U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2013 Progress Report:

Survival and initial feeding response of Delta Smelt larvae (*Hypomesus transpacificus)* exposed to Sacramento- San Joaquin Delta water obtained in April and May 2013.

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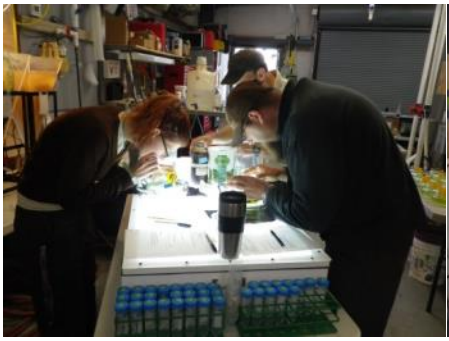
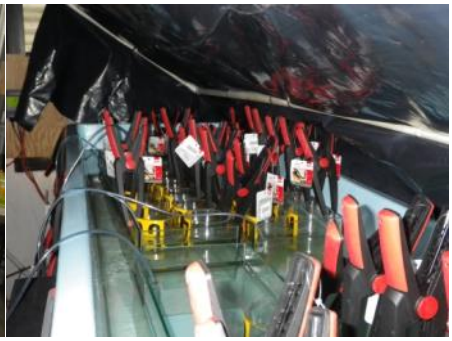

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Summary: Five trials (between 9April and 15May2013) were conducted with newly hatched delta smelt larvae reared for 6 days in delta water. Sacramento river water was collected from Cache slough, Deepwater shipping channel, and near the Rio Vista Ferry ramp. Reduced 6d survival and/or feeding response of larvae was observed in the 9April Cache slough and Deepwater shipping channel water and 25April Ferry ramp water. Three fungicides and 2 organophosphates were detected in the water samples however no consistent pesticide detection was associated with impaired survival or feeding response.

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Background – *The delta smelt (Hypomesus transpacificus) was listed as threatened under the Endangered Species Act in 1993 and is endemic to the upper San Francisco Bay Estuary – lower Sacramento and San Joaquin River delta (Moyle et al. 1992). Potential factors associated with these declines in delta smelt include reduction in freshwater flows, entrainment losses at diversions and power plants, inadequate food base and competition for food from exotic species, predation by exotic fishes and environmental contaminants.*

Spawning occurs from late February to June when water temperatures are between 7 and 15^{0}C. Egg incubation lasts about 11 to 13 days at temperatures between 14 and 16^{0}C and 8 to 10 days at temperatures between 15 and 17^{0}C. Optimal hatching and larval survival occurs at 15 to 17^{0}C in captivity. Larvae are most abundant in the wild from mid-April through May. Newly hatched yolk-sac larvae are phototaxic and swim continuously (Baskerville-Bridges et al. 2004). Size of yolk-sac larvae ranges from 4.5 to 6 mm total length at hatch to about 5 to 8 mm standard length when exogenous feeding begins at 4 to 8 days post hatch (Bennett 2005). First feeding larvae eat sub-adult cyclopid and calanoid copepods in the wild, but are fed rotifers in captivity. There is little information on losses of larval delta smelt, but further restrictions on recruitment may be imposed by pulses of pesticides and other chemicals resulting from urban and agricultural runoff during the larval rearing period (Kuivila and Foe 1995).The effects of toxic chemicals on larval survival are obscured by the potential for synergisms between chemicals, and the constantly changing types of chemicals involved (Moon et al. 2000); however, water collected from the Delta in recent years has been associated with detrimental effects in fish. Water collected from the confluence of Cache and Lindsey sloughs during April, 2009 decreased the survival of >30-day-old delta smelt larvae (Reece et al. 2009). During the spawning seasons of 1998 and 1999, eight to ten different pesticides were detected near the confluence of the Sacramento and San-Joaquin rivers, with as many as eight occurring in the same water sample. The most prevalent contaminants were thiocarbamate herbicides used on rice fields, including EPTC, molinate, and thiobencarb (Moon et al. 2000). Complex mixtures of toxic chemicals may kill delta smelt yolk-sac larvae or cause spinal deformities, neurotoxicity, genotoxic effects, or other physiological insults sufficient to impair feeding or growth. Fish larvae are sensitive to delays in initial feeding. Delaying first feedings to three days after opening of the mouth killed larval Chu's drum (Huang et al. 2005). Insufficient feeding may affect larval delta smelt indirectly by reducing growth rates and prolonging the vulnerable larval stage (Nobriga 2002).

The USFWS maintains a refugial population of delta smelt at the Livingston Stone National Fish Hatchery (LSNFH) near Shasta Dam on the Sacramento River, California pursuant to Recovery Permit #TE702631. The refugial population at LSNFH is secondary to the population maintained at the UC Davis Fish Conservation and Culture Laboratory in Byron, California. Progeny of the secondary refugial population at LSNFH are utilized for scientific research involving fish health, transportation, marking, and tagging. We used excess delta smelt yolk-sac larvae produced at LSNFH to conduct bioassays at the USFWS California Nevada Fish Health Center (CNFHC) in Anderson, California. In 2013, five trials were conducted between 9April and 21May.

General Methods

Sacramento River (delta) water was collected in the morning close to ebb tide. Collection sites included Cache Slough (N 38.24450°, W 121.68980°), the Sacramento Deep Water Channel (N 38.26851°, W 121.66409°), and on 25April the Rio Vista Ferry ramp (N 38.18642, W 121.66255)(Figure 1). Merz et. al (2011) report both adult and larvae are found in Cache slough and the Sacramento Deep Water Channel.

