

## NWRI SCSC Fellowship Progress Report October 2015

Allison Kupsco

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

**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:** 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 SeMet.

**Progress:** Exposures of Japanese medaka to four different varieties of saltwater have been performed. Embryos were treated at fertilization with: 1. Freshwater; 2. Artificial seawater (17, 35, 42, 56, and 70 parts per thousand (ppth)); 3. Brine from a desalination facility at Monterey Bay Aquarium, CA, diluted 75%, 50%, and 25% with 35ppth artificial seawater to simulate mixing (39, 42, 46 and 50ppth); 4. Artificial San Joaquin River prepared in the lab then water diluted 75%, 50%, and 25% with artificial seawater to simulate mixing (13, 19, 24, and 30ppth). Embryos were maintained in the saltwater until 3 days post hatch and hatch, day to hatch, deformities, swim bladder inflation and survival post hatch were recorded. Brine was requested but not obtained from Orange County Water District due to their refusal to provide it.

**Results:**

**Artificial Seawater:** A significant decrease in hatch was observed in fish exposed to 70ppth (22% hatch) and 56ppth (37% hatch) in artificial seawater. A significant decrease in survival post hatch was also observed for these concentrations (0% and 21% for 70 and 56ppth respectively). Meanwhile, an increase in deformities was observed in larvae treated with 56ppth and 42ppth (50% and 13%, respectively) and a decrease of swim bladder inflation was observed in fish treated with 56, 42, and 35ppth.

**Monterey Bay Aquarium Brine:** Embryos were treated in brine mixed with 35ppth artificial seawater to simulate disposal into marine environments. The concentrated brine sample was approximately 50ppth and was mixed 25%, 50% and 75% with 35ppth artificial seawater to generate 39ppth, 46ppth and 49ppth. 50ppth and 46ppth brine significantly decreased medaka hatch to 53% and 81% respectively. Survival was significantly decreased at 50ppth to 90% and deformities were increased at this concentration (23%). Swim bladder inflation was also decreased in 50 and 46ppth brine.

**Artificial San Joaquin River Water:** San Joaquin River (SJR) water was chosen to compare the effects of various ionic contents of saltwater to embryos. This water is composed primarily of sulfate, calcium and sodium, in contrast to seawater, which is primarily sodium and chloride. As with the Monterey Bay brine, this water was mixed to 25%, 50% and 75% its original concentration (13ppth) with seawater to simulate mixing. The final concentrations tested were 13, 19, 24 and 30ppth. The only observed effects were a decrease in hatch to 85% and a decrease in survival post hatch to 52% in embryos treated with 100% SJR.

**Significance of Results:** From the artificial seawater and Monterey bay brine results we calculated EC50 values for hatch, survival, and deformities. The EC50 values calculated ranged between 45-55ppth, 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 100% SJR suggests that these ions may be more toxic to larval stages of fish. Further studies need to be performed to confirm these effects.

**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.

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 $\mu$ M, 5 $\mu$ M and 50 $\mu$ 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 $\mu$ M, 5 $\mu$ M, and 50 $\mu$ 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 was just accepted for publication in *Environmental Toxicology and Chemistry*.

### **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 $\mu$ M and 5 $\mu$ M SeMet at stage 9 had significantly greater survival than all other stages. There were further differences between freshwater and saltwater treatments with 50 $\mu$ 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 $\mu$ M and 50 $\mu$ M SeMet. Differences between freshwater and saltwater were observed following treatment with 5 $\mu$ M and 50 $\mu$ M SeMet, particularly at stage 25.

**Deformities:** The total deformities increased with increasing concentrations of SeMet exposure. Embryos treated with 5 $\mu$ M and 50 $\mu$ 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.

**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 stages 9 and 17 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. 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:** The artificial San Joaquin River water will be concentrated 2x then diluted with freshwater to determine effect concentration thresholds in Japanese medaka embryos. These experiments will then be repeated in a species native to CA to determine species differences. We will continue the SeMet dose response studies with differing brines on Japanese medaka.