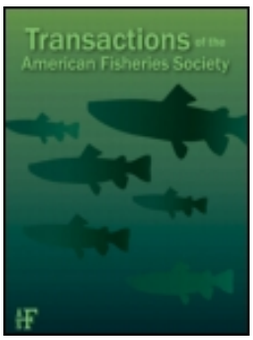

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ARTICLE

Detection of Threatened Delta Smelt in the Gut Contents of the Invasive Mississippi Silverside in the San Francisco Estuary Using TaqMan Assays

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Abstract

In the San Francisco Estuary, predation of the threatened delta smelt *Hypomesus transpacificus* by the invasive Mississippi silverside *Menidia audens* has been hypothesized but unconfirmed in the wild due to difficulties in reliably identifying egg or larval fish remains in gut contents. This study describes the use of TaqMan assays to examine the gut contents of wild Mississippi silversides for the presence of delta smelt DNA. The species-specific delta smelt assay was found to be highly sensitive and, in feeding trial experiments, capable of detecting delta smelt DNA in Mississippi silverside gut contents up to 36 h postingestion for some individuals. A substantial percentage (41%) of the 37 Mississippi silversides caught in the wild with midchannel trawling were positive for delta smelt DNA in their gut contents. Conversely, none of the 614 Mississippi silversides caught in the wild in nearshore beach seining contained delta smelt DNA in their gut contents.

Predator-prey interactions are a vital component of almost all natural ecosystems. Changes in predation patterns can occur for a variety of reasons, including nonnative species introductions (Ehrenfeld 2010), habitat alterations (Baeta et al. 2011), and environmental fluctuations (Hoegh-Guldberg and Bruno 2010). The use of DNA-based predation detection methods has become increasingly prevalent due to their ability to accurately detect very small quantities of potentially degraded prey DNA (Symondson 2002). These DNA-based techniques are particularly useful for situations involving cryptic predation, detection of prey lacking hard parts resistant to digestion, or studies conducting nonlethal sampling (Harper et al. 2005; Sheppard and Harwood 2005; Dunshea 2009).

The San Francisco Estuary (SFE), which includes the Sacramento-San Joaquin Delta (Delta), is an ecosystem in considerable flux due to many contributing environmental and anthropogenic factors (Nichols et al. 1986). It is perhaps the most invaded estuary in the world, with considerable ecosystem

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alteration due to exotic plants, clams, jellyfish, copepods, and fish species (Kimmerer et al. 1994; Mills and Sommer 1995; Cohen and Carlton 1998; Kimmerer 2002). Local examples of changes in predator-prey interactions have been observed as new species are introduced while other species become less abundant or extirpated (Kimmerer et al. 1994; Feyrer et al. 2003; Nobriga and Feyrer 2008). Shifting species abundance couples with environmental fluctuations (e.g., changes in salinity, flow, nutrients, temperature) to make predation patterns even more complex and often spatially and temporally divergent.

One of the threatened native fish species that has undergone a recent precipitous decline is the delta smelt *Hypomesus transpacificus* (Sommer et al. 2007). This pelagic fish is endemic to the upper SFE and is listed as a threatened species under the federal Endangered Species Act (USFWS 1993). Despite considerable research being done on the subject (Sommer et al. 2007; Grimaldo et al. 2009; Baxter et al. 2010; Glibert 2010; Mac Nally et al. 2010; Thomson et al. 2010), there is remaining uncertainty regarding the relative impacts of various stressors on delta smelt abundance, and it is likely that multiple seasonal stressors are responsible for the species' marked decline (Baxter et al. 2010). Predation has been implicated as one of the primary factors that can negatively affect recruitment success (i.e., survival of eggs or larvae to the next reproductive season) (Bailey and Houde 1989; Cushing 1990; Leggett and Deblois 1994). In particular, invertebrate and fish predation may be a major source of mortality for pelagic and demersal fish eggs and larvae (Swain and Sinclair 2000; Köter et al. 2003).