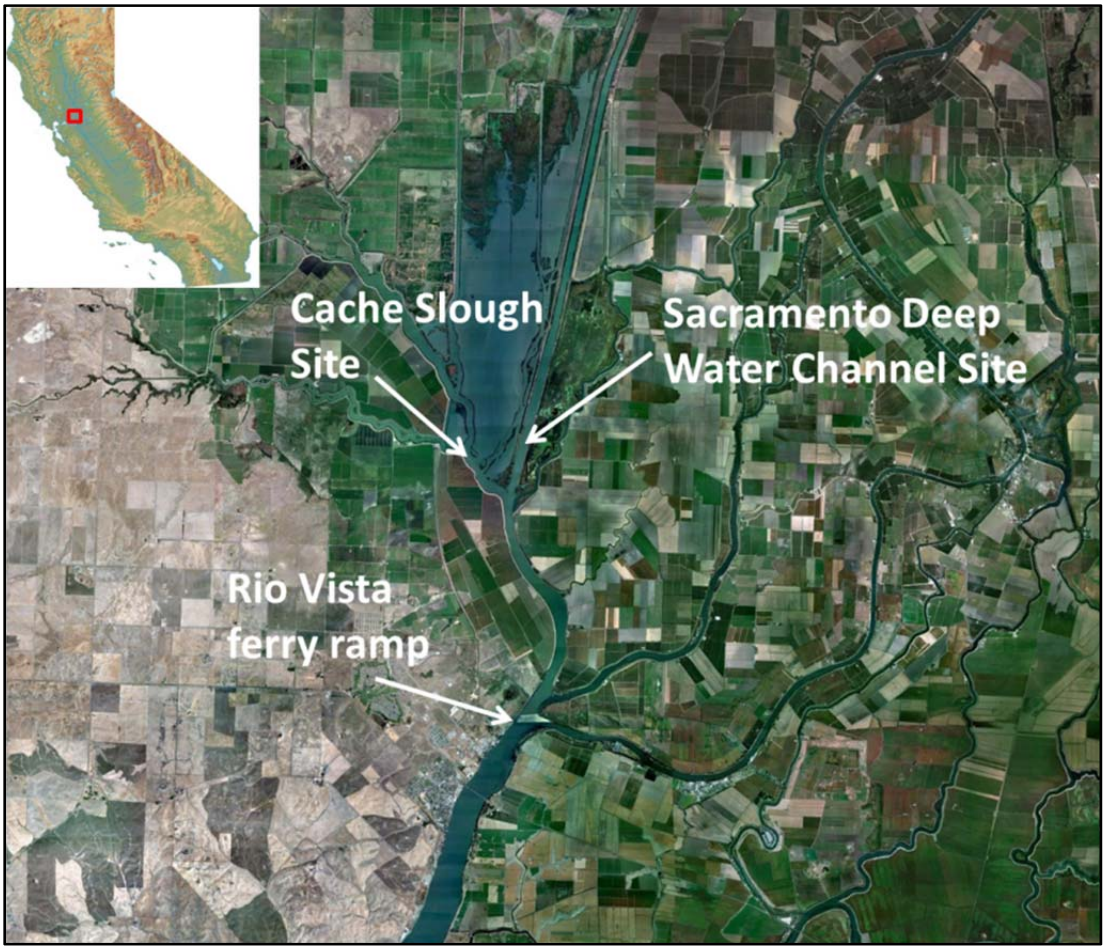

Figure 1. 2013 water sampling locations. The Cache Slough and Sacramento Deep Water Channel sites are the normal sampling locations. Sampling on April 25, 2013 was conducted at the Rio Vista ferry ramp due to adverse weather condition.

River water was obtained near the surface and placed into three 18.9-L (5 gal) chemically cleaned glass carboys held within an ice chest. All glassware was cleaned with 0.2% Liquinox, multiple distilled water rinses, and ASC methanol (USGS 2004). Three liter water samples were also collected for analysis of pyrethroids, fungicides, organochlorines, and organophosphates by the California Department of Fish and Game Water Pollution Control Laboratory in Rancho Cordova. Newly hatched larvae were placed into replicate containers containing either river ($\leq$ 6h post-collection) or laboratory control water for 6 days. Coleman National Fish Hatchery water with Instant Ocean^{tm} salt (enough to equal mean conductivity of the delta samples). Replicate containers were held under a black tarp to reduce ambient light to <20 lux with temperature maintained in a 15 - 17°C water bath.

On day 6 post-exposure (dpe), turbidity of each replicate container was adjusted to approximately 9 NTU by adding 40,000 rotifers in control water with Nanno 3600™ algal paste (Baskerville-Bridges et al. 2004). Replicates were fed sequentially and sampled 1 h later. Upon sampling, dead larvae were first counted and removed. Live larvae were anesthetized with MS222 prior to placement into zinc 10% formalin fixative (Zfix™, Anatech Ltd, Battle Creek,MI). These larvae were later microscopically examined to determine the number of rotifers in their gastro-intestinal tracts. The standard length, of 10 live anesthetized larvae from a replicate cylinder not examined for survival or feeding, was measured by an eyepiece reticle mounted on a stereo microscope. Ten individual larvae from the same cylinder were fixed in RNAlater for archival purposes. . Acetylcholinesterase activity of 5 pool larvae samples was assayed by the method of Wheelock et al. (2005). Briefly, larvae were frozen in (0.3mL) tank water on dry ice, stored at -70°C, defrosted and centrifuged (4,000xgx 5 min), water decanted and replaced with 200 µL of PBS –tritonX buffer, sonicated and centrifuged, and supernatant assayed for activity and total protein as per Wheelock et al. (2005). Activity (nmoles /min/mg protein) was determined from the mean Vmax (OD/min) value converted to moles/L/min by dividing the molar absorption coefficient for reduced Ellman reagent (5-thio-2-nitrobenzoic acid) and a final division by the protein content of the sample (Eyer et al. 2003).

