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Title of Research:

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

**Deliverable:
Effluent Discharge Point Water Collection (Task 6.1)
and Exposure (Task 6.2) Report**

August 13th, 2014

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Contract # E1183010

Effluent Discharge point water collection (Task 6.1) and Exposure (Task 6.2) Report

Background

Endocrine disrupting chemicals (EDCs) originate from a variety of sources and are widespread in the aquatic environment, but are particularly concentrated downstream of treated wastewater outfalls. We proposed to investigate EDC effects on individual gene and protein expression and link these to changes in reproductive performance in the silverside (*Menidia beryllina*), exposed to contaminants commonly present in treated wastewater and surface waters downstream of outfalls. Here we report water quality assessments and quantification the presence of two contaminants known to affect reproductive output in fish: bifenthrin, a pyrethroid pesticide, and ibuprofen, a non-steroidal anti-inflammatory pharmaceutical. We also report exposure survival data from 4 and 12 day exposures of 12 and 64 dph fish, respectively. Ambient waters were sampled from within 50 m radius of the Publicly Owned Treatment Works (POTW) effluent discharge points at Boynton Slough (Fairfield-Suisun POTW; +38° 12' 46.6086", -122° 3' 14.4498") and Carquinez Strait (Benicia POTW; +38° 2' 36.0378", -122° 9' 2.991"), and used to evaluate effect-based responses in pre-spawning adults (64 dph), and larval (12 dph) *M. beryllina* (Task 6.3 – current research)

Effluent Collection (Task 6.1)

Samples were collected from each site, by boat, in 5 gallon cubitainers, every four days for a period of 12 days; April 17, 21, and 25, 2014. Sampling times were selected to match tidal cycle for each time point so as to ensure similar salinities. Ambient water samples, and respective control waters, were kept in cubitainers, and refrigerated, until required for water renewal. Cubitainers were leached with DI water for 96 hours prior to each sample collection.

Chemical analyses

Bifenthrin: Within 24h, the samples were extracted by solid phase extraction (C18cartridges), and analyzed using gas chromatography electron capture negative ionization mass spectrometry (GC-ECNI-MS) on an Agilent 5973 series gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a split-splitless injector (280°C, splitless, 1.5-minute purge time) and a Supelco DB-5MS column (30 m x 0.25 mm with a 0.3 µm film thickness). Instrumental calibration was performed using nine sets of calibration standard solutions (purchased as 100 µg/ml solution in acetonitrile, Chem Service, West Chester, PA), a surrogate bifenthrin (EQ Laboratories, Atlanta, GA), and an internal standard; dibromooctafluorobiphenyl (Chem Service, West Chester, PA) in hexane. Quantity was calculated based on peak area, and comparison to standard curve. Quality-assurance/quality-control was conducted by analyzing a method blank of deionized water (Milli-Q), to ensure that no contamination occurred during sampling extraction and analysis. Dibromooctafluorobiphenyl was added to sample extracts before analysis in order to correct quantitative differences in extract volume as well as to monitor instrument conditions. The limit of detection for bifenthrin is 0.4 ng L⁻¹.

Ibuprofen: Samples were submitted to the California Department of Fish and Wildlife Water Pollution Control Laboratory (Rancho Cordova, CA) for quantification of ibuprofen, gemfibrozil, sulfamethazine and caffeine. These samples were extracted using solid phase extraction and analyzed by gas chromatography (Agilent 6890 plus; Agilent Technologies Inc., Santa Clara, CA) with dual columns (DB5 and DB7).

Results

Waters sampled at Carquinez Strait had a high salinity (range 11.4-16.2 ppt), while samples at Boynton Slough were low (range 1.0 – 1.3 ppt), consistent with specific conductance. Overall this will serve as a

good comparison between responses to salinity as well as differences due to exposures to contaminants present in waters affected by effluent discharge. Physicochemical parameters of receiving waters were relatively stable per site, and are summarized in table 1. Ammonium concentrations were variable; low at Carquinez Strait and elevated at Boynton Slough. pH remained stable at both sites, higher at Boynton Slough than at Carquinez Strait. Ammonia/um determination and pH is summarized in table 2.

