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ARTICLE

Examination of Predation on Early Life Stage Delta Smelt in the San Francisco Estuary Using DNA Diet Analysis

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Abstract
We examined predation by nonnative Mississippi Silversides *Menidia audens*, other small fishes, and invertebrates on the early life stages of the endangered Delta Smelt *Hypomesus transpacificus*, which is endemic to the Sacramento–San Joaquin Delta in California. Mississippi Silversides and other putative predators were collected primarily via boat electrofishing in the northern reaches of the upper San Francisco Estuary, an area targeted for substantial tidal wetland restoration to enhance habitat for Delta Smelt and other endangered fishes. Predators' digestive tracts were removed and analyzed for the presence of Delta Smelt DNA by using quantitative PCR TaqMan assays. Across all sites, 69 of 550 Mississippi Silversides tested positive for Delta Smelt DNA. The number of sampled Mississippi Silversides that were positive for Delta Smelt DNA was significantly greater in offshore habitats than in nearshore habitats. Delta Smelt DNA detection data indicated that a wide variety of other species were also predators of Delta Smelt. Additionally, we used generalized linear modeling to analyze the relationship between Delta Smelt predation detections in Mississippi Silversides and concurrently collected habitat parameters. Turbidity was identified as a significant predictor of predation, as Delta Smelt DNA was detected more often in Mississippi Silverside samples from clearer water. These results suggest that restoration efforts designed to increase turbidity in the estuary may be beneficial in reducing Mississippi Silversides' predatory impacts on Delta Smelt.

The Sacramento–San Joaquin Delta (hereafter, "Delta") is part of the largest estuary on the west coast of North America, and its watershed comprises 40% of California's land area (Nichols et al. 1986). Like many estuaries around the world (Carlton and Geller 1993), the Delta is a highly invaded system (Nichols et al. 1986; Cohen and Carlton 1998). One of the most abundant and

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successful invasive fish species in the Delta is the Mississippi Silverside *Menidia audens* (Nobriga et al. 2005; Brown and Michniuk 2007). Since the introduction of Mississippi Silversides into the Delta in approximately 1975 (Moyle 2002), they have dramatically increased in abundance (Brown and May 2006; Brown and Michniuk 2007). The proliferation of Mississippi Silversides and other invaders is associated with additional anthropogenic changes in the Delta ecosystem that have contributed to the precipitous decline of several native fish species (Sommer et al. 2007; Baxter et al. 2010; MacNally et al. 2010). One of these declining native fishes is the Delta Smelt *Hypomesus transpacificus*, a species that is endemic to the Delta and that is listed under both the California Endangered Species Act (Title 14, California Code of Regulations 670.5) and the federal Endangered Species Act (USFWS 1993). One of the many potential causes for the Delta Smelt's recent decline is predation by nonnative species (Sommer et al. 2007).

Predation is a major driver of fish population dynamics, and predation by nonnative species has been linked to reduced recruitment and subsequent declines in native fish populations (Taylor et al. 1984; Lever 1996). In several watersheds of the western United States, there is substantial evidence that small, nonnative planktivorous fishes are preying upon native fish larvae at significant levels (Ruppert et al. 1993; Dunsmoor 1995; Bestgen et al. 2006; Markle and Dunsmoor 2007; Carpenter and Mueller 2008). Nonnative planktivorous fishes often compete with the later life stages of native fishes as well and can therefore have compounding negative effects on native fish populations (Irigoien and de Roos 2011).

Mississippi Silversides are considered to be intraguild predators (Polis et al. 1989) of Delta Smelt, as the two species have similar diets and life histories (Moyle 2002) and consequently have a high degree of niche overlap. Laboratory experiments have shown that Mississippi Silversides are efficient competitors with Delta Smelt when fed brine shrimp *Artemia* nauplii (Bennett 2005). Mississippi Silversides readily consume larval (8–14-mm) Delta Smelt in captivity (B. M. Schreier, unpublished data) and are also known to consume Delta Smelt in the wild (Baerwald et al. 2012). The impacts of Mississippi Silverside predation on Delta Smelt are further exacerbated by the exceptionally high Mississippi Silverside densities that occur in Delta Smelt spawning and rearing areas (Bennett and Moyle 1996; Matern et al. 2002).

To understand the prevalence and habitat correlates of Mississippi Silverside predation on Delta Smelt, we used genetic techniques (Baerwald et al. 2011, 2012) to detect the presence of Delta Smelt DNA in the digestive tracts of wild Mississippi Silversides. The use of genetic tools to detect predation has become widespread (King et al. 2008) and is substantially more sensitive than traditional visual analyses. Although visual analysis can detect predation on larval fish up to 60 min after consumption (Schooley et al. 2008), genetic assays can detect larval fish predation up to 36 h after consumption (Baerwald et al. 2012).

We collected Mississippi Silversides and other predators of early life stage Delta Smelt from the northern Delta and Suisun Marsh (Figure 1), and we analyzed their digestive tracts for Delta Smelt DNA. Sampling was primarily focused on the northern portion of the Delta because Delta Smelt spawning is known to occur there and because larvae are commonly detected in that area (Sommer and Mejia 2013). Furthermore, Mississippi Silversides are locally abundant in the northern Delta, thereby maximizing our ability to detect patterns in predation. We addressed the following questions: (1) "Does predation on Delta Smelt differ among sampling regions within the northern Delta?"; (2) "Does predation on Delta Smelt vary with habitat variables?"; and (3) "How does predation compare among the different species of Delta Smelt predators?"