Statistical analyses

We modeled larvae survival and fed status as a binary random variables, $y_i \sim Bin(1, \pi_i)$, where y_i is 1 if larva$_i$ survived (or surviving larva$_i$ fed), and 0 if not, and π_i is the probability of larva surviving the experiment, and if surviving, the probability they fed . To assess the effects of water source and survival and fed status, while additionally accounting for correlation among responses from the same experimental tanks, we used a binomial generalized linear mixed model with a logit link function, and a random effect for trials where

$$E[Y \mid \delta] = g^{-1}(X\beta + Z\delta)$$

and **X** is a design matrix of treatment levels for water sources, **β** is a vector of regression coefficients, **Z** is a design matrix specifying the trial for each observation, **δ** is a vector of random effects parameters, and *g()* represents the logit link function. We assessed the significance of treatment effects via likelihood ratio tests. Model parameters were estimated with SAS software's GLIMMIX procedure using adaptive Guass-Hermite quadrature to approximate the log-likelihood (SAS Institute Inc., 2008).

Results:

General trends- There was consistent difference between pH measurements of delta water upon collection (YSI® 556 MPS Water Quality Meter) and 4 – 6 h later at the wetlab (Hach 40D meter). Measurements done at the time of collection from Cache slough were 0.38 to 2.07 pH units lower, Deepwater Ship Channel 0.42 – 0.86 lower, and Ferry ramp (25April) was 1.45 units lower than measurements taken the same day upon arrival at the wetlab. It is likely that variability in pH meters is responsible for the difference. Un-ionized ammonia estimates for day 1 were based on pH values recorded at the wetlab (Florida Department of Environmental Protection. 2011). Both NTU and ammonia dropped over the 6 day exposure. Oxygen was at saturation and temperatures were 16±1°C for all trials. Light levels ranged from 1 – 16 lux depending on the time of measurement. Except for trial 1, conductivity (µS/cm) of control was selected as the mean of the 2 delta water sources. Statistical data generated by the binomial generalized linear mixed model for survival and feeding response of all trials is listed in Table 1 and Table 2, respectively.

Table 1. Estimated probability (Prob) of survival for the five 2013 trials and four water sources (laboratory control = Control, mouth of Cache slough = Cache, Deep water Ship Channel = DWSC, and Sacramento R. at the Rio Vista Ferry ramp = Ferry).

Trial	Source	Prob(Survival)	Lower 95%	Upper 95%
1	Control	0.990	0.972	0.997
1	Cache	0.937	0.858	0.973
1	DWSC	0.912	0.785	0.967
2	Control	0.949	0.903	0.974
2	Cache	0.948	0.899	0.974
2	DWSC	0.937	0.882	0.968
3	Control	0.976	0.949	0.989
3	Ferry	0.939	0.895	0.966
4	Control	0.964	0.920	0.984
4	Cache	0.904	0.815	0.953
4	DWSC	0.948	0.878	0.979
5	Control	0.953	0.901	0.979
5	Cache	0.916	0.832	0.960
5	DWSC	0.968	0.927	0.986

Table 2. Estimated probabilities of surviving larvae being classified as “fed,” for the five 2013 trials and four water sources (laboratory control = Control, mouth of Cache slough = Cache, Deep water Ship Channel = DWSC, and Sacramento R. at the Rio Vista Ferry ramp = Ferry). No data is presented for trial 4 due to fixation problem.

Trial	Source	Prob(Fed)	Lower 95%	Upper 95%
1	Control	0.583	0.516	0.647
1	Cache	0.240	0.180	0.312
1	DWSC	0.272	0.200	0.366
2	Control	0.344	0.266	0.431
2	Cache	0.384	0.299	0.476
2	DWSC	0.493	0.403	0.583
3	Control	0.644	0.591	0.693
3	Ferry	0.279	0.233	0.331
5	Control	0.481	0.427	0.535
5	Cache	0.404	0.348	0.463
5	DWSC	0.434	0.382	0.489

Three fungicide and two organophosphate pesticides were detected in the delta water samples (Table 3). Laboratory control water was assayed from trial 1 with no pesticide detection. Azoxystrobin was detected in each delta sample with concentrations ranging from 0.009 – 0.020 µg/L. Tebuncconazole (0.005 and 0.007 µg/L) and disulfoton (0.113 and 0.185 µg/L) was detected in each of two collections. Chloropyriphos (0.028 µg/L) was detected in the 9April Cache Slough sample and tetraconazole (0.005 and 0.007 µg/L) in both 18April delta samples. These concentrations were 10^4 – 10^6X below the reported 96h LD_{50} values for bluegill (Azoxystrobin = USEPA Pesticide Fact Sheet, Tebuncconazole = PAN Pesticide database, Disulfoton = PAN Pesticide database, Chloropyriphos = National pesticide information center technical fact sheet, tetraconazole= USEPA Pesticide Fact Sheet).

Table 3. Pesticide detections in collected delta water.

Delta Smelt Contaminants Investigation: 2013 Water Sampling Results			
Water Sample Date	Site	Pesticide Detected	Concentration (µg/L)
4/9/2013	Cache Slough	Azoxystrobin	0.019
		Chlorpyriphos	0.028*
	Ship Channel	Azoxystrobin	0.02
4/18/2013	Cache Slough	Azoxystrobin	0.012
		Tetraconazole	0.005*
	Ship Channel	Azoxystrobin	0.015
		Tetraconazole	0.007*
4/25/2013	Rio Vista Ferry	Azoxystrobin	0.009*
		Tebuconazole	0.006*
		Disulfoton	0.185
5/8/2013	Cache Slough	Azoxystrobin	0.011
	Ship Channel	Azoxystrobin	0.013
5/15/2013	Cache Slough	Azoxystrobin	0.008
		Disulfoton	0.113
	Ship Channel	Azoxystrobin	0.014

** Analyte detected above dectection limit, but below quantification limit. Value is an estimate.*

No significant difference (P>0.05) in acetylcholinesterase activity was observed between 5 pooled whole larvae samples of the treatment groups (Table 4).