Bifenthrin was detected at Boynton Slough in samples from April 21st at relatively high concentrations of 2.894 ng/L (note: concentrations as low as 0.5 ng L⁻¹ have been shown to affect reproductive output in *M. beryllina*; Brander and Connon, current study), but in none of the other sampled ambient waters. No bifenthrin was detected in neither controls not method blanks.

Ibuprofen was not detected in any of the collected water samples, however gemfibrozil and sulfamethazine were detected in every sample, at both sites, at relatively consistent concentrations ranging from 83 to 123 µg L⁻¹, 71.8 to 94.0 µg L⁻¹, respectively. Caffeine was detected only at Boynton Slough, at concentrations ranging from 52 to 69 ng L⁻¹.

Effluent discharge point *Menidia beryllina* exposures (Task 6.2)

Two tests were conducted with ambient water samples from Carquinez Strait (Benicia) and Boynton Slough (Fairfield-Suisun) POTW effluent discharge points: a twelve day (chronic; 4/18/2014 to 4/30/2014) exposure using 64 dph; pre-spawning *Menidia beryllina*, and a four day (short-term; 4/26/2014 to 4/30/2014) exposure using 12 dph; larval *M. beryllina*. Physicochemical parameters of sampled waters are presented in table 1, Task 6.1. (See above).

Inland silversides were supplied by Aquatic Biosystems (Ft. Collins, CO, USA), fed *Artemia franciscana*, and were allowed four days to acclimatize to holding conditions at the Aquatic Toxicology Laboratory at the University of California Davis, which were designed to match the salinity of respective sample sites. Fish were received at a salinity of 18 ppt, and were acclimated, at approximately 3 ppt/day, down to salinities matching that of the ambient waters to be tested, prior to test initiation.

Sixty four dph fish were exposed in quadruplicate, in 2.0L beakers, containing 1.5L water with ten animals per replicate. Twelve dph fish were exposed in quadruplicate animals per replicate, in 600mL beakers, containing 400mL water with 10 animals per replicate. All exposures were conducted in waterbaths maintained at 21°C. Each beaker was gently aerated, and fish were fed *A. franciscana*, twice daily. Controls consisted of deionized water, modified to attain physicochemical parameters matching that of the sampled waters for each site. 80% of water was exchanged daily, at which time physicochemical parameters were measured. At test termination, fish were sacrificed in a lethal dose of tricaine methanesulfonate (MS-222) and whole fish were immediately frozen in liquid nitrogen and stored at -80°C until transcriptome analysis. The weight and length of fish from chronic exposures were recorded. Larval fish did not warrant weight and length determination, as it is unlikely to determine any significant growth effects over the short exposure period.

Results:

Chronic exposures (64 dph):

Water physicochemical parameters remained stable throughout each four-day sample collection batch, (Table 3; Carquinez Strait and 4; Boynton Slough), but varied among the three sample time point (see also Table 1), with salinity, and associated electrical conductivity ranging from 11.1 to 16.1 ppt, and 16.83 to 25.80 mS cm⁻¹ in Carquinez Strait exposures, and from 1.0 to 1.2 ppt, and 1.70 to 2.19 mS cm⁻¹

in Boynton Slough exposures, respectively. Control water adequately matched water samples from the POTW effluent discharge points.

There was no mortality associated with any of the ambient waters, nor controls, and there were no significant differences (One-way ANOVA) in fork length, nor weight of fish acclimated to low and high salinities at test initiation (Table 5), nor following the twelve day exposures (Table 6).

Short-term exposures (12 dph):

The last sampling event was used for the four-day larval exposures. Physicochemical parameters remained stable throughout the test duration (Table 7; Carquinez Strait and 8; Boynton Slough), with salinity, and associated electrical conductivity ranging from 11.1 to 11.5 ppt, and 16.80 to 17.80 mS cm⁻¹ in Carquinez Strait exposures, a salinity of 1.2 ppt, and conductivity range from 2.07 to 2.15 mS cm⁻¹ in Boynton Slough exposures, respectively. Control water adequately matched water samples from the POTW effluent discharge points.