The Mississippi silverside *Menidia audens* is a nonnative fish whose invasion and increasing abundance coincided with the decline of the delta smelt in the SFE (Bennett and Moyle 1996). Mississippi silversides have been shown to readily consume larval delta smelt in the laboratory (J. A. Hobbs and W. A. Bennett, University of California, Davis, unpublished data) and are abundant in shallow-water areas where delta smelt often spawn (Bennett and Moyle 1996). Adult Mississippi silversides are of a comparable size to adult delta smelt and therefore cannot consume them, but it has been hypothesized that Mississippi silversides may be preying on delta smelt larvae (5–12 mm or up to 30 d posthatch) in the wild, and it may also be possible that Mississippi silversides could prey on the demersal eggs of delta smelt (Bennett 2005). However, egg predation seems far less likely than larval predation given that Mississippi silversides are active, visual foragers feeding primarily higher in the water column and at the surface. Additionally, Mississippi silversides may have a dramatic impact on delta smelt abundance due to intraguild predation (Polis et al. 1989), consuming larval delta smelt in addition to competing for resources with adults (Bennett 2005).

The primary objective of our current study was to determine if invasive Mississippi silversides prey on larval delta smelt in the SFE. To achieve this objective, we used a previously developed delta smelt TaqMan assay (Baerwald et al. 2011) along with a newly developed Mississippi silverside TaqMan assay, both verified for species specificity. Quantitative PCR (qPCR) was performed on serial dilutions to identify the minimal amount of delta smelt DNA that can be detected, while a feeding trial experiment was conducted to determine how long after consumption delta smelt DNA can be detected in Mississippi silverside gut contents. Finally, wild Mississippi silversides were collected from several locations in the Delta and their guts were analyzed for the presence of delta smelt DNA.

METHODS

TaqMan Assay Development

The delta smelt TaqMan assay, comprised of primers (CytB-Htr-F and CytB-Htr-R) and a 6-FAM labeled probe (CytB-Htr-P), was previously designed, and details regarding assay development can be found in Baerwald et al. (2011). For the Mississippi silverside TaqMan assay development, a 485-base-pair region of the mitochondrial *cytochrome b* (*cyt-b*) gene was sequenced for three individuals collected from the Sacramento-San Joaquin Delta using conserved animal primers, H15149 and L14724 (Kocher et al. 1989; Irwin et al. 1991). The PCR was performed in a 20-μl total volume containing 2 μl of extracted genomic DNA, 1X GoTaq reaction buffer (Promega), 0.2 mM of each dNTP, 0.6 μM of each primer, and 0.75 units of GoTaq DNA polymerase (Promega). Thermal cycler conditions were as follows: 94°C for 15 min followed by 35 cycles of 94°C for 30 s, 52°C for 1.5 min, 72°C for 1 min, and ending with a final extension of 72°C for 10 min. The PCR products were purified using the Agencourt AmPure system (Beckman Coulter) according to manufacturer instructions and sequenced by the College of Agricultural and Environmental Sciences, Genomics Facility at University of California at Davis. Sequences were aligned using Sequencher ver. 4.8 (Gene Codes) and compared to the *cyt-b* sequences for 19 other fish species ($N = 3$–7 individuals per species) found in the San Francisco Bay-Delta (Table 1). Primer Express Oligo Design software (Applied Biosystems) was used for primer and probe design to specifically detect Mississippi silverside DNA. Table 2 shows the primer and probe sequences designed for both the Mississippi silverside and the previously reported delta smelt assay (Baerwald et al. 2011).

Mississippi Silverside Assay Species-Specificity

The ability of the designed TaqMan assay to reliably detect its target species was tested on 40 Mississippi silverside individuals. Specificity of the assay was tested on 19 potentially co-occurring fish species listed in Table 1 ($N = 3$–16 individuals per species). The DNA was extracted from fin clips using Qiagen's DNeasy Blood and Tissue kit. The PCR was performed in a 5-μl total volume containing 1 μl of DNA template, 1X TaqMan Universal Master Mix (Applied Biosystems), 1.8 μM final concentration of both forward and reverse primers (CytB-Mau-F and CytB-Mau-R), and 0.18 μM of final probe concentration (Cy5 labeled CytB-Mau-P). Thermal cycling occurred with

TABLE 1. Fish species tested to confirm Mississippi silverside TaqMan assay species-specificity.