Table 4. Acetylcholinesterase activity (nmol / min/ mg protein) of 5 larvae pools of Control, Cache slough, Deepwater Shipping Channel (DWSC), and Ferry ramp water sample groups in trials 1-3, and 5.

Trial	Control	Cache	DWSC	Ferry
13-1	120 (21)	121 (12)	104 (12)	NT
13-2	113 (20)	116 (20)	107 (10)	NT
13-3	118 (23)	NT	NT	125 (20)
13-5	126 (10)	156 (62)	171 (79	NT

NT = not tested

Trial 13-1, 9-15April 2013

Delta water was collected between 9:00 and 10:00 am from an out-going tide (discharge SRV gauge = 118, 562 cfs) with larvae going into water treatments beginning at 4:00pm. Control water conductivity was selected from 2:00 pm CDEC data at Rio vista and later found to be 97 – 153 µS/cm lower than the delta water (Table 6). Dissolved oxygen, temperature, ammonia, and pH of the 3 water sources were deemed acceptable for larval survival. Azoxystrobin (0.019 and 0.020 µg/L) was detected in each delta sample (Table 3). Chlorpyriphos (0.028 µg/L) was detected in the Cache slough sample.

There was strong evidence that survival from the control water source was higher than either Cache slough (p-value = 0.0065) or DWSC water (p-value = 0.0030). The odds of survival from the control water group was estimated to be 6.7 times higher (95% CI: 1.8 to 25 times higher) than Cache slough and 9.6 times higher (95% CI:2.3 to 40 times higher) than DWSC. Though the statistical evidence for higher survival from the control group is strong, the estimated probabilities of survival from each water source suggest marginal biological significance in the differences (Table 1). Mean survival ranged from 76 – 90% (Table 5). Similarly, a higher proportion of surviving larvae from the control group preyed upon rotifers (fed) compared to larvae from Cache slough (p-value < 0.0001) and DWSC water (p-value < 0.0001). The odds of eating for the control water source group was estimated to be 4.4 times higher (95% CI: 2.8 to 7 times higher) than those larvae from Cache slough and 3.7 times higher (95% CI: 2.2 to 6.3 times higher) than those from DWSC. The estimated proportions of fed from each group show potentially more biological significance than the survival data (Table 2). Mean percent fed ranged from 24 to 58% (Table 5). Mean (SD) standard length of 6dpe Control, Cache slough, and DWSC larval groups was 62 (3.7), 65 (3.0), and 63 (6.1) millimeters, respectively. No significant difference in length was detected by 1-way ANOVA (P=0.421).

Table 5. Mean (SD) percent survival and fed data for control, Cache slough, and Deep water shipping channel replicates (no.). Coefficient of variation (cv) is also reported.

%Survival	**Control**	**Cache**	**DWSC**
mean	90%	85%	76%
sd	14%	15%	17%
cv	15%	17%	23%
no.	15	7	7
%Fed			
mean	58%	24%	27%
sd	12%	12%	10%
cv	20%	52%	36%

Table 6. Water quality (temperature °C, pH, total ammonia nitrogen (NH4-N), Nephelometric Turbidity Units (NTU), electric conductivity (EC = µS/cm), dissolved oxygen (mg/L), and estimated un-ionized ammonia (NH3)) of the 3 water sources (Cache slough, Deep water Ship Channel, and control) over the 6 d trial.

Site	Cache Slough								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	9-Apr	9:00	15.2	ND*	ND	ND	317	9.6	
0	9-Apr	18:04	18.2	7.96	0.19	34.5	308	10.1	0.0063
1	10-Apr	7:40	17.1	8.22	0.23	28.0	310	10.1	0.0110
2	11-Apr	8:00	14.9	8.42	0.10	17.7	305	10.4	0.0057
3	12-Apr	7:50	15.5	8.36	0.18	14.8	306	10.3	0.0102
4	13-Apr	16:10	16.5	8.20	0.05	4.9	302	10.1	0.0023
5	14-Apr	9:15	16	8.30	0.08	3.6	308	10.4	0.0044
6	15-Apr	6:50	15.4	8.35	0.09	3.2	309	10.3	0.0051
mean			16.1	8.26	0.13	15.2	308	10.2	0.0064
SD			1.1	0.15	0.07	12.4	4	0.3	0.0031
*	on 4/9 9:00 was 5.92, suspect								
Site	Ship Channel								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	9-Apr	10:04	16.2	7.78	ND	ND	371	9.3	
0	9-Apr	18:04	18.8	8.22	0.13	13.2	364	10.1	0.0072
1	10-Apr	7:40	17.1	8.24	0.15	8.9	365	10.6	0.0072
2	11-Apr	8:00	15.1	8.13	0.11	13.9	362	10.0	0.0038
3	12-Apr	7:50	15.6	8.08	0.05	4.9	364	9.6	0.0017
4	13-Apr	16:10	16.5	8.21	0.05	1.4	357	9.5	0.0023
5	14-Apr	9:15	15.8	8.18	0.07	1.4	363	9.8	0.0031
6	15-Apr	6:50	15.4	8.00	0.11	1.0	366	9.8	0.0030
mean			16.3	8.11	0.10	6.4	364	9.8	0.0041
SD			1.2	0.15	0.04	5.6	4	0.4	0.0022
Site	Control								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	8-Apr	10:04	17.2	7.99	nd	nd	93.2	nd	nd
0									
1	10-Apr	7:40	17.1	7.98	0.09	2.6	210.6	9.35	0.0028
2	11-Apr	8:00	15.2	7.56	0.04	1.17	223	9.7	0.0004
3	12-Apr	7:50	15.5	7.62	0.06	1.68	220	9.6	0.0007
4	13-Apr	16:10	16.5	7.48	0.04	1.23	202	9.47	0.0003
5	14-Apr	9:15	15.9	7.68	0	1.16	215	9.71	0.0000
6	15-Apr	6:50	15.5	7.51	0.02	1.2	218	9.5	0.0002
mean			16.1	7.69	0.04	1.5	197	9.6	0.0007
SD			0.8	0.21	0.03	0.6	46	0.1	0.0010

Trial 13-2, 18-24April 2013

Delta water was collected between 9:54 and 10:36 am from an out-going tide (discharge SRV gauge = 33,542 cfs) with larvae going into water treatments beginning at 4:18pm. Dissolved oxygen, temperature, ammonia, and pH of the 3 water sources were deemed acceptable for larval survival Table 8). Azoxystrobin (0.012 and 0.015 µg/L) and tetraconazole (0.005 and 0.007 µg/L) was detected in each delta sample (Table 3).

