



**Connon Laboratory  
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Davis, California**

Title of Research:

***A Systems Biology Assessment of Endocrine Disrupting  
Contaminants in the Delta using the Inland Silverside  
(Menidia beryllina)***

**Deliverable: Task 7.1 –  
Menidia beryllina baseline spawning trials**

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To: Mitsuko Grube California Department of Fish and Wildlife  
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## **Task 7.1 – Baseline Spawning Trials:**

### **A. Baseline on 6 trials.**

The first round of baseline spawning trials for the Connon DFW ERP project was run from 25 January 2013 until 20 March 2013. During this time 6 trials, each with two replicates were run with 20-25 fish (*Menidia beryllina*) per replicate. Fish had been obtained from Aquatic Biosystems in Fort Collins, CO as juveniles and reared to reproductive maturity. All individuals were tagged with Visible Implant Alpha Tags (VI Alpha, Figure 1). They ranged in age from 9 – 18 months. Dates of the 6 trials were: 1/24 – 1/28, 1/28-2/1, 2/20-2/22, 2/25-2/27, 3/13-3/15, 3/18-3/20. With the exception of trials 1 and 2, fish were left in spawning tanks for 48 hours, as we realized following the first two trials that trial length could be shortened to increase efficiency without sacrificing data quality. After spawning, fish were given a 2-3 week recovery period between each trial.

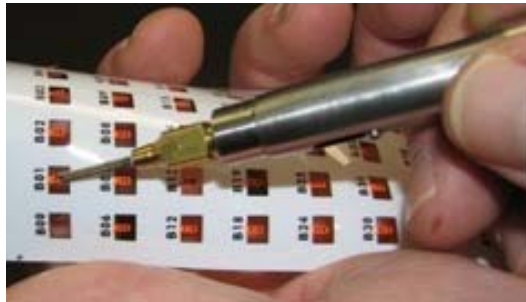


Figure 1. Visible Implant Alpha Tags and injector, manufactured by Northwest Marine Technology, Shaw Island, WA, United States.

For the first four trials, fish were randomly selected from a pool of 125 individuals and placed into tanks with spawning substrate (Figure 2). For the last two trials fish were selected based on size (large more likely to be female, small more likely to be male) to generate a wider distribution of sex ratios.



Figure 2. Spawning substrate consisted of yarn sterilized in a 10% bleach solution and thoroughly rinsed with filtered sea water prior to use. Seven clumps of 6 strands per clump were suspended mid-water column using ¼ inch diameter plastic tubing and clamps. Yarn was removed each day and replaced with new substrate. Eggs (2-3 mm diameter) were counted using a dissecting scope at 10x magnification.

Spawning trials were ended when egg production had declined, likely due to the age of some of the fish. All fish (85 remaining) were dissected to identify their sex. The organs (liver, brain, kidney, gonad, gill, heart) of 50 fish were snap-frozen on liquid nitrogen and are being stored at -80 degrees C for future analyses.

Egg production per female from the first round of trials was quantified, although a large number of the VI Alpha tags were lost during the trials, making it difficult to accurately assign fish to the trials they participated in. A significant relationship was not found between per capita egg production and proportion male, however a  $p$  value of 0.6 indicated that these findings would be strengthened with a larger sample size. As such, we ran a second round of baseline trials with a new group of younger fish (6-8 months at start) from June through October 2013.

### B. Baseline on ten trials.

Ten spawning trials with the same group of adult silversides were completed at the Center for Marine Science, conducted as described above. Spawning trials began in June, 2013, utilizing two groups of 25 fish, each spawned for 48 hours for each trial, with a break of 2 weeks in between spawning events. Tissues (liver, gonads, brain, gill) from fifty of these control fish were saved for future analyses.

Results indicate that the spawning functional response for *Menidia beryllina* is sigmoidal in shape, and that per capita female egg output actually increases as the proportion male increases (Figure 3). In effect, females are compensating for masculinization. However, although per capita reproductive output increases as proportion male increases, total reproductive output decreases as the proportion male exceeds 60% (not shown).

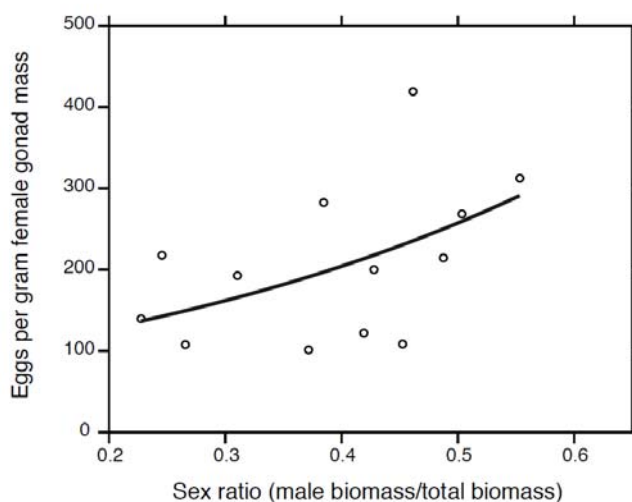


Figure 3. Per female egg production for unexposed fish that spawned during the second round of baseline trials.

Improvements made during the second round of baseline trials included the use of younger fish that are closer in age (6-8 months old), quantification of fertilization success (differentiating between fertilized and non-fertilized eggs), use of an alternative tagging location (shoulder instead of tail) which reduced tag loss, and a UV light provided by Northwest Marine Technology to better visualize tag numbers.