

NWRI SCSC Fellowship Progress Report April 2016

Project Title: Impacts of hypersalinity from brine disposal on selenium embryo toxicity in fish

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Introduction: Desalination of seawater and brackish groundwater in California could provide drinking water to millions of residents. However, reject from these plants is possibly harmful, and potential negative impacts of brine disposal need to be evaluated on aquatic organisms to ensure safe practices. Water restrained estuaries, such as the San Francisco Bay Delta (SFBD), may be at increased risk for brine toxicity due to decreased dispersal and turnover. While data from tests of brine on larval and adult aquatic organisms are available, chronic tests on vertebrate embryonic development are needed in order to fully assess brine toxicity for safe regulation. Furthermore, brine toxicity thresholds may be confounded by the multiple stressors that fish encounter in their environments.

Historical selenium contamination of many California waterways continues to impact developing fish and aquatic birds. Preliminary research has shown that hypersalinity can potentiate selenium toxicity by decreasing survival and increasing deformities. One tributary river of the SFBD is the San Joaquin River, which is naturally saline and with a different ionic content than seawater. Selenium concentrations in the San Joaquin river are elevated due to high inputs from agricultural drainage. Furthermore, desalination facilities proposed for the SFBD may draw source water from the San Joaquin River, and dispose of it back into the delta, altering the salinity or ionic composition.

This project aims to investigate the impacts of desalination brine on embryonic development and to characterize the potential interaction between the hypersalinity generated by desalination brine and selenium to inform regulatory decisions about brine disposal in California. Hypersalinity is defined as an increase in salinity over freshwater (1-2ppt) or over ambient conditions, since potential interactions may occur even at salinities of less than seawater (34ppt). This project will further examine differential effects between brine of varied ionic contents, as different ions have demonstrated different toxicity to aquatic organisms.

PART 1: Impacts of Desalination Brine on Embryonic Development

Hypothesis 1: Embryo hatch, fitness and survival will be increasingly impacted by increasing salinity of desalination brine and will vary with brine composition.

Specific Aim 1: Determine lethal and sublethal thresholds for brines of differing ionic contents on chronic vertebrate development. The project will work with two species of fish. First, exposures will be done with the model organism, Japanese medaka (*Oryzias latipes*). After work on the medaka is completed, similar experiments will be performed on a fish more relevant to California, the 3-spined stickleback (*Gasterosteus aculeatus*). Using both a model fish and a native, environmentally relevant fish will provide a more complete picture of the effects of hypersalinity and organic selenium in the form of selenomethionine (SeMet).

Progress: Experiments on impacts of brine on Japanese medaka embryos are complete. Embryos were exposed at fertilization to 5 different waters:

1. De-chlorinated freshwater (0 parts per thousand);
2. Instant Ocean artificial seawater (17, 35, 42, 56, and 70 ppt);
3. Reject brine from a desalination facility at Monterey Bay Aquarium, CA, diluted to 75%, 50%, and 25% with 35ppt artificial seawater to simulate mixing (39, 42, 46 and 50ppt);
4. A lab preparation of artificial San Joaquin River water (CA, USA) (9, 13, and 17 ppt); and
5. Artificial San Joaquin River water diluted to 75%, 50%, and 25% with artificial seawater to simulate estuarine mixing in the San Francisco Bay (13, 19, 24, and 30 ppt).

Hatch, survival post hatch, deformities, swim bladder inflation, and day to hatch were recorded to calculate EC50 and NOEC values. Statistical analyses were performed to assess significant differences between treatments. This data is in preparation for publication.

Results:

Hatch: SJR water and SJR mixed with seawater had no significant effect on hatch at any salinity. In contrast, artificial seawater decreased hatch in 56ppt seawater (160% full strength) and in 70ppt (200%; EC50 52.3 ppt). Similar decreases in hatch were observed with 50ppt RO brine (100% effluent; EC50 55.9 ppt).

