Hyper-Salinity Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols

Final Report

Prepared by:

Bryn M. Phillips, Brian S. Anderson, Katie Siegler, Jennifer P. Voorhees, Scott Katz, Lydia Jennings, and Ron S. Tjeerdema

University of California, Davis, Department of Environmental Toxicology

Prepared for:

California State Water Resources Control Board

Agreement Number 11-133-250

July 2012

Introduction

A growing number of coastal cities in California are exploring the potential for ocean desalination as a means to augment freshwater supplies. There are currently a number of desalination facilities proposed or operating in California. The process of desalination most commonly utilizes reverse osmosis (RO) to remove salts from groundwater, reclaimed water, and seawater. This process results in production of hypersaline reject brine. This reject brine may be discharged into the marine environment, sometimes in combination with discharge from a sewage treatment plant or power plant effluent. Typical RO reject brine produced using 100% seawater can have approximately twice the original salt content of seawater.

In order to ensure that brine discharges do not pose risk to coastal receiving waters, it is anticipated that desalination plants will be required to monitor effluent toxicity. Monitoring requirements will likely include testing for effluent toxicity using some combination of the short-term chronic toxicity test protocols listed in the 2009 California Ocean Plan (SWRCB, 2009).

While the tests listed in the Ocean Plan have been the subject of ongoing research, no comprehensive studies of the effects of hyper-salinity have been conducted with all seven toxicity test organisms (nine protocols when including sand dollar and urchin fertilization endpoints). The results from these tests will allow determination of the toxicity of hypersaline brine in the absence of any additional toxic constituents that may be produced during the desalination process. These results provide resource managers with background information to facilitate interpretation of toxicity test results from desalination plant effluent testing and permitting of desalination facilities. The results will also be used in the development of an amendment to the California Ocean Plan that addresses impacts to marine life from intakes and discharges from desalination facilities.

Methods

Hyper-saline Brine Toxicity Tests

The seven organisms assessed in this project are listed in the California Ocean Plan (SWRCB, 2009). All toxicity tests followed U.S. Environmental Protection Agency (U.S. EPA) west coast methods (U.S. EPA, 1995), with the exception of the mysid test (U.S. EPA, 2002). Short-term chronic larval development tests were conducted with the bay mussel (*Mytilus galloprovincialis*, Carlsbad Aquafarms), the purple sea urchin (*Strongylocentrotus purpuratus*, Marine Pollution Studies Laboratory - Granite Canyon (MPSL)), the sand dollar (*Dendraster excentricus*, San Diego population supplied by Dave Gutoff, and local population supplied by MPSL), and the red abalone (*Haliotis rufescens*, MPSL, Monterey Abalone Co., and Cultured Abalone Co.). The purple sea urchin and the sand dollar were also tested with the fertilization endpoint. Giant kelp (*Macrocystis pyrifera*, MPSL) was tested with the germination and germ tube growth endpoints. Topsmelt (*Atherinops affinis*, Aquatic Bio Systems) was tested with the survival and biomass endpoints, and mysid shrimp (*Americamysis bahia*, Aquatic Bio Systems) was tested with the survival and growth endpoints. All organisms were acclimated to ambient marine salinity (34‰) prior to test initiation. Brief descriptions of the toxicity tests are below.

Larval development tests were conducted in 20 mL scintillation vials containing 10 mL of test solution. Abalone and mussel tests were conducted for 48 hours, whereas urchin and sand dollar tests were conducted for 72 hours. Fertilization tests were conducted for 40 minutes in scintillation vials containing 5 mL of test solution. Giant kelp tests were conducted in 250 mL crystallizing dishes containing 200 mL of solution. Topsmelt tests were conducted in one-liter beakers containing 200 mL of test solution and mysid tests were conducted in 600 mL beakers containing 200 mL of test solution.