There was no evidence that survival differed by water source (p-value = 0.8811). Accordingly, the estimated survival probabilities for each group are quite similar (Table 1). Mean survival was similar ranging from 82 - 89% (Table 7). There was moderate evidence that a lower proportion of surviving larvae from the control group fed than those from DWSC water 2 (p-value = 0.022), but no evidence of a difference between the control source survivors and those from Cache slough water (p-value = 0.515). The odds of being fed was estimated to be 1.85 times higher (95%CI: 1.1 to 3.1 times higher) for the survivors from DWSC water than those from the control group, differences reflected in the estimated probabilities of being fed (Table 2). Mean percent fed ranged from 35 to 52% (Table 7). Mean (SD) standard length of 6dpe Control, Cache slough, and DWSC larval groups was 65 (0.7), 66, (1.4), and 65 (0.9) millimeters, respectively. No significant difference in length was detected by 1-way ANOVA (P=0.307).

Table 7. Mean (SD) percent survival and fed data for control, Cache slough, and Deep water shipping channel replicates (no.). Coefficient of variation (cv) is also reported.

%Survival	Control	Cache	DWSC
mean	89%	82%	86%
sd	10%	23%	13%
cv	11%	28%	15%
%Fed	Control	Cache	DWSC
mean	35%	40%	52%
sd	16%	18%	16%
cv	46%	46%	31%

Table 8. Water quality (temperature °C, pH, total ammonia nitrogen (NH4-N), Nephelometric Turbidity Units (NTU), electric conductivity (EC = µS/cm), dissolved oxygen (mg/L), and estimated un-ionized ammonia (NH3)) of the 3 water sources (Cache slough, Deep water Ship Channel, and control) over the 6 d trial.

			date	time	cfs				
	riovista discharge		18-Apr	10:00	33542		lux range	1	8
			18-Apr	10:30	15266				
Site	#1 Cache Slough								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	18-Apr	9:54	15.6	6.12	ND	ND	235	9.44	
0	18-Apr	3:35	18	8.19	0.24	12.78	232	9.88	0.0124
1	19-Apr	8:12	15.9	8.1	0.29	16.5	233	10.10	0.0104
2	20-Apr	9:30	15.8	8.28	0.21	14.97	230	10.33	0.0117
3	21-Apr	9:32	15.8	8.25	0.17	8.43	231	10.07	0.0095
4	22-Apr	8:14	15.8	8.25	0.13	6.31	231	9.80	0.0072
5	23-Apr	8:25	15.7	8.13	0.11	4.53	231	9.59	0.0039
6	24-Apr	6:55	15.7	8.05	0.04	3.47	232	8.64	0.0014
mean			16.0375	7.92	0.17	9.6	232	9.7	0.0081
SD			0.8	0.73	0.08	5.2	2	0.5	0.0041
Site	2 Ship Channel								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	18-Apr	10:36	15.4	7.31	ND	ND	274	ND	
0	18-Apr	3:35	18.3	8.17	0.22	21.11	267	9.83	0.0113
1	19-Apr	8:12	15.8	8.18	0.23	20.64	266	10.10	0.0103
2	20-Apr	9:30	15.7	8.24	0.20	14.64	264	10.29	0.0089
3	21-Apr	9:32	15.7	8.28	0.15	10.31	265	9.98	0.0083
4	22-Apr	8:14	15.9	8.23	0.1	7.30	265	9.27	0.0045
5	23-Apr	8:25	15.7	8.14	0.06	5.56	266	9.25	0.0021
6	24-Apr	6:55	15.7	8.14	0.06	0.86	261	9.70	0.0021
mean			16.0	8.09	0.15	11.5	266	9.8	0.0068
SD			0.9	0.32	0.07	7.7	4	0.4	0.0038
Site	Control								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	18-Apr	3:35	16.4	8.31	0.00	1.13	255	10.96	0.0000
0	19-Apr	8:12	16.4	8.12	0.00	0.65	256	10.20	0.0000
1	20-Apr	9:30	15.8	8.36	0.06	0.95	252	10.29	0.0035
2	21-Apr	9:32	15.9	8.31	0.02	0.63	260	10.07	0.0011
3	22-Apr	8:14	15.7	8.26	0.03	0.47	256	9.78	0.0017
4	23-Apr	8:25	15.7	8.14	0.06	0.86	261	9.70	0.0021
5	24-Apr	6:55	15.6	8.24	0.02	0.44	262	9.47	0.0009
mean			15.9	8.25	0.03	0.7	257	10.1	0.0013
SD			0.3	0.09	0.02	0.3	4	0.5	0.0013

Trial 13-3, 25April-1May 2013

High winds prevented boat operations on 25April and the delta water sample was obtained at 9:40 am from the edge of the river at the Rio Vista Ferry ramp (Figure 1). Delta water was collected from an out-going tide (discharge SRV gauge = 113,898 cfs) with larvae going into water treatments beginning at 3:30 pm. Dissolved oxygen, temperature, ammonia, and pH of the 2 water sources were deemed acceptable for larval survival Table 10). Initial ammonia concentration (0.0150 NH_3-N, mg/L) of the Ferry ramp water sample was the highest level seen in all trials however it is much lower than the effect level of 0.05 mg/L) reported by Reinbold and Pescitelli (1982) for larval fish growth. Azoxystrobin (0.009 µg/L) , tebuconazole (0.006 µg/L), and disulfoton (0.185 µg/L) was detected in the sample (Table 3).