Acknowledgments

We thank Linda Deanovic and Marie Stillway, and others at the UC Davis Aquatic Toxicology Laboratory for assistance in conducting fish exposures, and Dennis Cocherell, Department of Wildlife, Fish, and Conservation Biology, for assistance with water collections. This research was approved by the University of California Davis Institutional Animal Care and Use Committee (IACUC #16845).

Table 1: Physicochemical parameters of receiving water sampled from Carquinez Strait and Boynton Slough POTW discharge points.

Date	Carquinez Strait					Boynton Slough				
	Temperature (°C)	Specific Conductance (µS/cm)	Salinity (ppt)	Hardness (mg/L as CaCO ₃)	Alkalinity (mg/L as CaCO ₃)	Temperature (°C)	Specific Conductance (µS/cm)	Salinity (ppt)	Hardness (mg/L as CaCO ₃)	Alkalinity (mg/L as CaCO ₃)
4/17/2014	12.6	26050	15.8	2844	106	14.8	2148	1.1	348	246
4/21/2014	15.4	26280	16.2	2880	106	15.4	1948	1.0	312	248
4/25/2014	13.4	20060	11.4	2400	96	15.4	2543	1.3	360	220
Maximum	15.4	26280	16.2	2880	106	15.4	2543	1.3	360	248
Minimum	12.6	20060	11.4	2400	96	14.8	1948	1.0	312	220

Table 2: Ammonia/um determination of water sampled from Carquinez Strait and Boynton Slough POTW discharge points.

Date	Carquinez Strait					Boynton Slough				
	Temperature (°C)	pH	Electrical Conductivity (µS/cm)**	Ammonia Nitrogen (mg/L as NH ₃ -N)	Unionized Ammonia (mg/L as NH ₃ -N)	Temperature (°C)	pH	Electrical Conductivity (µS/cm)	Total Ammonia (mg/L as NH ₃ -N)	Unionized Ammonia (mg/L as NH ₃ -N)
4/17/2014 *	23.5	7.81	25230	0.23	0.006	23.5	8.17	1992	0.06	0.004
4/21/2014 †	19.4	7.90	24520	0.36	0.008	17.8	8.56	2191	0.41	0.046
4/25/2014 †	20.2	7.84	22810	0.52	0.011	20.4	8.51	1696	0.31	0.038
4/30/2014 §	19.8	7.99	19320	0.27	0.008	19.9	7.98	2100	0.26	0.010
Maximum	23.5	7.90	25230	0.52	0.011	23.5	8.56	2191	0.41	0.046
Minimum	19.4	7.81	22810	0.23	0.006	17.8	8.17	1696	0.06	0.004

* The initial water chemistry of which the fish were being exposed to during the test set up

† The final water chemistry of the specified date in which the fish has been exposed to for a 24-hour period

§ All water parameters were measured during the test take down as final water chemistry

† To calculate unionized ammonia, we need temperature, pH, electrical conductivity and total ammonia nitrogen. Our ammonia kit reads only ammonia nitrogen and thus we need to convert to total ammonia nitrogen for proper calculation of unionized ammonia. This can be achieved by multiplying 1.21 to the measured ammonia value for total ammonia. See "Unionized NH₃" tab for raw calculations.

** Electrical conductivity of water is not measured after the 24-hour period, but is measured in the initial water before a water renewal on specified date except 4/30/2014. Electrical conductivity should not change drastically in the 24-hour period

Table 3: Summary of physicochemical parameters from water sampled at Carquinez Strait, during a 12-day, 64 dph *Menidia beryllina* exposure.