Name	Sample size[a]	Collection location[b]	*Cyt-b* Genbank accession
American shad *Alosa sapidissima*	3 (5)	CQS, SB	JN008736
Bigscale logperch *Percina macrolepida*	3 (5)	YB	JN008743
Black crappie *Pomoxis nigromaculatus*	3 (5)	YB	JN008741
Bluegill *Lepomis macrochirus*	3 (3)	YB	JN008739
Common carp *Cyprinus carpio*	3 (6)	YB	JN008746
Channel catfish *Ictalurus punctatus*	3 (5)	YB	JN008747
Delta smelt *Hypomesus transpacificus*	6 (16)	CS, HB, SJR, SM, SR	HQ667171
Largemouth bass *Micropterus salmoides*	3 (3)	YB	JN008737
Longfin smelt *Spirinchus thaleichthys*	3 (11)	CI, LW	JN008751
Mississippi silverside *Menidia audens*	3 (40)	DWSC	JN008748
Pacific herring *Clupea pallasii*	3 (5)	SFB	JN008735
Prickly sculpin *Cottus asper*	3 (3)	SM	JN008740
Splittail *Pogonichthys macrolepidotus*	3 (5)	YB	JN008738
Sacramento sucker *Catostomus occidentalis*	3 (5)	YB	JN008744
Shimofuri goby *Tridentiger bifasciatus*	3 (5)	SM	JN008749
Striped bass *Morone saxatilis*	7 (5)	SB, YB	JN008752
Threadfin shad *Dorosoma petenense*	3 (3)	SB, SM, YB	JN008742
Wakasagi *Hypomesus nipponensis*	4 (10)	SR, YB	HQ667170
White catfish *Ameiurus catus*	3 (5)	YB	JN008745
White crappie *Pomoxis annularis*	3 (9)	YB	JN008750

[a]Sample size denotes the number of individuals sequenced, while the number of individuals tested with the Mississippi silverside TaqMan assay is in parentheses.
[b]Abbreviations are as follows: CI = Chipps Island, CS = Cache Slough, CQS = Carquinez Strait, DWSC = Sacramento Deep Water Ship Channel, HB = Honker Bay, LW = Lake Washington, SB = Suisun Bay, SFB = San Francisco Bay, SJR = San Joaquin River, SM = Suisun Marsh, SR = Sacramento River, and YB = Yolo Bypass.

Bio-Rad's Chromo4 real-time detector under the following conditions: initial enzyme activation of 10 min at 95°C, 40 cycles of 15 s denaturation at 95°C, 1 min annealing and extension at 63°C. Cycle threshold (Ct) values were quantified using Opticon Monitor software (3.1; Bio-Rad). Eight no-template controls were included per plate, and the amplification threshold line was set above background fluorescence for each reporter dye.

Delta Smelt Assay Sensitivity

Serial dilutions.—Fivefold serial dilutions of delta smelt DNA, with amounts ranging from 0.000001 ng to 10 ng, were amplified using the delta smelt TaqMan assay. A constant amount of predator Mississippi silverside DNA (100 ng) was also included in all reactions, and each dilution was replicated 10 times. Additionally, a second fivefold serial dilution series was conducted to determine a maximum reliable Ct cut-off for defining positive reactions. The same delta smelt DNA amounts as mentioned above (0.000001 ng to 10 ng) were amplified across four plates with each plate containing eight replicates of each dilution ($N = 32$ replicates/dilution). The lowest delta smelt DNA amount that had ≥95% of samples amplifying (i.e., pinpointing the concentration that produces reliable amplification across replicates) was determined and the corresponding Ct value was set as the Ct cut-off (Burns and Valdivia 2008; Caraguel et al. 2011). For the amplifications described directly

TABLE 2. Probe and *Cyt-b* primer sequences used in Mississippi silverside and delta smelt TaqMan assays (delta smelt assay originally described in Baerwald et al. [2011]).