There was moderate evidence that survival was higher in the control group than those larvae from Ferry ramp water (p-value = 0.0399). The odds of survival in the control group was estimated to be 2.6 times higher (95%CI: 1.05 to 6.5 times higher) than Ferry ramp water. Similar to the results from Trial-1, the despite the statistical evidence of higher survival, the biological significance may be marginal (Table 1). Mean survival was 92% for controls and 85% for Ferry ramp water larvae (Table 9). There was strong evidence that the proportion of survivors being fed from the control water source group was higher than those from Ferry ramp water (p-value < 0.0001). The odds of being fed was estimated to be 4.7 times higher (95%CI: 3.3 to 6.5 times higher) for the control group, and the biological significance again appears to be greater than the estimated surviving proportion (Table 2). Mean percent fed was 64% for controls compared to 28% for Ferry ramp water larvae (Table 9). Mean (SD) standard length of 6dpe Control and Ferry ramp larval groups was 61 (1.9) and 60 (4.0 millimeters, respectively. No significant difference in length was detected by t-test (P=0.169) however variance was considered high in the Ferry ramp group.

Table 9. Mean (SD) percent survival and fed data for control and Ferry ramp water replicates (no.). Coefficient of variation (cv) is also reported.

%Survival	Control	Ferry ramp
mean	92%	85%
sd	10%	9%
cv	10%	11%
%Fed	Control	Ferry ramp
mean	64%	28%
sd	9%	8%
cv	14%	30%

Table 10. Water quality (temperature °C, pH, total ammonia nitrogen (NH4-N), Nephelometric Turbidity Units (NTU), electric conductivity (EC = µS/cm), dissolved oxygen (mg/L), and estimated un-ionized ammonia (NH3)) of the 2 water sources (Ferry ramp and control) over the 6 d trial.

			date	time	cfs				
	riovista discharge		25-Apr	9:45	113898		lux range	0	10
Site	#1 Ferry ramp Sac river (too windy for boat ops)								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	25-Apr	9:40	17.55	6.69	nd	nd	211	8.92	
0	25-Apr	3:07	19.2	8.14	0.34	38.59	230	9.29	0.0150
1	26-Apr	9:45	15.8	8.22	0.04	37.91	203	10.20	0.0019
2	27-Apr	9:00	15.9	8.19	0.02	37.71	205	10.13	0.0009
3	28-Apr	8:50	15.9	8.2	0.00		205	9.94	0.0000
4	29-Apr	6:30	16.0	8.19	0.04	3.17	205	9.84	0.0019
5	30-Apr	9:10	16.0	8.25	0.02	2.95	204	9.55	0.0011
6	1-May	6:25	15.7	8.01	0.00	3.06	206	8.52	0.0000
mean			16.50625	7.99	0.07	20.6	209	9.5	0.0030
SD			1.2	0.53	0.12	19.2	9	0.6	0.0053
Site 2	no sample								
Site	Control								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	25-Apr	3:07	17.0	8.26	0.01	2.61	212	10.12	0.0006
1	26-Apr	9:45	16.0	8.30	0.00	2.21	210	10.06	0.0000
2	27-Apr	9:00	16.2	8.20	0.00	2.02	212	10.08	0.0000
3	28-Apr	8:50	16.1	8.26	0.00		211	9.64	0.0000
4	29-Apr	6:30	16.3	8.22	0.03	0.69	211	9.67	0.0013
5	30-Apr	9:10	16.2	8.36	0.02	1.12	212	9.49	0.0012
	1-May	6:25	15.7	7.91	0.01	0.46	217	9.6	0.0002
mean			16.2	8.22	0.01	1.5	212	9.8	0.0005
SD			0.4	0.14	0.01	0.9	2	0.3	0.0006

Trial 13-4, 8-14May 2013

Delta water was collected between 9:48 and 10:12 am on out-going tide (discharge SRV gauge = 93,205 cfs) with larvae going into water treatments beginning at 3:30pm. Dissolved oxygen, temperature, ammonia, and pH of the 3 water sources were deemed acceptable for larval survival Table 12). Azoxystrobin (0.011 and 0.013 µg/L) was detected in each delta sample (Table 3).

There was no evidence that survival differed by water source (p-value = 0.1653). Accordingly, the estimated survival probabilities for each group are quite similar (Table 1). Mean percent survival ranged from 81 to 91% (Table 12). A procedural change, 10% buffered formalin in place of Zfix, resulted in poor fixation of gut contents and low confidence in rotifer identification within fixed larvae. No analysis was performed on percent fed data. Mean (SD) standard length of 6dpe Control, Cache slough, and DWSC larval groups was 62 (0.7), 61, (0.7), and 58 (1.6) millimeters, respectively. No significant difference in length was detected by Kruskal-Wallis 1-way ANOVA on ranks (P=0.197).

Table 12. Mean (SD) percent survival and fed data for control, Cache slough, and Deep water shipping channel replicates (no.). Coefficient of variation (cv) is also reported.