Date	Water Chemistry	Control @ Carquinez Straight Salinity					Carquinez Straight				
		Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (mS/cm)	Salinity (ppt)	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (mS/cm)	Salinity (ppt)
4/18/2014	Initial	23.4	8.04	8.3	23.73	15.9	23.5	7.81	8.1	25.23	15.4
	Final	--	--	--	--	--	--	--	--	--	--
4/19/2014	Initial	23.7	8.15	7.6	24.56	15.4	23.7	8.00	7.7	24.76	15.8
	Final	22.1	8.10	7.7	--	--	22.3	7.96	8.0	--	--
4/20/2014	Initial	21.4	8.05	8.2	24.48	15.5	21.7	7.87	8.1	25.53	16.1
	Final	20.2	7.98	7.1	--	--	20.3	7.94	7.4	--	--
4/21/2014	Initial	22.4	8.05	9.0	23.98	15.3	23.0	7.88	8.5	24.52	15.6
	Final	20.9	7.92	7.5	--	--	20.9	7.87	7.9	--	--
4/22/2014	Initial	20.0	8.14	7.9	23.66	16.0	19.1	7.88	8.0	23.41	15.9
	Final	19.5	8.13	7.4	--	--	19.4	7.90	8.3	--	--
4/23/2014	Initial	19.5	8.14	8.2	22.45	15.4	19.6	7.97	8.0	25.80	15.7
	Final	19.3	8.06	7.3	--	--	19.5	8.01	7.8	--	--
4/24/2014	Initial	20.2	8.15	8.5	22.83	15.5	20.1	8.02	8.4	23.64	15.6
	Final	20.1	8.03	8.3	--	--	20.0	7.91	8.4	--	--
4/25/2014	Initial	18.6	8.12	8.8	22.44	15.7	18.6	7.79	8.7	22.81	15.9
	Final	19.8	7.98	7.9	--	--	19.8	7.96	8.7	--	--
4/26/2014	Initial	19.7	8.12	8.4	17.98	11.8	19.6	7.98	8.2	16.83	11.5
	Final	20.2	8.00	7.5	--	--	20.2	7.84	7.6	--	--
4/27/2014	Initial	20.8	8.16	8.1	17.84	11.6	21.2	8.08	8.0	17.74	11.4
	Final	19.1	8.03	8.4	--	--	19.1	8.00	8.2	--	--
4/28/2014	Initial	20.6	8.17	8.6	17.53	11.5	20.3	7.99	8.6	17.08	11.1
	Final	20.7	7.95	8.1	--	--	20.5	7.82	7.8	--	--
4/29/2014	Initial	20.6	8.12	8.3	17.66	11.6	20.5	7.96	8.3	17.56	11.3
	Final	21.0	7.91	7.7	--	--	20.9	7.87	8.0	--	--
4/30/2014	Initial	--	--	--	--	--	--	--	--	--	--
	Final	19.6	8.03	7.9	17.75	11.9	19.8	7.99	8.2	19.32	11.4
Initial Maximum		23.7	8.17	9.0	24.56	16.0	23.7	8.08	8.7	25.80	16.1
Initial Minimum		18.6	8.04	7.6	17.53	11.5	18.6	7.79	7.7	16.83	11.1
Final Maximum		22.1	8.13	8.4	17.75	11.9	22.3	8.01	8.7	19.32	11.4
Final Minimum		19.1	7.91	7.1	17.75	11.9	19.1	7.82	7.4	19.32	11.4

Table 4: Summary of physicochemical parameters from water sampled at Boynton Slough, during a 12-day, 64 dph *Menidia beryllina* exposure.