Species	Primer or probe	Sequence (5′–3′)	Reporter	Quencher
Mississippi silverside	CytB-Mau-F	CCGTTTGCATGCATATTTCG		
	CytB-Mau-R	CCTTTTCGTCTGTTGCACACA		
	CytB-Mau-P	AAGCCACCCGTAGTTTACATCCCGACA	Cy5	BHQ-2
Delta smelt	CytB-Htr-F	AATGGCCAACCTTCGGAAA		
	CytB-Htr-R	GARATATTRGAGGGTGCAGG		
	CytB-Htr-P	CCCATCCCCTCCTGAAAATTACCAACG	6FAM	MGB

above, reaction components and conditions were identical to those for the Mississippi silverside cross-reactivity amplifications except the Mississippi silverside primers and probes were replaced with 1.8 μM each of CytB-Htr-F and CytB-Htr-R primers and 0.06 μM of CytB-Htr-P delta smelt probe.

Laboratory feeding trial.—For the feeding trial, all delta smelt sacrificed were from the Fish Conservation and Culture Lab at University of California at Davis, which maintains a refugial population of delta smelt for research and potential future reintroduction purposes (Fisch et al. 2009). Tissue from adult delta smelt was used since only this life stage was available at the time of the feeding experiment. Wild Mississippi silversides were captured via beach seine from the Sacramento-San Joaquin Delta and housed in a single 350-L aquaria at 16 ± 0.5°C with filtration and aeration. Mississippi silversides were acclimated to captivity for 1 week, during which time they were shifted from live food to frozen brine shrimp. For the feeding trial, silversides were fasted for 48 h and then fed 2-mm 2-mm tissue pieces from adult delta smelt en masse, with uneaten tissue immediately siphoned from the bottom of the aquaria. Immediately after feeding, nine Mississippi silversides were sacrificed, and their guts were visually examined for evidence of delta smelt tissue consumption. Given our inability to verify that every Mississippi silverside ingested delta smelt tissue, this initial examination was used as a proxy to determine feeding success of the whole group. At each subsequent time step (1, 3, 6, 9, 24, 36, 48, 60, and 72 h), 12 Mississippi silversides were euthanized and their guts were immediately dissected and preserved in 80% ethanol for genetic analysis. Body weight, gut weight, and length measurements were recorded for all fish.

Entire gut samples were completely homogenized in 1 mL of ATL lysis buffer (Qiagen) and 8.3 μL of proteinase K (Qiagen) using the TissueLyser II (Qiagen) and digested overnight at 56°C. The DNA was extracted from 200-μL digest aliquots using the animal tissue protocol of Qiagen's DNeasy Blood and Tissue Kit. These samples, along with negative (extraction, PCR, nontarget species DNA) and positive (target species DNA) controls, served as the template for qPCR TaqMan assays. The Mississippi silverside assay was performed one time per sample for quality assurance (i.e., to ensure that the sampled predator was the correct species and that DNA extractions were successful). The PCR and thermal cycling conditions were identical to those described for Mississippi silverside assay specificity. For delta smelt detection, all samples were assayed in duplicate using the PCR and thermal cycling conditions previously described for the delta smelt TaqMan assay (see serial dilution methods). Any sample testing positive in at least one of the replicates was assayed an additional two times.

Study Site and Sample Collections

Mississippi silversides were collected from two midchannel sites in the Sacramento Deep Water Ship Channel by the California Department of Fish and Game's Spring Kodiak trawl survey on April 7, 2010 and at 14 sites along the Deep Water Ship Channel from April 14–29, 2010, via beach seining (Figure 1). The trawl collection was done to allow for a contrast between the gut contents of Mississippi silversides captured in open-channel versus nearshore habitats and was conducted in the morning (0930 and 1030 hours). The collection locations and sampling dates were chosen to coincide with the peak of the delta smelt spawning season (Wang 1986) and thus the highest densities of larval delta smelt. Beach seine sampling was conducted during daylight hours (0700-1700 hours) and across all tidal stages.