%Survival	**Control**	**Cache**	**DWSC**
mean	91%	81%	89%
sd	5%	16%	9%
cv	5%	20%	8%
%Fed **			
mean	22%	17%	39%
sd	14%	9%	16%
cv	63%	52%	40%
** poor fixation limited confidence to observed ingested prey			

Table 11. Water quality (temperature °C, pH, total ammonia nitrogen (NH4-N), Nephelometric Turbidity Units (NTU), electric conductivity (EC = µS/cm), dissolved oxygen (mg/L), and estimated un-ionized ammonia (NH3)) of the 3 water sources (Cache slough, Deep water Ship Channel, and control) over the 6 d trial.

			date	time	cfs				
	riovista discharge		8-May	9:45	93205		lux range	0	11
			8-May	10:15	86998				
Site	#1 Cache slough								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	8-May	9:48	19.07	6.16			213	8.17	
0	8-May	3:25	19.1	8.01	0.19	29.21	208	8.61	0.0068
1	9-May	6:54	16.1	7.78	0.18	19.57	208	10.08	0.0033
2	10-May	6:45	15.9	8.21	0.20	12.88	209	10.07	0.0089
3	11-May	8:00	16.3	8.27	0.18	7.99	209	9.91	0.0100
4	12-May	7:40	16.1	8.08	0.12	5.52	207	9.79	0.0043
5	13-May	10:40	16.2	8.02	0.07	4.56	207	9.90	0.0020
6	14-May	6:42	15.9	8.09	0.04	4.16	208	10.08	0.0014
mean			16.83375	7.83	0.14	12.0	209	9.6	0.0052
SD			1.4	0.69	0.06	9.4	2	0.7	0.0034
Site	#2 ship channel								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	8-May	10:12	19.51	7.57			300	8.1	
0	8-May	3:25	19	8	0.13	27.63	297	8.70	0.0046
1	9-May	6:54	16	7.95	0.11	20.71	299	10.07	0.0032
2	10-May	6:45	15.9	8.11	0.11	11.49	299	10.06	0.0033
3	11-May	8:00	16.4	8.22	0.08	8.61	299	10.06	0.0037
4	12-May	7:40	16.2	7.94	0.02	5.94	296	9.75	0.0005
5	13-May	10:40	16.2	8.06	0.03	4.55	298	9.82	0.0009
6	14-May	6:42	16	7.94	0.01	4.39	299	10.10	0.0002
mean			16.9	7.97	0.07	11.9	298	9.6	0.0023
SD			1.5	0.19	0.05	9.0	1	0.8	0.0018
Site	Control								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	8-May	1:06	16.9	7.43	0.02	0.85	255	10.16	0.0012
1	9-May	6:54	16.4	7.53	0.00	0.46	258	10.15	0.0000
2	10-May	6:45	15.9	8.26	0.00	0.41	257	10.08	0.0000
3	11-May	8:00	16.4	8.28	0.00	0.30	259	10.09	0.0000
4	12-May	7:40	16.2	7.89	0.00	0.45	255	10.24	0.0000
5	13-May	10:40	16.2	7.76	0.03	0.24	254	9.77	0.0018
6	14-May	6:42	16.5	7.89	0	0.08	256	10.4	0.0000
mean			16.4	7.86	0.01	0.4	256	10.1	0.0004
SD			0.3	0.33	0.01	0.2	2	0.2	0.0007

Trial 13-5, 15-21May 2013

Delta water was collected between 9:15 and 10:15 am on out-going tide (discharge SRV gauge = 60,062 cfs) with larvae going into water treatments beginning at 3:00 pm. Dissolved oxygen, temperature, ammonia, and pH of the 3 water sources were deemed acceptable for larval survival Table 14). The day 0 ammonia (0.0133 mg/L estimated NH_3) observed in the DWSC water sample was the second highest value observed for all 5 trials. Azoxystrobin (0.008 and 0.014 µg/L) was detected in each delta sample (Table 3). Disulfoton (0.113 µg/L) was detected in the Cache Slough sample.

There was no evidence that survival differed by water source (p-value = 0.2055). Accordingly, the estimated survival probabilities for each group are quite similar (Table 1). Mean percent survival ranged from 79 to 87% (Table 13). There was weak evidence that a higher proportion of surviving larvae from the control group were fed than those from Cache slough water (p-value = 0.0591), but no evidence of a difference between the control group and those from DWSC water (p-value = 0.2278). The odds of being fed was estimated to be 1.4 times higher (95% CI: 0.98 to 1.9 times higher) for the control group survivors compared to those from Cache slough water. The estimated probabilities of being fed for each group reflect the rather benign estimated differences (Table 2). Mean percent fed ranged from 39 to 48% (Table 13). Mean (SD) standard length of 6dpe control, Cache slough, and DWSC larval groups was 59 (4), 60(3), and 60(2) respectively. No significant difference in length was detected by 1-way ANOVA (P=0.511).

Table 13. Mean (SD) percent survival and fed data for control, Cache slough, and Deep water shipping channel replicates (no.). Coefficient of variation (cv) is also reported.

%Survival	**Control**	**Cache**	**DWSC**
mean	85%	79%	87%
sd	14%	18%	15%
cv	17%	23%	17%
%Fed			
mean	48%	39%	44%
sd	10%	13%	10%
cv	20%	33%	21%

Table 13. Water quality (temperature °C, pH, total ammonia nitrogen (NH4-N), Nephelometric Turbidity Units (NTU), electric conductivity (EC = µS/cm), dissolved oxygen (mg/L), and estimated un-ionized ammonia (NH3)) of the 3 water sources (Cache slough, Deep water Ship Channel, and control) over the 6 d trial.