Sample Preparation

Brine was prepared by freezing pristine MPSL seawater that was filtered to 1 µm. The seawater source at MPSL-Granite Canyon is isolated from sources of ambient contamination, and this seawater is used by numerous other west coast toxicity testing laboratories. Brine produced using the freezing method is recommended for effluent salinity adjustments in the USEPA Guidance document (U.S. EPA, 1995). Seawater was frozen overnight and the brine was separated from the frozen freshwater component. Resulting brine had a salinity of approximately 70‰. Range-finder tests consisted of six salinities ranging from 34‰ (ambient) to as high as 70‰, depending on the organism (Table 1). Definitive tests were organism-specific and were designed based on the result of the range-finding tests. Salinities used in the second definitive test were sometimes different from the first definitive test, in order to better resolve the statistical endpoints. Salinities were also sometimes unevenly spaced in order to focus the dilution series around the effect concentration. The test salinities listed in Table 1 are measured salinities from the actual exposures.

Three tests were also conducted with RO reject brine discharged from the desalination facility at the Monterey Bay Aquarium. Brine discharge from this facility ranged in salinity from 51-55‰. The brine discharge was tested in a dilution series with the larval topsmelt, mussel development, and giant kelp protocols. Test concentrations were 0, 20, 40, 60, 80, and 100% effluent, which roughly equated to salinities of 34, 38, 42, 46, 50, and 54‰.

All tests consisted of a range of seawater samples adjusted with hypersaline brine to give a range of salinities and a negative control consisting of 1-um filtered seawater. Controls for the brine addition were also tested. These ambient salinity controls consisted of the same volume of brine used in the highest salinity concentration. In some cases the volume of brine added to create very high salinity treatments designed to affect the more salinity-tolerant test organisms exceeded the volume needed to create a high-salinity brine control. In these cases, a 50% brine control was created by combining equal parts brine (70‰) and Nanopure water (0‰), resulting in a brine control at approximately ambient salinity (35‰). Definitive tests and effluent tests were also accompanied by copper, cadmium, or zinc reference toxicant tests, as appropriate for each protocol.

The salinity of the test solutions was measured with a Fisher Accumet electrode standardized to ambient salinity (34‰). If the dilution series contained samples with salinities greater than 55‰, the electrode could not be used because hypersaline calibration standards were not available. Samples from these dilution series were measured with a refractometer. Accuracy of the refractometer was confirmed at salinities less than 55‰ using the Accumet electrode. Dissolved oxygen concentration and pH were also measured using a Fisher Accumet water quality meter and appropriate electrodes. Temperature was measured at the initiation and termination of the test using a standard thermometer, and throughout the test using a continuous temperature recorder.

Data Analysis

Dilution series data from the salinity tolerance tests were analyzed using Comprehensive Environmental Toxicity Information System software (CETIS 1.8.4.14, Tidepool Software, McKinleyville, CA). No observed effect concentrations (NOECs), lowest observed effect concentrations (LOECs), and median effect concentrations (EC50s for survival, development and germination) or 25% effect concentrations (EC25s for biomass, growth and germ tube length) were calculated for range-finder and definitive tests. 95% confidence limits (CL) were calculated for EC50s and EC25s. Brine controls were statistically compared to dilution seawater controls using a separate-variance t-test to determine if the brine had a significant effect on the test organism. Dilution series data from the reference toxicant tests were analyzed for the same statistics as the salinity tolerance tests. Median effect concentrations and EC25s were plotted in control charts to determine if organism responses to reference toxicants were within the range of historic tests.

Results

Quality Assurance

Control responses for all tests were greater than U.S. EPA test acceptability criteria. Two brine control responses were significantly different from their respective dilution seawater control responses, indicating that the hypersaline brine caused an effect beyond the alteration of the sample salinity. The brine control was used in both statistical analyses. Reference toxicant tests were conducted with each definitive test, and control charts were constructed for protocols that had at least three historical tests in addition to the two current tests. In cases where at least five reference toxicant tests were available for evaluation, effect concentrations (EC50 and EC25) were all within two standard deviations of a running mean (Appendix A). For the red abalone development endpoint, sand dollar endpoints, and mysid shrimp endpoints, all effect concentrations were comparable to published values (Lussier et al., 1985; Dinnel et al., 1989; Hunt and Anderson, 1989; Bailey et al., 1995).