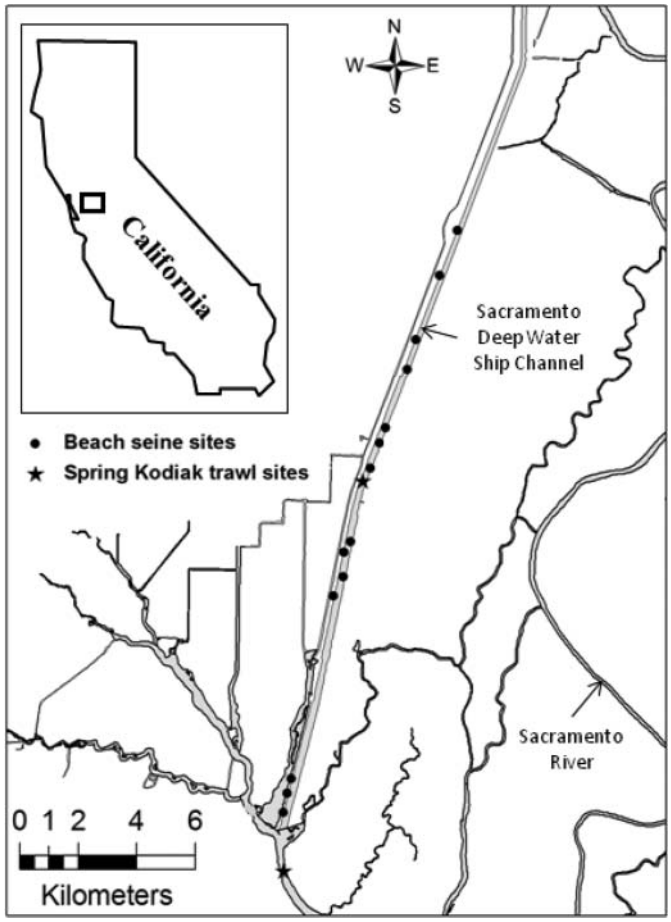

FIGURE 1. Sampling locations for collection of wild delta smelt predators.

Mississippi silversides were rinsed with deionized water, preserved whole in 95% ethanol, and stored on dry ice for transportation back to the laboratory. Water samples were collected from the purse of the seine to test for the presence of ambient delta smelt DNA. Only Mississippi silversides greater than 50 mm FL were saved for analysis, and a maximum of 40 Mississippi silversides were preserved from each seine to increase the geographic distribution of samples. For ease of field collection, Mississippi silversides were preserved communally, by seine, and not individually. The day after collection, all Mississippi silversides were individually rinsed in ethanol and dissected, using DNA sterile techniques, and their entire guts

were removed and stored in 80% ethanol at –20°C. Samples of ethanol (2 mL) from the preservation containers were taken to examine the presence of delta smelt DNA cross-contamination between Mississippi silversides. While the primary focus of the field sampling, and the study as a whole, was on Mississippi silversides as predators of larval delta smelt, we identified additional putative larval delta smelt predators a priori that were opportunistically sampled and analyzed. These additional predators included largemouth bass *Micropterus salmoides*, striped bass *Morone saxatilis*, threadfin shad *Dorosoma petenense*, fall-run Chinook salmon *Oncorhynchus tshawytscha*, yellowfin goby *Acanthogobius flavimanus*, and bluegill *Lepomis macrochirus*. Wet weight of both whole fish and dissected guts, along with fork length measurements, were recorded for all fish.

The DNA extraction, Mississippi silverside TaqMan assay, and initial duplicate delta smelt TaqMan assays were conducted as described for the captive feeding trial. If at least one of the duplicate reactions tested positive for delta smelt, the assay was replicated an additional eight times. Samples were deemed true positives for delta smelt DNA if 7 of the 10 replicates tested positive.

RESULTS

Mississippi Silverside TaqMan Assay Development and Species-Specificity

The Mississippi silverside assay amplified all 40 Mississippi silverside individuals and did not cross-amplify any of the 19 other co-occurring fish species when using a positive identification cut-off of Ct $\leq$ 35, although some species did weakly amplify after this cut-off. The average Ct value with the Cy5 dye for the Mississippi silverside samples was 17.