Date	Water Chemistry	Control @ Boynton Slough Salinity					Boynton Slough				
		Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (µS/cm)	Salinity (ppt)	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (µS/cm)	Salinity (ppt)
4/18/2014	Initial	23.3	8.00	8.3	2138	1.2	23.5	8.17	8.1	1992	1.1
	Final	--	--	--	--	--	--	--	--	--	--
4/19/2014	Initial	23.8	8.09	8.2	2318	1.1	24.1	8.25	8.1	2065	1.1
	Final	21.9	7.96	8.1	--	--	21.9	8.48	8.3	--	--
4/20/2014	Initial	22.1	8.06	7.9	2145	1.2	21.4	8.28	8.1	2115	1.1
	Final	20.2	8.10	7.6	--	--	20.3	8.60	8.1	--	--
4/21/2014	Initial	22.7	8.05	8.9	2034	1.1	22.9	8.06	8.6	2191	1.2
	Final	20.4	8.09	8.6	--	--	20.8	8.09	8.8	--	--
4/22/2014	Initial	20.1	8.07	8.6	1837	1.0	20.7	8.05	8.4	1755	1.0
	Final	20.7	7.93	7.4	--	--	17.8	8.56	8.3	--	--
4/23/2014	Initial	19.7	8.19	8.0	1815	1.0	19.9	8.32	8.1	1737	1.0
	Final	19.8	8.01	8.6	--	--	19.8	8.63	8.8	--	--
4/24/2014	Initial	20.4	8.13	8.4	1801	1.0	20.3	8.41	8.3	1747	1.0
	Final	20.2	7.96	8.5	--	--	20.3	8.47	8.4	--	--
4/25/2014	Initial	19.8	8.01	9.5	1739	1.0	19.7	7.94	9.3	1696	1.0
	Final	19.7	8.01	8.2	--	--	19.7	8.46	7.8	--	--
4/26/2014	Initial	19.4	8.11	9.0	2301	1.3	19.6	8.30	9.0	2098	1.2
	Final	20.3	7.93	8.3	--	--	20.4	8.51	8.5	--	--
4/27/2014	Initial	21.2	8.24	8.4	2388	1.3	20.9	8.30	8.4	2153	1.2
	Final	19.2	8.10	8.6	--	--	19.5	8.50	8.4	--	--
4/28/2014	Initial	20.4	8.09	8.9	2163	1.2	20.4	8.42	8.9	2074	1.2
	Final	20.6	7.93	8.5	--	--	20.7	8.42	8.4	--	--
4/29/2014	Initial	20.6	8.07	8.7	2185	1.2	20.4	8.39	8.7	2118	1.2
	Final	20.8	7.96	8.3	--	--	20.8	8.42	8.2	--	--
4/30/2014	Initial	--	--	--	--	--	--	--	--	--	--
	Final	19.9	8.44	8.6	2259	1.3	19.9	7.98	8.5	2100	1.2
Initial Maximum		23.8	8.24	9.5	2388	1.3	24.1	8.42	9.3	2191	1.2
Initial Minimum		19.4	8.00	7.9	1739	1.0	19.6	7.94	8.1	1696	1.0
Final Maximum		21.9	8.44	8.6	2259	1.3	21.9	8.63	8.8	2100	1.2
Final Minimum		19.2	7.93	7.4	2259	1.3	17.8	7.98	7.8	2100	1.2

Table 5: Summary of fork length and wet weight of 64 dph *Menidia beryllina* acclimated salinities of 3 and 12.5 ppt, prior to a 12-day exposure to water sampled at Carquinez Strait and Boynton Slough.

Fish	Low Salinity--3 ppt		High Salinity--12.5 ppt	
	Fork Length (cm)	Wet Weight (g)	Fork Length (cm)	Wet Weight (g)
1	2.0	0.08581	1.9	0.07120
2	1.9	0.06036	1.8	0.06419
3	1.8	0.05391	1.8	0.06814
4	1.8	0.05435	1.8	0.05927
5	2.0	0.06840	2.2	0.10594
6	1.9	0.06801	1.7	0.05455
7	2.0	0.07783	2.1	0.07911
8	2.0	0.08974	1.7	0.05252
9	2.0	0.08037	2.0	0.08610
10	1.9	0.06577	1.7	0.04691
11	2.3	0.12259	1.9	0.07419
12	2.2	0.10372	2.0	0.10401
13	2.4	0.12264	2.0	0.07920
14	2.0	0.07852	1.9	0.05761
15	2.1	0.08807	1.9	0.07519
16	2.0	0.07545	1.9	0.06226
Minimum	1.8	0.05391	1.7	0.04691
Maximum	2.4	0.12264	2.2	0.10594
Mean	2.0	0.08097	1.9	0.07127
Standard Error	0.0	0.00525	0.0	0.00425

Table 6: Summary of fork length and wet weight of 64 dph *Menidia beryllina* following a 12-day exposure to water sampled at Carquinez Strait and Boynton Slough.