Salinity Tolerance

The California Ocean Plan toxicity test protocols demonstrated a wide range of salinity tolerances, with the euryhaline topsmelt and mysid shrimp, and the giant kelp being the most tolerant to elevated salinities (Table 1). The most sensitive protocols were the marine larval development tests. Red abalone, purple sea urchin, and sand dollar were more sensitive than mussels, which also occur in estuarine environments. The marine fertilization endpoints (purple urchin and sand dollar) were less sensitive than their corresponding development endpoints, but the sand dollar fertilization test was more sensitive than the mussel larval development test. Mysid shrimp survival was less sensitive than the echinoderm embryo development and fertilization protocols. A mysid growth effect was not observed at the same concentrations tested for mysid neonate survival because complete mortality occurred before a growth effect registered. The giant kelp germination endpoint was less sensitive than the mysid shrimp survival endpoint, but the kelp germ tube EC25 was comparable to the mysid EC50. Both topsmelt survival and biomass endpoints produced comparable summary statistics (EC50 for survival or EC25 for biomass) of approximately 60‰.

Salinity tolerance values from these experiments are corroborated by results of other researchers. Pillard et al. (Pillard et al., 1999) used a modified U.S. EPA artificial seawater (GP2) formula to produce hypersaline brine for 48 hour exposures using *Americamysis bahia (*formerly *M. bahia)*. The 48 hour LC50 for *A. bahia* was 43.03‰. Bay and Greenstein (Bay and Greenstein, 1994) assessed salinity tolerance of giant kelp and purple sea urchin development using test solutions produced from frozen seawater brine. Solutions of 33.5 - 43‰ did not affect kelp spore germination or germ tube growth. Sea urchin development was significantly reduced at 36.5‰, a lower value than observed in the current tests.

Brine Effluent Toxicity

Seawater RO desalination discharge effluent from the Monterey Bay Aquarium was tested with mussels, giant kelp and topsmelt protocols. The salinity of the three undiluted effluent samples ranged from 51-55‰ (Table 2). The sensitivities of the topsmelt endpoints were both greater than the salinity of the highest concentration of effluent, so no effect from the effluent was observed on topsmelt survival or growth , (NOEC=50.8; LOEC and EC50 > 50.8‰). These results are within the range of effluent tests reported elsewhere using topsmelt larvae exposed to RO effluent. For example, Weston Solutions (2007) conducted 96-hour larval topsmelt tests with RO concentrate from a desalination plant. In this experiment, 15 day-old topsmelt larvae were exposed to a series of RO dilutions with salinities ranging from 36 to 60‰. The NOEC, LOEC, and LC50 from these experiments were 42‰, 44‰, and 58.6‰, respectively.

The sensitivity of the giant kelp germination endpoint was also greater than the salinity of the highest effluent concentration, but the germ tube growth endpoint was not. The germ tube growth EC25 (based on effluent salinity) was 51.8, whereas the mean EC25 from the frozen seawater brine tolerance tests was 47.3. These results demonstrate that the effluent did not have any greater effect on the growth of germ tubes beyond that exerted by salinity. Bay and Greenstein (1994) also assessed toxicity of a RO reject brine (from the Diablo Canyon Power Plant), and found no effects on kelp germination and growth, and no effect on sea urchin fertilization in solutions of 10% RO brine mixed with seawater.

The mussel salinity tolerance was lower than the salinity of the undiluted effluent, and a significant effect was observed in the effluent test, but the effluent EC50 (calculated from the salinity of the effluent concentrations) was the same as the mean EC50 from the salinity tolerance experiments (43.3‰). This indicates that salinity was the only component of the effluent that contributed to the observed toxicity.