Delta Smelt TaqMan Sensitivity: Dilution Series and Captive Feeding Trial

For the fivefold DNA dilution series, using the starting 10 ng as a reference point, we successfully amplified a 10,000-fold dilution of delta smelt DNA (0.0001 ng) in 100 ng of Mississippi silverside DNA with 100% success (i.e., all 10 replicates) with a median Ct of 32 when the threshold was set to 0.005 based on no-template controls. Further delta smelt dilutions, down to 0.000001 ng, also sporadically amplified in a few of the replicates, but amplification was not reliable for these DNA amounts (0.00003–0.000001 ng). The PCR efficiency was estimated to be 99% based on the slope of the standard curve. The second fivefold dilution series also showed that 0.0001 ng consistently amplified all 32 of the replicates while only 44% of the replications amplified in the next dilution (0.00002 ng). To reduce issues with unreliable amplification across replicates, we set a Ct cut-off of $\leq$ 35.03, based on the limit of detection, to define a reaction as a "positive" for delta smelt detection.

For the captive feeding trial, all nine initial Mississippi silverside guts visually inspected to verify consumption of smelt tissue were found to contain tissue fragments, thus indicating that the rate of initial consumption of delta smelt tissue was high. No regurgitation was observed in either the holding tank or the euthanasia container. There were no significant differences in the median length or weight of Mississippi silversides across the duration of the trial or between fish that tested positive or negative for delta smelt DNA (Kruskal–Wallis, $P > 0.05$). Figure 2 displays the percentage of Mississippi silversides positive for delta smelt DNA at each time point postingestion. Delta smelt DNA was consistently detected (i.e., 100% of Mississippi silverside samples tested positive for delta smelt DNA) in Mississippi silverside guts up to 6 h postingestion and was commonly detected (92% amplification) 9 h postingestion. Detection rates were considerably reduced at 24 and 36 h, with 25% and 17% delta smelt amplification at these respective time points. Delta smelt DNA was not detected in Mississippi silverside guts after 36 h postingestion. Using logistic regression, the median detection time when 50% of the Mississippi silversides are estimated to test positive for delta smelt is 17.5 h postingestion.

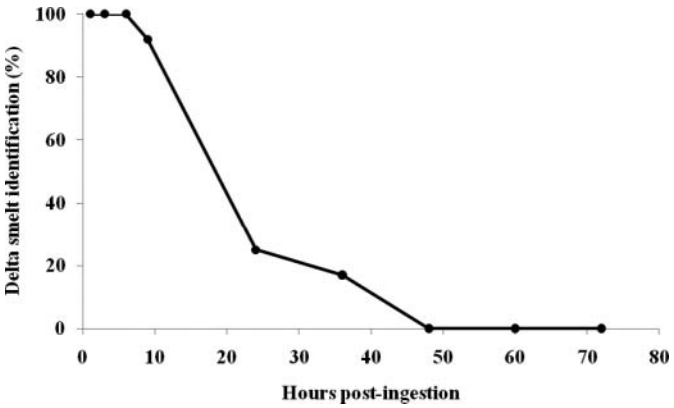

FIGURE 2. Results from the time course experiment during the captive feeding trial showing the percentage of Mississippi silverside guts at each time point postingestion that had detectable delta smelt DNA using TaqMan assays.

Wild Mississippi Silverside Field Sampling and Gut Content Analysis

Gut content results for predators captured in the wild is shown in Table 3. Through beach seine sampling, we captured 614 Mississippi silversides. Of these, zero Mississippi silversides were positive for the presence of delta smelt DNA. All of the samples tested positive for the presence of Mississippi silverside DNA however, indicating that all DNA extractions were successful. From the Spring Kodiak trawl sampling, 37 Mississippi silversides were captured in the Deep Water Ship Channel over two tows on 1 d. Of these, 15 were positive for the presence of delta smelt DNA and all samples were positive for Mississippi silverside DNA. While median lengths of Mississippi silversides caught in the Spring Kodiak trawl were significantly shorter than those caught via beach seining (Mann–Whitney, $P < 0.001$), the Spring Kodiak trawl length distribution is completely encapsulated by the beach seine length distribution. Weight and length

TABLE 3. Detection of delta smelt in the gut contents of wild predators captured in the Sacramento Deep Water Ship Channel using TaqMan assays.