Replicate	Fish	DIEPAMH @ CS ppt		Carquinez Strait		DIEPAMH @ BS ppt		Boynton Slough	
		Fork Length (cm)	Wet Weight (g)						
A	1	2.2	0.10203	2.1	0.07685	2.2	0.08775	2.0	0.06103
	2	1.9	0.07096	2.1	0.08193	2.1	0.07198	2.1	0.08675
	3	1.8	0.05434	2.1	0.07404	2.2	0.08637	2.2	0.08209
	4	2.1	0.09028	2.0	0.07108	2.2	0.08062	2.0	0.07030
	5	2.1	0.07805	2.0	0.07595	2.1	0.07098	2.0	0.06405
	6	1.8	0.05358	1.9	0.06598	2.2	0.08789	2.3	0.09687
	7	2.2	0.09474	1.9	0.06241	2.1	0.06447	1.9	0.06371
	8	2.0	0.07628	2.0	0.07124	2.2	0.09481	2.0	0.06740
	9	--	--	2.0	0.07609	2.0	0.07308	2.3	0.11350
B	1	2.1	0.07789	2.2	0.08609	2.1	0.08649	1.8	0.05867
	2	2.2	0.08557	1.9	0.05903	1.9	0.05594	2.0	0.07517
	3	2.15	0.08037	2.1	0.08278	2.1	0.08171	2.0	0.07158
	4	2.1	0.08091	2.2	0.09587	2.0	0.06550	2.0	0.07490
	5	2.0	0.06949	1.8	0.06057	2.0	0.06798	1.8	0.05880
	6	1.9	0.05556	2.2	0.08971	2.1	0.06927	2.3	0.10694
	7	2.1	0.07434	2.1	0.07270	2.1	0.07126	2.2	0.09623
	8	2.3	0.10537	2.1	0.07153	2.1	0.07590	2.1	0.07375
	9	2.0	0.06576	1.8	0.05652	1.9	0.06331	2.2	0.09409
C	1	2.2	0.08850	2.0	0.07912	2.4	0.10661	1.9	0.06087
	2	2.1	0.07820	2.1	0.07604	2.3	0.09565	1.9	0.06655
	3	1.9	0.06561	2.3	0.11012	2.0	0.07195	2.2	0.08289
	4	2.2	0.07826	2.1	0.08273	2.1	0.07734	2.1	0.08406
	5	2.1	0.07173	2.0	0.07986	2.5	0.13179	2.1	0.07539
	6	2.0	0.07147	2.0	0.07764	2.1	0.07179	2.0	0.06208
	7	2.0	0.05760	2.5	0.11845	2.2	0.08604	2.1	0.08158
	8	2.2	0.09977	2.2	0.09342	2.0	0.06958	2.1	0.07751
	9	1.8	0.05923	2.3	0.09258	2.0	0.06073	2.1	0.08777
D	1	2.2	0.08854	1.9	0.06951	2.1	0.08130	2.1	0.08333
	2	2.0	0.06779	2.2	0.08390	2.0	0.07196	2.0	0.06815
	3	2.1	0.07436	2.1	0.07270	1.9	0.05853	2.1	0.08292
	4	1.9	0.05937	2.0	0.07542	2.1	0.08229	1.9	0.06352
	5	2.0	0.07507	1.9	0.06566	2.0	0.06628	2.4	0.12492
	6	2.0	0.07489	1.7	0.04672	2.2	0.09673	2.2	0.08778
	7	2.1	0.07920	2.2	0.08423	2.1	0.07507	2.1	0.08692
	8	2.2	0.08432	1.9	0.06882	2.0	0.06629	2.3	0.11593
	9	2.0	0.06717	1.9	0.07250	2.3	0.10327	2.0	0.06905

Table 7: Summary of physicochemical parameters from water sampled at Carquinez Strait, during a 4-day, 12 dph *Menidia beryllina* exposure.