Table 1. No observed effect (NOEC), lowest observed effect (LOEC), and median effect concentration (EC50) or 25% effect concentration (EC25) for range-finder and definitive tests. 95% confidence limits (CL) are presented for the EC50 and EC25. Mean EC is the average of the two definitive test results. All results are based on measured salinities.

Protocol	Endpoint	Test	Measured Test Solution Salinities	NOEC	LOEC	EC50	95% CL	Mean EC
Red Abalone	Development	Range Finder	35, 42, 49, 56, 63, 70	34	>34	37.8	NA	
		Definitive 1	34, 35, 36, 37, 38, 39, 40	34.9	35.6	36.4	36.4-36.5	
		Definitive 2	34, 35, 36, 37, 38, 39, 40	34.9	35.6	37.1	37.0-37.2	36.8
Purple Urchin	Development	Range Finder	34, 40, 47, 55, 62, 70	34	40	36.9	36.9-36.9	
		Definitive 1	34, 35, 36, 37, 38, 39, 40, 41, 42	35.5	36.8	37.9	37.8-37.9	
		Definitive 2	34, 35, 36, 37, 38, 39, 40, 41, 42	37.4	38.6	38.4	38.4-38.4	38.1
Sand Dollar	Development	Range Finder	34, 42, 49, 56, 63, 70	<43	43	37.8	NA	
		Definitive 1	34, 35, 36, 37, 38, 39, 40, 41, 42	37.7	38.6	39.5	39.5-39.6	
		Definitive 2	34, 35, 36, 37, 38, 39, 40, 41, 42	38.1	38.7	39.7	39.7-39.8	39.6
Sand Dollar	Fertilization	Range Finder	34, 42, 49, 56, 63, 70	<43	43	39.0	38.7-39.3	
		Definitive 1	35, 38, 39, 41, 43, 45, 47, 48, 50	37.6	39.5	41.2	41.1-41.3	
		Definitive 2	34, 36, 38, 40, 41, 43, 45, 46, 48	37.6	39.5	39.5	39.4-39.6	40.3
Mussel	Development	Range Finder	34, 36, 37, 39, 41, 43, 44, 46, 48	41	42	42.3	42.3-42.4	
		Definitive 1	34, 40, 41, 42, 43, 44, 45, 46, 47	<40.2	40.2	42.2	42.1-42.3	
		Definitive 2	35, 40, 41, 42, 44, 45, 46, 47, 48	42.2	43.9	44.3	44.3-44.4	43.3
Purple Urchin	Fertilization	Range Finder	34, 40, 47, 55, 62, 70	40	47	43.3	43.3-43.4	
		Definitive 1	34, 36, 38, 39, 41, 43, 45, 46, 48	41.1	43.0	44.4	44.3-44.5	
		Definitive 2	34, 38, 41, 42, 43, 44, 45, 46, 47	41.6	41.9	44.0	43.9-44.1	44.2
Mysid Shrimp	Survival	Range Finder	35, 43, 49, 56, 64, 70	43	49	50.1	48.8-51.4	
		Definitive 1	35, 41, 45, 50, 56, 61	44.9	50.2	48.0	47.1-48.8	
		Definitive 2	37, 42, 45, 49, 53, 56	45.8	49.2	47.7	46.9-48.4	47.8
						EC25		
	Growth	Range Finder	35, 43, 49, 56, 64, 70	49	>49	>49	NA	
		Definitive 1	35, 41, 45, 50, 56, 61	50.2	>50.2	>50.2	NA	
		Definitive 2	37, 42, 45, 49, 53, 56	49.2	>49.2	>49.2	NA	>49.7
Giant Kelp	Germination	Range Finder	35, 42, 48, 55, 63, 70	49	57	59.1	58.8-59.5	
		Definitive 1	34, 45, 49, 54, 59, 64	49	54	55.8	55.4-56.1	
		Definitive 2	35, 44, 49, 54, 59, 65	44	49	55.2	54.8-55.6	55.5
						EC25		
	Growth	Range Finder	35, 42, 48, 55, 63, 70	49	57	52.7	50.2-54.4	
		Definitive 1	34, 45, 49, 54, 59, 64	<45	45	48.3	44.6-51.2	
		Definitive 2	35, 44, 49, 54, 59, 65	<44	44	46.3	43.7-48.2	47.3
Topsmelt	Survival	Range Finder	34, 42, 48, 55, 62, 69	56	63	60.2	58.5-62.0	
		Definitive 1	35, 45, 50, 55, 60, 65, 70	55	60	60.4	58.8-62.1	
		Definitive 2	35, 44, 50, 54, 60, 65, 70	60	65	63.4	62.0-64.9	61.9
						EC25		
	Biomass	Range Finder	34, 42, 48, 55, 62, 69	56	63	57.3	34-59.7	
		Definitive 1	35, 45, 50, 55, 60, 65, 70	55	60	57.3	53.3-58.7	
		Definitive 2	35, 44, 50, 54, 60, 65, 70	60	65	61.2	56.1-63.1	59.3