METHODS

Collection of Delta Smelt predators.—The primary target of our sampling efforts was the Mississippi Silverside, although other fish species that were large enough to be larval fish predators were identified a priori and retained for analysis (Table 1). Some predators had adult sizes that were large enough to target adult Delta Smelt; therefore, to prevent detections of adult Delta Smelt predation from confounding our results, predators over 200 mm FL were not retained for analysis (Table 1). Limiting the size of collected predators also reduced the likelihood of positive detections due to secondary predation. In addition to the a priori (targeted) predator species, other species (e.g., shrimp) that were capable of consuming early life stage Delta Smelt were saved in an ad hoc manner as circumstances allowed (Table 1). These ad hoc predator collections primarily consisted of species that were hypothesized to prey upon early life stage Delta Smelt but that could not be effectively collected by our sampling methods.

We primarily sampled predators at randomly selected points within four main sampling areas in the northern Delta (Lindsey Slough, Cache Slough, Liberty Island, and the Sacramento Deep Water Ship Channel [SDWSC]; Figure 1). Sampling effort was scaled among the four areas in an attempt to collect roughly equal numbers of predators from each area; that is, areas with lower predator catches received more sampling effort. Additional sampling was also conducted in the Sacramento River and Suisun Marsh (Table 2). Sampling was carried out biweekly from March 9 to June 29, 2011, coinciding with the presence of Delta Smelt larvae in the northern Delta, as determined by the 20-mm larval Delta Smelt survey conducted by the California Department of Fish and Wildlife (CDFW; www.dfg.ca.gov/delta/projects.asp?ProjectID=20 mm).

The primary collection method for predators was boat electrofishing (5.49-m [18-ft] Smith-Root electrofishing boat equipped with a Smith-Root 5.0 Generator-Powered Pulsator). Transects were recorded by using a Trimble GeoXM GPS data recorder. Electrofishing was conducted

TABLE 1. Numbers of individuals from each predator species that were analyzed for the presence of Delta Smelt DNA, along with predator FL range and mean, from samples obtained in the upper San Francisco Estuary (CS = Cache Slough; SDWSC = Sacramento Deep Water Ship Channel; LI = Liberty Island; LS = Lindsey Slough; SM = Suisun Marsh; SR = Sacramento River). Asterisks denote nonnative species.

Species	FL range analyzed (mm)	Mean FL analyzed (mm)	Number of individuals analyzed by area						Total number analyzed	Number positive for Delta Smelt DNA
			CS	SDWSC	LI	LS	SM	SR		
A priori (targeted) predators										
Mississippi Silverside *Menidia audens**	25–102	65	119	73	159	119	74	6	550	69
Black Crappie *Pomoxis nigromaculatus**	72–118	85	3	1	0	2	0	0	6	0
Bluegill *Lepomis macrochirus**	28–114	100	0	1	0	5	0	0	6	1
Largemouth Bass *Micropterus salmoides**	61–198	162	12	0	0	14	0	0	26	1
Sacramento Pikeminnow *Ptychocheilus grandis*	55–194	102	7	5	18	8	1	0	39	2
Spotted Bass *Micropterus punctulatus**	69	69	0	0	0	1	0	0	1	0
Striped Bass *Morone saxatilis**	32–198	133	0	19	6	1	31	0	57	1
Yellowfin Goby *Acanthogobius flavimanus**	50–116	70	1	1	2	1	0	0	5	0
Ad hoc predators										
American Shad *Alosa sapidissima**	123–145	134	0	0	1	1	0	0	2	0
Chinook Salmon *Oncorhynchus tshawytscha*	71–108	81	0	14	0	2	0	0	16	1
Golden Shiner *Notemigonus crysoleucas**	66–158	95	4	0	0	6	0	0	10	1
Prickly Sculpin *Cottus asper*	64–75	70	2	0	0	0	0	0	2	0
Redear Sunfish *Lepomis microlophus**	153–221	179	2	0	0	12	0	0	14	0
Sacramento Sucker *Catostomus occidentalis*	58	58	1	0	0	0	0	0	1	0
Shimofuri Goby *Tridentiger bifasciatus**	50–80	62	0	1	19	0	0	0	20	1
Shrimp *Exopalaemon* sp.*	N/A	N/A	0	1	3	0	0	0	4	1
Threadfin Shad *Dorosoma petenense**	67–153	88	1	11	4	5	0	0	21	1
Threespine Stickleback *Gasterosteus aculeatus*	34	34	0	0	0	1	0	0	1	1
Tule Perch *Hysterocarpus traskii*	29–115	53	2	1	0	3	0	0	6	1
Total			154	128	212	181	106	6	787	81
Percent positive for Delta Smelt DNA			5.6	16.3	9.5	9.2	7.5	50	9.8	