			date	time	cfs				
	riovista discharge		15-May	9:15	60062		lux range	0-16 (afternoon highe	
			15-May	10:15	106333				
Site	#1 Cache slough								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	15-May	9:15	20.6	7.58			171	8.01	
0	15-May	2:48	19.7	7.96	0.22	14.95	169.5	8.25	0.0081
1	16-May	8:05	16.2	7.59	0.23	6.97	165	9.55	0.0027
2	17-May	8:00	16.0	7.78	0.23	6.08	165.9	9.58	0.0042
3	18-May	12:20	16.4	7.61	0.21	3.99	165.5	9.44	0.0025
4	19-May	6:15	16.0	7.52	0.22	2.72	166	9.59	0.0020
5	20-May	9:00	16.5	7.51	0.11	1.69	165.6	9.42	0.0011
6	21-May	6:21	16.2	7.43	0.10	1.67	165.6	9.22	0.0007
mean			17.2	7.62	0.19	5.4	167	9.1	0.0031
SD			1.8	0.17	0.06	4.7	2	0.6	0.0025
Site	#2 ship channel								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	15-May	10:15	20.2	7.67			385	8.4	
0	15-May	2:48	20.1	8.09	0.29	31.22	374	8.75	0.0133
1	16-May	8:05	16.2	7.87	0.09	16.55	375	8.94	0.0021
2	17-May	8:00	16	7.84	0.06	12.47	375	9.08	0.0011
3	18-May	12:20	16.3	7.76	0.05	9.66	375	8.34	0.0009
4	19-May	6:15	15.9	7.66	0.06	8.41	375	8.08	0.0009
5	20-May	9:00	16	7.58	0.04	4.38	375	7.31	0.0005
6	21-May	6:21	16.1	7.51	0.04	3.26	375	7.35	0.0004
mean			17.1	7.75	0.09	12.3	376	8.3	0.0027
SD			1.9	0.18	0.09	9.5	4	0.7	0.0047
Site	Control								
Day	**Date**	**Time**	**Temp**	**pH**	**NH4-N**	**NTU**	**EC**	**DO**	**NH3 est.**
0	15-May	2:22	17.2	7.66	0.01	1.66	280	10.20	0.0002
1	16-May	8:05	16.6	7.32	0.05	2.04	286	9.79	0.0003
2	17-May	8:00	16.1	7.29	0.01	1.15	287	9.34	0.0001
3	18-May	12:20	16.1	7.38	0.02	2.59	275	8.13	0.0001
4	19-May	6:15	16.2	7.25	0.01	0.80	288	9.08	0.0001
5	20-May	9:00	16.7	7.20	0.03	0.25	283	8.91	0.0002
6	21-May	6:21	16.4	7.30	0.02	0.08	286	8.9	0.0001
mean			16.5	7.34	0.02	1.2	284	9.2	0.0001
SD			0.4	0.15	0.01	0.9	5	0.7	0.0001

Discussion: Reduced 6d survival and/or feeding response was observed for larvae reared in the 9April water collections (both delta sites) and 25April Ferry ramp collection. No consistent pesticide detection was associated with the impaired response groups. It is possible that either synergistic effects of low concentration pesticides or undetermined toxicants produced the observed effects. Other measured water parameters (temperature, pH, conductivity, ammonia, and dissolved oxygen) of the delta water collections were considered adequate for larval survival. The limitation of a single grab sample to represent the impacts of variable delta water quality on delta smelt larval survival could be overcome with in-situ exposures.

Author contribution: JS Foott, R. Stone and J. Jacobs performed the exposures and microscopic examination of fixed larvae. J. Jacobs transported water to the wetlab . JS Foott prepared this progress report; K Aceituno prepared the final funding proposal, obtained permits, collected water in the delta, transported water samples to the CDFG Water Pollution Laboratory, and reviewed this progress report. N. Som performed the statistical analysis of both survival and feeding responses.

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Reference:

Baskerville-Bridges, B, JC Lindberg and SI Doroshov. 2004. The effect of light intensity, alga concentration, and prey density on the feeding behavior of delta smelt larvae. Early Life History of Fishes in the San Francisco Estuary and Watershed, pp. 219-227. American Fisheries Society Symposium, Volume 39.

Bennett, WA. 2005. Critical assessment of the delta smelt population in the San Francisco Estuary, California. San Francisco Estuary and Watershed Science 3:1-71.

Eyer P, F Worek, D Kiderlen, G Sinko, A Stuglin, V Simeon-Rudolf, and E Reiner. 2003. Molar absorption coefficients for the reduced Ellman reagent: reassessment. Analytical BioChemistry 312: 224 - 227.

Florida Department of Environmental Protection. 2011. Chemistry Laboratory Methods Manual, Calculation of un-ionized ammonia in fresh water.

Huang, L, Y Xie, G Zhang and J Hu. 2005. Effects of delayed initial feeding on foraging, growth and survival of Chu's drum (Nibea chui) larvae. Journal of Dalian Fisheries University 20:300-303.

Kuivila, KM and CG Foe. 1995. Concentration transport and biological effects of dormant spray pesticides in the San Francisco Estuary, California. Environmental Toxicology and Chemistry 14:1141-1150.

Merz JE, S Hamilton, PS Bergman, and B Cavallo. 2011. Spatial perspective for delta smelt: a summary of contemporary survey data. California Fish and Game 97(4):164 – 189.

Moon, GE, KM Kuivila, C Ruhl and DH Shoelhamer. 2000. Exposure of delta smelt to dissolved pesticides in 1998 and 1999. Interagency Ecological Program Newsletter 13:27-33.

Moyle, PB, B Herbold, DE Stevens and LW Miller. 1992. Life history and status of delta smelt in Sacramento-San Joaquin Estuary, California. Transactions of the American Fisheries Society 121:67-77.

Nobriga, ML. 2002. Larval delta smelt diet composition and feeding incidence: environmental and ontogenetic influences. California Fish and Game 88:27-33.

Reece, C, D Markiewicz, L Deanovic, R Connon, B Sebastian, M Stillway and I Warner. 2009. Pelagic Organism Decline (POD): acute and chronic invertebrate and fish toxicity testing in the Sacramento-San Joaquin Delta 2008-2010, Progress Report. Aquatic Toxicology Laboratory, School of Veterinary Medicine, University of California, Davis.

Reinbold KA and SM Pescitelli. 1982. Effects of exposure to ammonia on sensitive life stages of aquatic organisms. EPA Contract report 68-01-5832/A. Center for Aquatic Ecology Illinois Natural History Survey, Champaign IL.

USGS. 2004. Chapter A3. Cleaning of equipment for water sampling, In: National field manual for the collection of water-quality data, Book 9 Handbooks for water-resource investigations, ed F.D. Wilde, http://pubs.water.usgs.gov/twri9A.