. More than one copy of the sheets may be used.

6. Click on the tab labeled *For Chem, no A&B* if Tests 4A &4B and 5A & 5B will not be necessary. Click on the tab labeled *For Chem, A&B* if Tests 4A &4B and 5A & 5B are necessary.

Worksheet *For Chem, no A&B*
or
Worksheet *For Chem, A&B*

1. Print whichever of these sheets is appropriate and include them with samples being analyzed to confirm the final major seawater ion concentrations. All values are approximate and are generated automatically.

These follow-up analyses are necessary to confirm the major seawater ion concentrations in the final solutions and the information on these sheets will assist the chemists in determining appropriate dilutions prior to analysis.

Note: these values are approximations.

2. Click on the tab labeled *Follow-up Chem*.

Worksheet Follow-*up Chem*

1. When the results of the follow-up analytical chemistry are received, enter them into the appropriate cells of this worksheet. Blocks of cells where data can be entered are blue surrounded by a bold black border.

Carbonate concentrations are calculated by the spreadsheet from alkalinity values.

2. Beside the columns of data for the various solutions are calculations comparing the various solutions to each other or to seawater of an appropriate salinity. Cells containing these calculations are light yellow.

Good lab methods and good analytical chemistry should provide final ion concentrations within approximately 10% of the goals.

Results too far from those expected probably indicate laboratory errors in synthesis or analysis.

3. Click on the tab labeled *Tox Results*.

Worksheet *Tox Results*

1. Enter the results of the toxicity tests into the appropriate places on this worksheet.

The cells that are editable are blue.

This sheet is intended to juxtapose the toxicity test results as an aid to interpretation. This sheet performs no calculations.

2. Compare test results to interpret cause(s) of toxicity. This interpretation requires a thorough understanding of the test methodology and rationale. Read *Protocols for Determining Major-Seawater-Ion Toxicity in Membrane-Technology Water-Treatment Concentrate* for more information.

Protocols for Determining Major-Seawater-Ion Toxicity in Membrane-Technology Water-Treatment Concentrate is available from the Florida Department of Environmental Protection, Central Biology Laboratory, Tallahassee.