Date	Water Chemistry	Control @ Carquinez Straight Salinity					Carquinez Straight				
		Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (mS/cm)	Salinity (ppt)	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (mS/cm)	Salinity (ppt)
4/26/2014	Initial	19.7	8.12	8.4	17.98	11.8	19.6	7.98	8.2	16.83	11.5
	Final	--	--	--	--	--	--	--	--	--	--
4/27/2014	Initial	20.8	8.16	8.1	17.84	11.6	21.2	8.08	8.0	17.74	11.4
	Final	19.5	8.04	7.9	--	--	19.4	7.92	8.1	--	--
4/28/2014	Initial	20.6	8.17	8.6	17.53	11.5	20.3	7.99	8.6	17.08	11.1
	Final	20.7	8.01	7.5	--	--	20.6	7.87	7.6	--	--
4/29/2014	Initial	20.6	8.12	8.3	17.66	11.6	20.5	7.96	8.3	17.56	11.3
	Final	20.9	7.94	7.0	--	--	20.8	7.79	7.4	--	--
4/30/2014	Initial	--	--	--	--	--	--	--	--	--	--
	Final	19.6	8.08	7.9	--	--	19.6	7.97	7.8	--	--
Initial Maximum		20.8	8.2	8.6	18.0	11.8	21.2	8.1	8.6	17.7	11.5
Initial Minimum		19.7	8.1	8.1	17.5	11.5	19.6	8.0	8.0	16.8	11.1
Final Maximum		20.9	8.1	7.9	0.0	0.0	20.8	8.0	8.1	0.0	0.0
Final Minimum		19.5	7.9	7.0	0.0	0.0	19.4	7.8	7.4	0.0	0.0

Initial water physicochemical parameters measured before a water renewal

Final water physicochemical parameters measured at water renewal, after a 24-hour period of fish exposure

Table 8: Summary of physicochemical parameters from water sampled at Boynton Slough, during a 4-day, 12 dph *Menidia beryllina* exposure.

Date	Water Chemistry	Control @ Boynton Slough Salinity					Boynton Slough				
		Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (µS/cm)	Salinity (ppt)	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Electrical Conductivity (µS/cm)	Salinity (ppt)
4/26/2014	Initial	19.4	8.11	9.0	2301	1.3	19.6	8.30	9.0	2098	1.2
	Final	--	--	--	--	--	--	--	--	--	--
4/27/2014	Initial	21.2	8.24	8.4	2388	1.3	20.9	8.30	8.4	2153	1.2
	Final	19.5	7.90	8.0	--	--	19.6	8.42	7.8	--	--
4/28/2014	Initial	20.4	8.09	8.9	2163	1.2	20.4	8.42	8.9	2074	1.2
	Final	20.3	7.84	7.7	--	--	20.4	8.35	7.8	--	--
4/29/2014	Initial	20.6	8.07	8.7	2185	1.2	20.4	8.39	8.7	2118	1.2
	Final	20.8	7.82	7.8	--	--	20.9	8.40	7.7	--	--
4/30/2014	Initial	--	--	--	--	--	--	--	--	--	--
	Final	19.6	8.04	8.3	--	--	19.6	8.46	7.9	--	--
Initial Maximum		21.2	8.2	9.0	2388.0	1.3	20.9	8.4	9.0	2153.0	1.2
Initial Minimum		19.4	8.1	8.4	2163.0	1.2	19.6	8.3	8.4	2074.0	1.2
Final Maximum		20.8	8.0	8.3	0.0	0.0	20.9	8.5	7.9	0.0	0.0
Final Minimum		19.5	7.8	7.7	0.0	0.0	19.6	8.4	7.7	0.0	0.0

Initial water physicochemical parameters measured before a water renewal

Final water physicochemical parameters measured at water renewal, after a 24-hour period of fish exposure