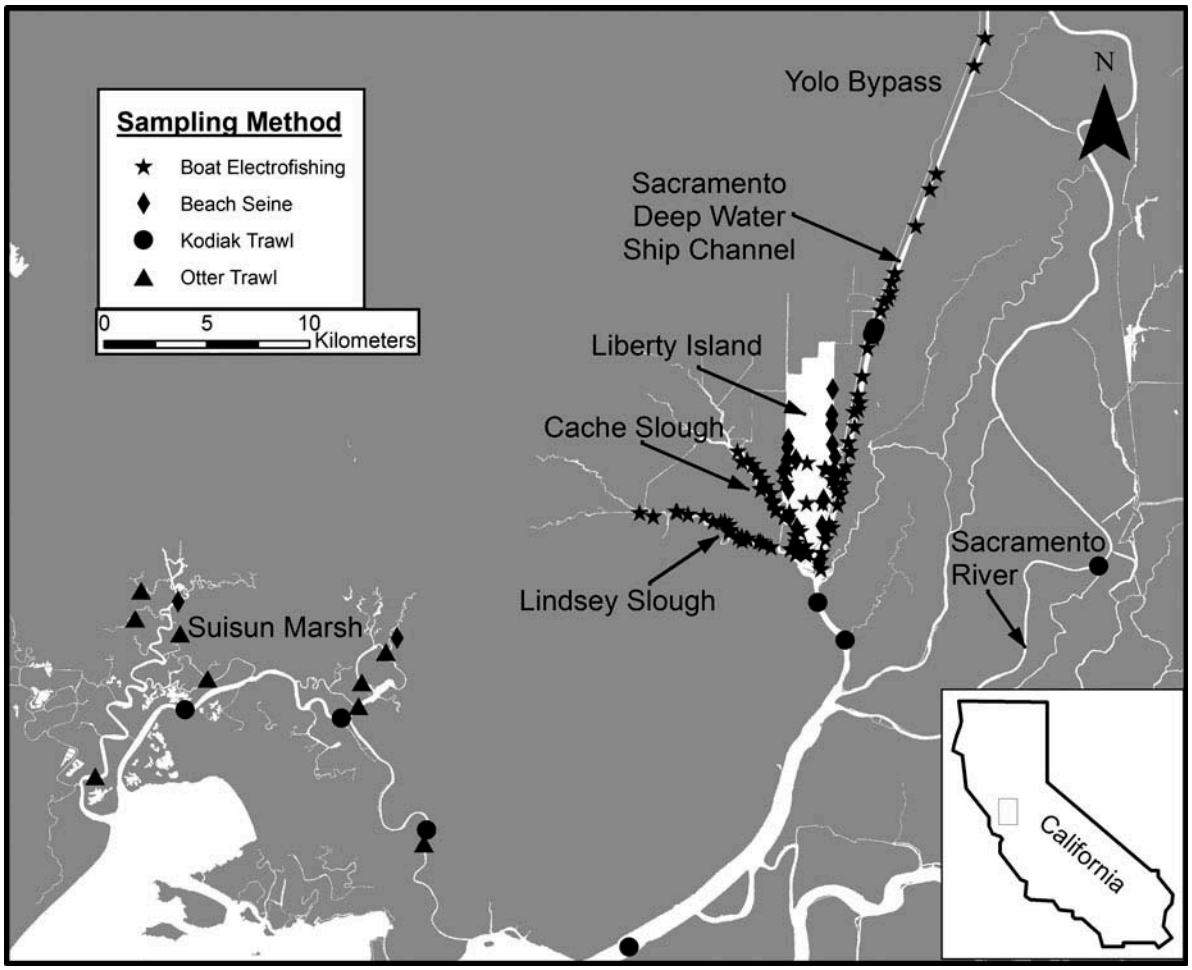

FIGURE 1. Map of the study area in the upper San Francisco Estuary, California, where predation on early life stage Delta Smelt was evaluated. Major sampling areas are labeled.

at 5–11 A (15–80% of range). Two netters on the bow captured stunned fish by using electrofishing nets (2.44-m [8-ft] handles; 1.27-cm [0.5-in] mesh), and collected fish were placed in the onboard live well. To minimize the chance of DNA contamination, any captured Delta Smelt were immediately placed in a separate aerated bucket, and the net that was used to collect them was sterilized with a 20% bleach solution before re-use.

Before sampling began at each site, we recorded the Secchi depth (cm) and measured surface pH, water temperature (°C), turbidity (NTU), dissolved oxygen concentration (mg/L), and specific conductance (µS/cm) with a multiparameter YSI Model 6600 sonde (Yellow Springs Instrument Company, Inc.). At the end of each transect, all fish were identified to species and measured for FL (nearest mm). Delta Smelt were measured first so as to minimize stress to the fish. To avoid DNA contamination, Delta Smelt were processed with a separate set of dip nets, buckets, and measuring boards, all of which were sterilized with bleach after each transect. All predators were placed alive in sealed, labeled buckets and were transported to the field dissection laboratory. The vessel's live well was drained after each transect, and all items of equipment that might have come into contact with the fish were sterilized with bleach at the end of each day.

To obtain broader geographic coverage of the Delta, we also collected predators that were sampled during long-term fish monitoring programs (Table 2; Figure 1). The programs used otter trawling and beach seining in Suisun Marsh (University of California–Davis, Suisun Marsh fish monitoring program; O'Rear and Moyle 2010); Kodiak trawling in the Sacramento River and Suisun Marsh (CDFW, spring Kodiak trawl program; www.dfg.ca.gov/delta/projects.asp?ProjectID=SKT); and beach seining at Liberty Island (U.S. Fish and Wildlife Service [USFWS], Delta juvenile fish monitoring program; www.fws.gov/stockton/jfmp/libertyisland.asp). These additional sampling methods encompassed a diverse array of habitat types, including open-water (otter trawling and Kodiak trawling), shoreline (beach seining), and littoral (boat electrofishing) habitats.