Deformities: Total deformities (spinal, facial, fin, cardiac and yolk sac) were quantified in live hatched larvae. Artificial seawater increased deformities at 42ppt and 56 ppt, whereas reject brine increased deformities at 50 ppt. SJR water mixed with seawater resulted in no increase in deformities. 17ppt SJR water alone caused 53% deformities, which is in contrast to 17ppt seawater exposures, which did not cause deformities. EC50 values are indicative the difference: 58.2ppt for seawater, 54.6ppt for RO brine, and 18.7ppt for SJR.

Swim Bladder Inflation: The swim bladder maintains fish buoyancy and balance in the water column and without proper inflation swimming may be impaired. A salinity dependent increase in the failure of the swim bladder to inflate was observed for all water types except for SJR water mixed with seawater. EC50 values were calculated at seawater: 60.1 ppt, brine: 45.1 ppt, and SJR: 18.2 ppt. Based on salinity alone, this further suggests that SJR water is more toxic to developing fish than seawater.

Larval Survival: Larval survival was assessed for 3 days post hatch. Larval survival decreased with increasing salinity of artificial seawater beginning at 42ppt. RO brine treated larvae survival decreased in 50ppt. No decrease in survival was observed in SJR water mixed with seawater. SJR water alone was larval toxic at all tested salinities. EC50 values for survival could only be calculated for artificial seawater (49 ppt) because not enough significance was observed at the tested salinities of RO brine and due to the U-shaped nature of the dose response curve for SJR water.

Day to hatch: No alteration in time to hatch was observed in SJR water or SJR mixed with seawater. However, RO brine significantly increased day to hatch from approximately 9 days in freshwater to 10 days at all concentrations tested. Artificial seawater also significantly increased hatch beginning at 17 ppt to 10.25 dpf, at 42 ppt to 10.4 dpf, at 50ppt to 11 dpf, and at 70ppt to 14.5 dpf.

Conclusions and Significance: From the artificial seawater and Monterey bay brine results we can calculate EC50 values for hatch, survival, and deformities. The EC50 values calculated

ranged between 45-55ppt, and no difference was detected between the seawater and the brine. This indicates that current regulations would be protective of Japanese medaka embryo development assuming brine discharge into the open ocean with proper mixing. However, the increased mortality in embryos treated with SJR suggests that these ions may be more embryo and larval toxic.

Future Research: This work represents an important step towards understanding the effects of desalination on fish. Future research will expand these results into species endemic to CA, such as the 3-spined stickleback or the Delta smelt. It will also investigate desalination brines from different sources, including those from brackish water, in order to ascertain effects of different ion mixtures.

PART 2: Interactions between salinity and selenium

Hypothesis 2: The addition of environmentally relevant levels of selenomethionine (SeMet) to desalination brine will significantly decrease embryo hatch, fitness and survival in comparison to SeMet in freshwater.

Specific Aim 2: The second part of the project will involve the addition of SeMet to the saltwater. Typically, oviparous females transfer selenium to their embryos prior to fertilization in the organic form of selenomethionine. Thus, in order to obtain a dose response for Japanese medaka embryo susceptibility to SeMet and hypersalinity, treatment with Se will begin at 0 hours post fertilization and end at hatch. SeMet exposures will be waterborne, as previous research suggests that SeMet is absorbed through the chorion and into the embryo.

Since previous research on the interactions between hypersalinity and selenium toxicity to fish have focused only on very early life stages, the second part of specific aim 2 will isolate a window of susceptibility to SeMet and hypersalinity from desalination brine. Treatments with 0.5 μ M, 5 μ M and 50 μ M of SeMet in fresh or saltwater will begin at each day post fertilization (dpf) and last for 24hrs (e.g. 0-24hpf, 24-48hpf, etc.). Then, embryos will be replaced into fresh or saltwater and allowed to develop to hatch.

