

NWRI SCSC Fellowship Progress Report April 2015

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, 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. 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. 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.

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: The project will work with two species of fish. First, exposures will be done with Japanese medaka (*Oryzias latipes*). Medaka are a good model organism for this work because they are euryhaline, they develop rapidly (9-12 days to hatch) and they have a clear chorion for imaging. Furthermore, they have shown to be previously sensitive to SeMet and hypersaline conditions. 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 SeMet.

Embryos will be collected into four dilutions of four types of saltwater: artificial seawater, a lab preparation of San Joaquin River Valley saltwater, wastewater brine and desalination brine from Monterey Bay. Throughout development, embryos will be monitored for mortality. The embryos will remain in the water until 3 days post hatch. Percent hatch, median day to hatch, percent deformities and percent survival post hatch will be measured as indicators of the saltwater's effects.

Progress: Exposures of Japanese medaka to range finding dilutions of artificial seawater and San Joaquin Rivers Valley saltwater have begun, however, no conclusions have been reached. We have also begun work to obtain desalination brine from OCWD and Monterey Bay Aquarium.

Hypothesis 2: The addition of environmentally relevant levels of 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 Se to their embryos prior to fertilization. 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. Anecdotal range finding for SeMet toxicity suggests that SeMet doses will range from 0.05 μ M, 0.5 μ M, 5 μ M, and 50 μ M, which correspond to approximately 1 μ g/L, 0.01mg/L, 0.1mg/L, and 1mg/L respectively. Previous experiments for preliminary data used 50 μ M SeMet to represent the upper limit of SeMet bioaccumulation after 12hr of exposure.

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. The same endpoints examined in Specific Aim 1 will be examined. From these values, thresholds for SeMet embryotoxicity can be determined for mortality, survival, and deformities.

Progress: Experiments investigating the window of susceptibility for Japanese medaka in San Joaquin River Valley saltwater are almost 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.

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. We also examined swim bladder inflation. The swim bladder is important for fish to maintain buoyancy and balance, and while fish without swim bladders can survive, they may be more susceptible to predation. We observed a greater incidence of failed swim bladders in fish with increasing concentrations of SeMet. Furthermore, embryos treated at earlier stages had significantly more failed swim bladder than at later stages, suggesting that SeMet interferes with swim bladder formation rather than swim bladder inflation.

Conclusions: The data suggest that embryos at stage 9, which is the early blastula stage, are less sensitive to Se induced mortality. This is in contrast to dogma that developmental toxicants tend to be more toxic at earlier stages. However, embryos treated at stage 9 did have a significant increase in deformities and a wider range of types of deformities, suggesting that SeMet is acting at this stage. Stage 17, the early neurulation stage was most susceptible to SeMet toxicity in both fresh and salt water. 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. This suggests that site-specific regulation of desalination brine and Se may be necessary. 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: In the future, we will continue the dose response studies of differing brines on Japanese medaka and once we have final concentrations, we will repeat them in 3-spined stickleback to account for species differences. From this, we will be able to calculate the salinities that will be protective of developing fish embryos.

Once the dose response studies on brine alone are complete, we will add a dose response of selenium to each of the brines and do analytical chemistry to determine toxicity thresholds for Se in different types of saltwater for both medaka and stickleback.