TABLE 2. Information on the spatial and methodological distribution of sampling effort, with associated catches of targeted (a priori) predators (see Table 1) and the number of individual predators that were positive for Delta Smelt DNA (SDWSC = Sacramento Deep Water Ship Channel). Sampling that was conducted by regular monitoring programs includes the associated organization (USFWS = U.S. Fish and Wildlife Service; UCD = University of California–Davis; CDFW = California Department of Fish and Wildlife).

Sampling area	Number of sites	Number of fish caught	Number of predators caught/ dissected	Number positive for Delta Smelt DNA
		Boat electrofishing		
Cache Slough	28	411	152	9
SDWSC	70	314	104	4
Liberty Island	55	171	36	5
Lindsey Slough	25	493	181	17
Total	178	1,389	473	35
		Beach seining		
Liberty Island (USFWS)	42	5	176	17
Suisun Marsh (UCD)	3	196	66	3
SDWSC	1	66	5	1
Total	46	267	247	21
		Kodiak trawling		
Cache Slough (CDFW)	6	46	2	0
SDWSC (CDFW)	3	124	19	17
Sacramento River (CDFW)	15	74	6	3
Suisun Marsh (CDFW)	18	268	11	5
Total	42	512	38	25
		Otter trawling		
Suisun Marsh (UCD)	12	29	29	0
Total	12	29	29	0
Grand total, all methods	278	2,197	787	81

Predators collected at Liberty Island (beach seining) and in Suisun Marsh (otter trawling and beach seining) were dissected in a manner identical to that used for predators captured by electrofishing. All of the predators captured via electrofishing were delivered alive to a field dissection laboratory that consisted of an enclosed trailer stationed in the field. The trailer provided a controlled, sterile environment where predators could be dissected and their gut contents could be preserved as soon as possible after capture, thus minimizing the postcapture digestion time. Predators that were sampled by Kodiak trawls were processed inside the trawling vessel's cabin immediately after capture.

Removal and analysis of gut contents.—Before processing fish from a given site, we sterilized all work surfaces, nets, and instruments with sequential rinses of 20% bleach, distilled water, and 95% ethanol. Predators were kept in their transfer buckets with aeration until they were processed at the dissection location. To minimize cross-contamination, only fish from a single sampling site were processed at any given time. In the rare instance that a predator died in transport or was observed to be substantively injured or impaired by heavy parasite loads, that individual was not saved for analysis. Before handling each predator, staff changed gloves and sterilized all work surfaces and dissecting instruments.

In an attempt to detect any contamination of a given site's predators by environmental DNA (eDNA) from Delta Smelt, water samples were collected from each transfer bucket after the predators were processed. A sterile eye dropper was used to sample 0.5 mL of agitated bucket water; these samples were subject to the same DNA extraction, quantitative PCR (qPCR), and analysis methods as the predator gut samples.

Predators were individually euthanized in a 250-mg/L solution of tricaine methanesulfonate (MS-222), which was prepared fresh for each bucket of predators. During euthanasia, fish were observed for evidence of prey regurgitation. Euthanized predators were rinsed with distilled water, weighed, measured (mm FL), and rinsed with a 95% solution of ethanol, and their digestive tracts (esophagus to vent) were removed. Digestive tracts were preserved in sample vials that were pre-filled with 1.0 mL of ATL lysis buffer (Qiagen) and

8.3 μL of proteinase K (Qiagen). All samples were homogenized by using the TissueLyser II (Qiagen), were digested overnight at 56°C, and were then placed in –80°C for storage.

The DNA was extracted from 200-μL digest aliquots by using the animal tissue protocol of the Qiagen DNeasy Blood and Tissue Kit. These samples, along with the negative controls (extraction, PCR, bucket water, and nontarget species DNA) and positive controls (target species DNA), served as the template for qPCR TaqMan assays.

The Mississippi Silverside and Delta Smelt TaqMan assays (designed with the mitochondrial cytochrome-*b* gene) used during qPCR were described in detail by Baerwald et al. (2011). Reaction components and thermal cycling conditions were detailed by Baerwald et al. (2012). The Mississippi Silverside assay was used as a control to ensure that species identification in the field was correct and that extractions and amplification were successful. In contrast to the methods of Baerwald et al. (2012), each sample was amplified five times (i.e., five technical replicates) to test for the presence of Delta Smelt DNA. If any one of the five replicates was amplified above background fluorescence prior to 40 cycles, then the sample was considered positive for Delta Smelt DNA. This approach was based on cloning results for seven Mississippi Silversides that sporadically tested positive for Delta Smelt DNA. For all seven samples, including when only one technical replicate amplified Delta Smelt DNA, cloning results showed that the amplified product was indeed Delta Smelt DNA rather than a nonspecific amplification product. Given these results, we opted to take a less-conservative approach in order to avoid an inflated rate of false positives.