Table 2. No observed effect (NOEC), lowest observed effect (LOEC), and median effect concentration (EC50) or 25% effect concentration (EC25) for Monterey Bay Aquarium seawater RO brine effluent tests. 95% confidence limits (CL) are presented for the EC50 and EC25. Statistics were calculated based on measured effluent salinities.

Protocol	Endpoint	Measured Test Solution Salinities	NOEC	LOEC	EC50	95% CL
Mussel	Development	35, 39, 43, 45, 49, 53	38.8	42.7	43.3	43.2-43.3
Giant Kelp	Germination	36, 41, 44, 48, 52, 55	53.0	>53.0	>53.0	NA
	Growth	36, 41, 44, 48, 52, 55	53.0	>53.0	51.8	48.3-NA
Topsmelt	Survival	34, 37, 40, 45, 49, 51	50.8	>50.8	>50.8	NA
	Biomass	34, 37, 40, 45, 49, 51	50.8	>50.8	>50.8	NA

Further Research

The current project determined the tolerance of Ocean Plan organisms to hypersaline brine as measured by salinity. The seawater used to prepare the brine was from a pristine source, and the brine was generated by freezing the seawater. Further research topics could include testing brine prepared from other sources of water. For ocean disposal, discharge from some desalinization facilities will likely be combined with effluent discharges from publicly owned treatment works and power plants to facilitate dilution. Additional testing should be conducted with various brine effluent mixtures. The whole effluent toxicity (WET) protocols used in the current research were designed to provide short-term indications of chronic toxicity. Because there is some concern over the chronic effects of brine effluent on marine receiving systems, longer-term chronic toxicity studies should be conducted to confirm the WET protocols are adequately protective of ocean receiving systems impacted by hypersalinity. Of the most sensitive organisms in the current results, the red abalone is the most amenable to chronic effects evaluation. The red abalone larval development test can be extended to a settling and metamorphosis endpoint, which provides a more sensitive assessment of chronic effects. The giant kelp and topsmelt protocols can also be extended to longer-term exposures to assess chronic effects.

In addition to concerns about elevated salinity in ocean receiving systems, studies are also needed to assess brine discharges in estuarine receiving systems. Because of the potential for reduced brine dilution and dispersal in these systems there is likely a greater potential for impacts on estuarine water column and benthic species in estuaries. Additional toxicity assessments could be conducted using estuarine species sensitive to salinity gradients (e.g., migrating salmonids), and using estuarine benthic infaunal species which may be impacted by negatively buoyant brine discharge plumes.

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Appendix A – Reference Toxicant Control Charts