Progress: Experiments investigating the window of susceptibility for Japanese medaka in San Joaquin River Valley saltwater are complete. Medaka embryos were placed in freshwater or saltwater at fertilization and staged for one of 6 stages (stage 9 (5 hours post fertilization (hpf)), stage 17 (24hpf), stage 25 (48hpf), stage 29 (72hpf), stage 34 (170hpf) and stage 38 (192hpf)). At each stage, embryos were treated in a dose response of SeMet in freshwater or saltwater of 0.5 μ M, 5 μ M, and 50 μ M for 24hours. Following treatment, embryos were removed from the SeMet treatment and replaced into freshwater or saltwater to continue development. Embryos were monitored for survival, hatch, days to hatch, deformities, type of deformities and severity of deformities. This work has been accepted for publication in *Environmental Toxicology and Chemistry* (DOI: 10.1002/etc.3268) and the figures below are taken from that publication. Full results are available.

Results:

Survival: As hypothesized, decreased survival of medaka embryos was observed at increasing concentrations of SeMet at all stages. However, significant differences were observed between

stages. Embryos treated with 50 μ M and 5 μ M SeMet at stage 9 had significantly greater survival than all other stages. There were further differences between freshwater and saltwater treatments with 50 μ M SeMet. Specifically, embryos treated at stage 25 in saltwater had 2% survival, while embryos treated in freshwater had 40% survival.

Hatch: Embryo hatch following exposures showed similar patterns as survival. Stage 17 was identified as the most sensitive stage to 5 μ M and 50 μ M SeMet. Differences between freshwater and saltwater were observed following treatment with 5 μ M and 50 μ M SeMet, particularly at stage 25.

Deformities: The total deformities increased with increasing concentrations of SeMet exposure. Embryos treated with 5 μ M and 50 μ M SeMet at stages 17 and 25 had significantly greater deformities than those treated at stages 29 and 34. In addition to total deformities, we also examined different types of deformities, including spinal, cardiac, cranio-facial and fin. Spinal deformities were the most common type of deformity caused by SeMet, while pectoral fin deformities were the least common. There were no significant differences between dose or water type, however, embryos treated at stage 9 had significantly more cardiac and cranio-facial abnormalities than embryos treated at stages 17 and 25.

Se tissue content: Se tissue concentrations were measured in order to determine the precise exposure dose and to correlate observed effects to exposure concentrations. Se uptake ranged between 50 and 5 μ g/g dry weight. Se content did not vary between freshwater and saltwater, suggesting that observed differences in effects were not due to differences in Se uptake. However, Se uptake was stage dependent, and later stages assimilated more Se than earlier stages. For instance, no significant uptake in Se was observed at stages 9 and 17 (approximately 7 μ g/g dry weight), however, embryos treated with 0.5 μ M at stages 25-38 assimilated approximately 10-20 μ g/g and those treated with 5 μ M SeMet assimilated approximately 25-50 μ g/g dry weight. This may be due to a gradual weakening of the chorion (egg shell), throughout development. Correlations between Se content and total deformities were determined for stages 25-38.

Conclusions: Se tissue concentrations were variable by stage and they were dose dependent. Embryos at treated at stages 9 and 17 assimilated less Se than those treated at later stages. Taking into account the Se content at each stage, we can conclude that earlier stages were more sensitive to SeMet toxicity than other stages in both saltwater and freshwater. The peak of the interaction between freshwater and saltwater in SeMet toxicity can be identified to occur at around stage 25. At stage 25, the liver is beginning to form, as are the osmoregulatory cells. The liver is the major site of xenobiotic metabolism, and most likely plays a key role in SeMet toxicity. Furthermore, development of active osmoregulation could impact embryonic salinity regulation.

Significance of Results: These results indicate that SeMet and hypersalinity do interact at stage 25 to cause embryo lethality and deformities. Following analytical chemistry of the tissue samples, we will be able to identify stage specific thresholds for Se toxicity under both freshwater and saltwater conditions.

Future work: Unfortunately, we were unable to obtain stickleback in order to complete these studies with a species more relevant to California. Thus, future research will need to include species endemic to California to fully determine the necessary level of protection against selenium and hypersalinity.