Data analysis.—Due to the use of multiple sampling methodologies with differing capture efficiencies, we examined the proportion of Mississippi Silversides that tested positive for Delta Smelt DNA rather than examining the CPUE of Mississippi Silversides at each sampling location. The goal of these analyses—across all three of our study questions—was not to estimate the proportion of the Delta Smelt population that was consumed by Mississippi Silversides but rather to understand whether the proportion of Mississippi Silversides that were positive for Delta Smelt DNA was distributed randomly or was correlated with any habitat variable(s).

We used a chi-square goodness-of-fit test in Minitab version 16 to assess whether the proportion of Delta Smelt DNA-positive Mississippi Silversides differed among the sampling areas. To examine the influence of habitat variables on predation, the effects of environmental parameters (water temperature, conductivity, and turbidity) and Mississippi Silverside FL were analyzed by use of generalized linear modeling (Zuur et al. 2010) with a binomial distribution and a logit link function (i.e., logistic regression, with individual Mississippi Silversides being positive or negative for Delta Smelt DNA) in R software (R Development Core Team 2010). To simplify the interpretation of model results and to avoid overfitting, no interaction terms were included in the model. Mississippi Silverside FL was included to test for an ontogenetic effect of predator size. Collinearity among covariates was detected by calculating variance inflation factor (VIF) scores in R; collinearity was indicated if the VIF exceeded a threshold value of 3. Model selection was conducted using a forward/backward stepwise approach. The best model was selected based on Akaike's information criterion (AIC), with an AIC difference (ΔAIC) threshold of –2.0. The 95% confidence intervals (CIs) for model parameters were calculated by using the MASS package in R. For model analyses, measured water temperature was converted to relative water temperature to account for seasonal changes in temperature over the 3 months of the study. Relative water temperature was calculated by taking the median water temperature for the day of sampling and subtracting it from the water temperature measured at a given site. Thus, our analysis tested whether predation on Delta Smelt occurred in relatively cold or relatively warm parts of the study area rather than whether predation was correlated with absolute temperature.

To compare the proportion of individuals that were positive for Delta Smelt DNA across predator species, we present a qualitative analysis of the various predator species examined. Sample sizes were very low for the majority of predator species, thereby precluding any quantitative statistical analysis.

RESULTS

Field Sample Collection

In total, 278 sites were sampled during March–June 2011 (Table 2). Boat electrofishing was the most common sampling method (143 sites) and captured the most predators (n = 473; 60% of all predators caught). Sampling effort for electrofishing was not evenly distributed among the four main study areas: Cache Slough (a total of 372 m fished) and Lindsey Slough (260 m) were fished the least, whereas the SDWSC (1,916 m) and Liberty Island (12,966 m) received significantly more effort.

Mississippi Silversides comprised the bulk of the predators collected by electrofishing (68% of predators caught). Other targeted predators, such as the Largemouth Bass (6%), Sacramento Pikeminnow (5%), and Striped Bass (5%), were collected in much smaller numbers. The remaining four species of targeted predators collected by electrofishing comprised an insignificant proportion (3% total) of the samples that were tested for Delta Smelt DNA. Ad hoc predators collectively comprised the remaining 13% of predators analyzed from electrofishing.

Additional predators were collected via Kodiak trawling, otter trawling, and beach seining. Of these, beach seining comprised the most effort (46 sites) and captured the most predators (n = 257). As with electrofishing, Mississippi Silversides comprised the bulk (81%) of the predators caught. Sacramento Pikeminnow (5%) and Shimofuri Goby (7%) were also regularly captured. Kodiak trawling and otter trawling

had lower effort (42 and 12 sites, respectively) and correspondingly smaller catches of predators. Kodiak trawl sampling yielded mostly Mississippi Silversides (95% of predators); in contrast, otter trawl samples primarily consisted of Striped Bass (93% of predators).

Genetic Results

Across all sampling methods, 81 of 787 (10.3%) predators tested positive for Delta Smelt DNA in their digestive tracts (Table 1). Among the tested predators, Mississippi Silversides made up the majority of the positive detections (85%). Overall, 12.5% of all Mississippi Silversides tested positive for Delta Smelt DNA in their digestive tracts. The monthly proportion of Mississippi Silversides that tested positive for Delta Smelt DNA ranged from 6.3% (June) to 16.5% (April). Fish that tested positive were obtained in all sampling months. Among the bucket water samples, one sample tested positive for Delta Smelt DNA: the sample was from a bucket containing predators that also tested positive for Delta Smelt DNA. All predators from that bucket were removed from further analysis.

Mississippi Silversides that were positive for Delta Smelt DNA were found at all sampling areas and were collected by all sampling methods except otter trawling, which had by far the lowest sample size (n = 1 Mississippi Silverside). Separated by method of collection and sampling area, three groups of sites resulted in the collection of more than 100 Mississippi Silversides: (1) electrofishing in Cache Slough, (2) electrofishing in Lindsey Slough, and (3) beach seining at Liberty Island. Mississippi Silversides in Lindsey Slough and at Liberty Island had similar positive detection rates (10.9% and 10.4%, respectively), whereas Mississippi Silversides in Cache Slough had roughly half the positive detection rate (5.9%) of the other two collections.

The regional distribution of Mississippi Silversides that tested positive for Delta Smelt DNA was significantly different from the null expectation based on the distribution of total Mississippi Silverside catch, both by sampling area (χ^2 = 24.36, P < 0.0001) and by sampling method (χ^2 = 99.46, P < 0.0001). The SDWSC contributed most to this difference by having more than double the expected number of positive detections due to a high proportion of positive detections among Mississippi Silversides collected via Kodiak trawling.

Our analysis of habitat variables that were correlated with predation based on logistic regression identified the best model as one in which turbidity (coefficient = –0.013, P = 0.0462; Table 3) was the only covariate. We calculated the 95% CI for the final model's parameter coefficient, and that interval did not overlap with zero. The next-best model included turbidity and relative water temperature, but the 95% CI for temperature bounded zero, further indicating that the most appropriate model was the one that included only turbidity as a covariate. The negative coefficient for turbidity indicated that Mississippi Silversides testing positive for Delta Smelt predation were more likely to be collected in areas where turbidity was low (i.e., water was clearer) relative to other sampling sites.

Predator species other than Mississippi Silversides were only collected in low numbers; when examining differences in predation among species, we found that positive detections of Delta Smelt DNA in those other predators were rare (Table 1). Of the seven targeted predator species other than the Mississippi Silverside (i.e., Black Crappie, Bluegill, Largemouth Bass, Sacramento Pikeminnow, Spotted Bass, Striped Bass, and Yellowfin Goby), three species each had one positive detection, and one species had two positive detections (Table 1). Due to the low catch and subsequent low detection rates for the other targeted predators, further analysis was not feasible, although we include the results here for reference. Despite very low sample sizes, analysis of ad hoc predators produced a number of positive detections as well (Table 1). Some of these detections are noteworthy, such as the detection of Delta Smelt DNA in the gut of an *Exopalaemon* shrimp. We collected four shrimp for analysis, and one was positive for Delta Smelt DNA.

DISCUSSION

Our study's primary finding was that a wide variety of species consume early life stage Delta Smelt, including the highly invasive *Exopalaemon* shrimp (Brown and Hieb 2014). We determined that a higher proportion of Mississippi Silversides preyed on Delta Smelt in the offshore habitats sampled by Kodiak trawling (Table 2), which was consistent with results from a previous study (Baerwald et al. 2012). Additionally, we identified turbidity as having a significant negative effect on the occurrence of Delta Smelt predation by Mississippi Silversides.

An important caveat to our conclusions is that a positive detection of Delta Smelt DNA in a predator's digestive tract was assumed to be representative of predation on Delta Smelt. Aside from the possibility of contamination, there are other potential explanations for the presence of Delta Smelt DNA in the gut contents. For example, some of the detections could reflect carrion foraging, ingestion of eDNA, or secondary predation. However, the most parsimonious reason for positive detections—particularly with regard to Mississippi Silversides—is predation on the early life stages of Delta Smelt. For instance, secondary predation by Mississippi Silversides is unlikely, as larval Delta Smelt represent one of the largest prey items they are capable of ingesting; furthermore, Mississippi Silversides have not been documented to feed on carrion. The ingestion of eDNA and its detectability in the gut have not been quantified; eDNA ingestion remains a hypothetical source of positive detections but is likely rare enough to have a negligible effect on the conclusions drawn from our data. Furthermore, Mississippi Silverside predation probably focuses on the planktonic larvae of Delta Smelt rather than on the demersal eggs, as other Mississippi Silverside diet work in

TABLE 3. Model selection results for generalized linear models that were used to identify habitat variables affecting the detection of Delta Smelt DNA (i.e., predation) in Mississippi Silversides (AIC = Akaike's information criterion; ΔAIC = AIC difference; Turb = turbidity, NTU; Temp = relative water temperature, °C; FL = fork length, mm; Cond = conductivity, μS/cm). The null model is listed at the bottom; subsequent covariates were added in a stepwise manner. Parameter coefficients are listed under each covariate, and *P*-values are indicated by asterisks (*$P < 0.05$). The best model is shown in bold italics.

			Covariate			
Model	AIC	ΔAIC	Turb	Temp	FL	Cond
Turb + Temp + FL + Cond	385.31	1.86	–0.018*	–0.354	–0.020	<–0.001
Turb + Temp + FL	383.45	–0.57	–0.017*	–0.376*	–0.019	
Turb + Temp	384.02	–1.66	–0.017*	–0.318		
Turb	***385.68***	***–4.21***	***–0.013****			
Null	389.89					

San Francisco Estuary marshes has produced no evidence of fish egg predation (Howe et al. 2014). Other visual analyses of the diets consumed by Mississippi Silversides from open-water habitats in the upper estuary indicated that they mainly consume copepods and cladocerans, with relatively minor contributions from amphipods, insects, and other zooplankton (S. Slater, CDFW, personal communication). Moreover, our observation that a higher proportion of Mississippi Silversides positive for Delta Smelt DNA was found in offshore habitats indicates that larval predation is the most likely source of detections, as larval Delta Smelt are primarily found in those habitats (Grimaldo et al. 2004).

Regional and Habitat Effects

Our first question was whether Mississippi Silverside predation on Delta Smelt varied among the different sampling regions and methods. Consistent with the results reported by Baerwald et al. (2012), Mississippi Silversides that were collected via Kodiak trawling were much more likely to be positive for Delta Smelt DNA than Mississippi Silversides collected by other methods. This result may be attributable to a high degree of co-occurrence between Mississippi Silversides and larval Delta Smelt in the trawled habitats, even though Mississippi Silversides are typically associated with nearshore areas (Moyle 2002). Kodiak trawling was the only method that effectively sampled pelagic habitat, which is the primary habitat of larval Delta Smelt (Grimaldo et al. 2004). Additionally, a recent synthesis of Delta Smelt ecology determined that Mississippi Silverside catch was generally higher in springtime Kodiak trawls conducted in the SDWSC region, where springtime Delta Smelt densities are among the highest in the estuary (IEP 2015). Interestingly, given that Delta Smelt DNA is detectable in the digestive tract for up to 36 h after ingestion (Baerwald et al. 2012) and given that Mississippi Silversides are known to conduct inshore-to-offshore diel migrations in Clear Lake, California (Wurtsbaugh and Li 1985), we expected similar results between electrofishing (inshore) and Kodiak trawling (offshore) samples due to a presumed homogeneity of Mississippi Silversides in inshore and offshore habitats.

At Liberty Island, beach seining and electrofishing collected 22 predators (17 and 5, respectively) that were positive for Delta Smelt DNA. Liberty Island afforded a unique opportunity to compare predator and prey distributions, as monthly larval fish samples were collected by the USFWS during the same period as our sampling (L. Smith, USFWS, personal communication). Larval Delta Smelt were collected by USFWS only during May and June, yet our sampling detected predation by Mississippi Silversides in all 4 months (March–June; Figure 2). Some of this temporal disparity could reflect detections of egg predation, but Delta Smelt eggs typically hatch in 9–13 d (Moyle 2002), so it is unlikely that the difference can be fully explained by egg predation. Assuming that Mississippi Silverside predation affects substantially less than 100% of larval Delta Smelt present, these results indicate that (1) current monitoring may be missing substantial proportions of early life stage Delta Smelt, and (2) utilizing genetic assays on Mississippi Silversides has the potential to enhance our knowledge of predation on early life stage Delta Smelt as well as their general presence and distribution.

Effects of Environmental Variables

With respect to our question concerning the habitat parameters associated with Mississippi Silversides positive for Delta Smelt DNA, turbidity was the only covariate included in the best model. The coefficient for turbidity was negative, indicating that Mississippi Silverside predation on Delta Smelt was more likely to take place in clearer water. The effect of turbidity is logical: clearer water allows visual predators to see and catch prey more easily. The effects of turbidity on predation in aquatic environments have been well documented in laboratory experiments (Abrahams and Kattenfeld 1997; Carter et al. 2010), including evidence that turbidity negatively affects the foraging success of piscivorous fish to a significantly greater extent than planktivorous fish (De Robertis et al. 2003). Data from

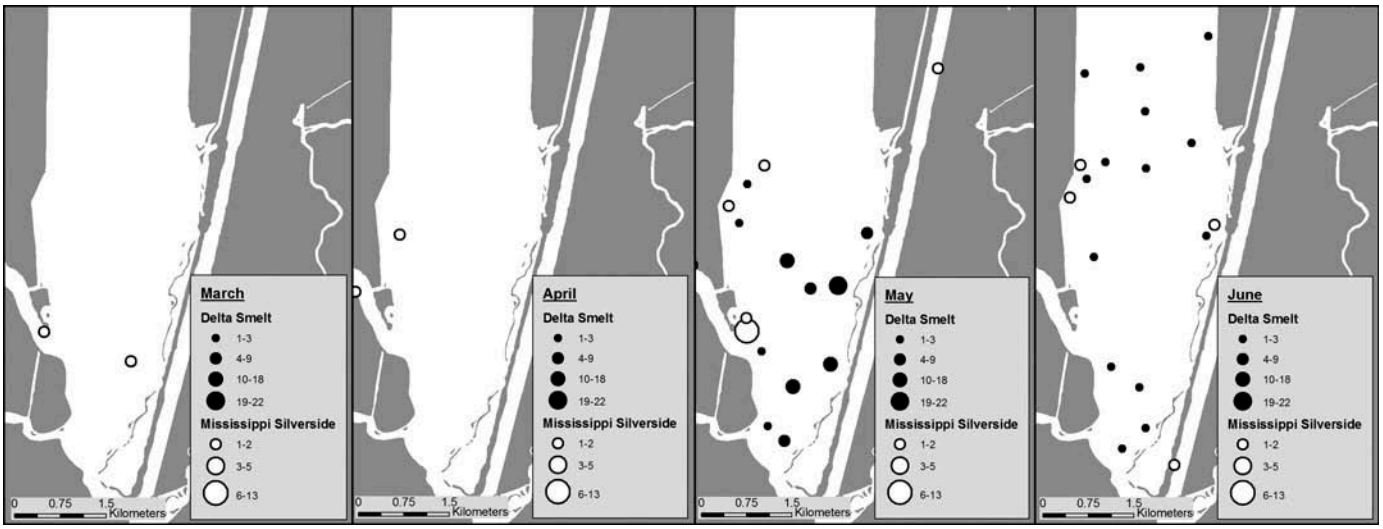

FIGURE 2. Results of fish sampling at Liberty Island in the upper San Francisco Estuary, March–June 2011. Black-shaded circles represent the number of larval Delta Smelt that were sampled by the U.S. Fish and Wildlife Service's fish monitoring survey. Open circles represent the number of Mississippi Silversides (from all sampling methods) that tested positive for the presence of Delta Smelt DNA in their digestive tracts.

the wild are lacking; higher turbidity has been correlated with increased recruitment in some freshwater ecosystems (Reichert et al. 2010), but the mechanism for this effect is not clear.

Larval Delta Smelt are associated with high-turbidity water (Sommer and Mejia 2013), and their feeding efficiency improves with increased turbidity (Baskerville-Bridges et al. 2004). In contrast, Mississippi Silversides are diurnal, visual feeders (Moyle 2002) whose feeding efficiency is likely affected negatively by water clarity. Our results suggest that management actions to create higher-turbidity habitat may reduce rates of predation on larval Delta Smelt as well as benefiting the initiation of first feeding (Baskerville-Bridges et al. 2004). Our results further reinforce that turbidity is an important consideration for resource managers with respect to tidal wetland restoration, especially given the long-term decline in suspended sediments (Wright and Schoellhamer 2004) and the increased water clarity (Feyrer et al. 2007) within the Delta.

Differences among Predator Species

Approximately 70% of the predators that were analyzed for Delta Smelt DNA were Mississippi Silversides. Although small sample sizes limit the conclusions that can be drawn regarding the variation in Delta Smelt predation pressure among predator species, the remaining 30% of predator samples provided some insights into the general scope of the predation experienced by early life stage Delta Smelt. We found DNA evidence of Delta Smelt predation in 11 of 18 predator species (other than Mississippi Silversides), suggesting that most species of the appropriate size will opportunistically feed on early life stage Delta Smelt. This observation is not surprising (Bax 1998), but it is notable that we detected Delta Smelt DNA in many species represented by only a small number of sampled individuals: five species in which 10 or fewer individuals were analyzed had at least one positive detection.

The preponderance of Mississippi Silversides in our predator sampling agrees with data from other sampling conducted in the northern Delta. Fish monitoring in the Yolo Bypass (Figure 1), which empties into the northern Delta, recorded Mississippi Silversides as the most abundant species (Feyrer et al. 2006). Additionally, data from USFWS beach seining efforts at Liberty Island in 2011 revealed that Mississippi Silversides comprised 80% of all fish sampled (L. Smith, USFWS, personal communication). Our results indicate that Mississippi Silversides are feeding on Delta Smelt across the entire northern Delta. By virtue of their high abundance in this region of the Delta, Mississippi Silversides are likely to be the predators that are most frequently encountered by larval Delta Smelt. Mississippi Silversides are also highly abundant across much of the San Francisco Estuary (Matern et al. 2002; Nobriga et al. 2005), indicating that early life stage Delta Smelt may be presented with predation pressure that is similar across much of their range.

Invasive *Exopalaemon* shrimp were not targeted by our sampling, and electrofishing is not an adequate sampling method for their collection. Our detection of Delta Smelt predation by an *Exopalaemon* shrimp in this study was insufficient to indicate a broader prevalence of such predation, but it does show that *Exopalaemon* are capable of preying upon Delta Smelt. This potential predation is noteworthy because *Exopalaemon* can be extremely abundant in Delta Smelt spawning areas; episodic catches in a rotary screw trap within the Yolo Bypass have exceeded 20,000 individuals in a 24-h period (J.

Frantzich, California Department of Water Resources, personal communication). However, *Exopalaemon* are not well sampled by existing monitoring programs, so much about their life history in the San Francisco Estuary remains unknown (Brown and Hieb 2014).

Predation by invasive species on early life stages of native fish has the potential to drastically impact the recovery of endangered native fish species, even with improvements in foraging and habitat conditions. Emphasis has been placed on the need for increased tidal marsh restoration to benefit native species in the Delta, potentially (and most likely indirectly) including the Delta Smelt (Brown 2003; Bennett 2005). However, Mississippi Silversides may also benefit from an increase in shallow-water habitat (Cohen and Bollens 2008; Gewant and Bollens 2011), and their encounter rates with larval Delta Smelt may increase near these restored habitats. Our results suggest that increased turbidities could benefit Delta Smelt larvae via a reduction in predation by Mississippi Silversides, and the San Francisco Estuary has seen long-term declines in suspended sediment—the primary source of turbidity (Wright and Schoellhamer 2004). Increases in shallow-water habitat may allow for higher turbidities through mechanisms like wind–wave resuspension, but the invasion of these habitats by nonnative species of submerged aquatic vegetation could reduce turbidity by limiting sediment resuspension processes (Yarrow et al. 2009; Hestir et al., in press). Furthermore, to fully elucidate the Mississippi Silverside's impact as an intraguild predator of Delta Smelt, more work must be done to understand the life histories of both species within the San Francisco Estuary, including their diets and their population responses to various environmental characteristics.

Ultimately, as nonnative fishes often have myriad impacts on native species, many impacts can be difficult to predict and quantify. The Delta affords a unique opportunity to study the effects of nonnative species on native species due to the large amount of monitoring data that are collected in the estuary and due to the introductions of numerous highly successful invasive species in recent history (Kimmerer 2004). Our study also highlights the utility of highly sensitive genetic techniques for identifying predation on early life stages of endangered species, which may be rare and patchy in the environment. Although the effects on early life stages of native fishes may be easily overlooked, particularly in complex estuarine environments, an understanding of these impacts may be crucial to the recovery of threatened and endangered species whose populations are at very low abundance.

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