Species	Sampling method	Dates collected	Predator size range (mm)	Fish collected	Guts positive for delta smelt
Mississippi silverside	Spring Kodiak trawl	April 7, 2010	51–90	37	15
Mississippi silverside	Beach seine	April 14–29, 2010	50–95	614	0
Bluegill	Beach seine	April 28, 2010	87	1	0
Fall-run Chinook salmon	Beach seine	April 19, 2010	73	2	0
Largemouth bass	Beach seine	April 14, 2010	77	1	0
Striped bass	Beach seine	April 14–28, 2010	79–135	13	0
Threadfin shad	Beach seine	April 29, 2010	60	1	0
Yellowfin goby	Beach seine	April 19–29, 2010	93–125	11	0

were not significantly different (Mann–Whitney, $P > 0.05$) between Mississippi silversides positive versus negative for delta smelt DNA.

During the course of our beach seine sampling, we captured six additional (i.e., non–Mississippi silverside) potential larval delta smelt predator species for gut contents analysis. None of these fish tested positive for the presence of delta smelt DNA based on our Ct ($\leq$35.03) and replication (7 out of 10 positive) cut-offs (Table 3). One of the two fall-run Chinook salmon individuals collected did amplify weakly for delta smelt DNA (replicate Ct values = 34–38) but only 4 out of 10 replicates were considered positive, and therefore the sample was not considered a true positive.

For all samples, TaqMan assays of the ethanol aliquots from euthanization jars resulted in no detectable amount of delta smelt DNA, indicating an absence of cross-contamination between fishes. Water samples collected during beach seining were also negative for delta smelt DNA. Additionally, extraction and water negative controls did not amplify, further verifying a lack of sample cross-contamination.

DISCUSSION

We have demonstrated the utility of TaqMan assays for the molecular identification of a SFE invasive fish species, the Mississippi silverside, and its threatened native prey, delta smelt, in both laboratory and wild settings. Both Mississippi silverside and delta smelt assays are species-specific in the SFE with no positive reactions for the tested co-occurring fish species, including the wakasagi and longfin smelt species. However, since Mississippi silverside were only collected from the SFE and not the entire species range, confirmation of assay specificity would be needed before using the Mississippi silverside assay for populations outside the SFE.

The captive feeding trial compares well with other molecular detection assays for fish predation, such as postingestion detection of 24 h for plaice *Pleuronectes platessa* fed to brown shrimp *Crangon crangon* and shore crab *Carcinus maenas* (Albaina et al. 2010) and 12 h for Atlantic cod *Gadus morhua* fed to Atlantic mackerel *Scomber scombrus* (Rosel and Kocher 2002). These assays are considerably more sensitive than visual identification studies, with almost all larval or juvenile fish prey unidentifiable 1–2 h postingestion (Schooley et al. 2008; Legler et al. 2010). In our study, individual differences in detection at later time points, after 6 h, may be due to variation in digestion rates or differences in total amount of prey tissue initially consumed. Alternatively, some Mississippi silversides collected at the later time points may not have consumed delta smelt. Lack of consumption, however, seems unlikely given that 100% of the predators sampled at time zero and 1–6 h postingestion ($N =$ 40 individuals total) had consumed delta smelt.

The temperature used for the feeding trial (16°C) is the average water temperature of the Deep Water Ship Channel during the peak delta smelt spawning period. However, fluctuations in water temperature, along with other variables not examined in this preliminary feeding trial (e.g., fish life stage), may alter digestion rates (Albaina et al. 2010; Legler et al. 2010; Carreon-Martinez et al. 2011). Future work could expand the feeding trial to examine the effects of these factors on digestion rates, along with other environmental and technical (e.g., preservation method, nonlethal sampling) parameters, which will be valuable for interpreting results of future spatially and temporally variable field sampling.

While an assay for the predator DNA is not absolutely essential, it is recommended (King et al. 2008) and allowed us to have greater confidence in stating that guts negative for delta smelt were due to biological reasons and not technical error (e.g., issues with DNA extractions or TaqMan assays or incorrect species identification). In fact, we removed a few samples from analysis that did not amplify predator DNA and even discovered that one sample was not a Mississippi silverside but another fish species. While researchers do their best to limit errors, a few technical errors are practically unavoidable in studies with large sample sizes and the more safe guards employed, such as the use of a predator control, the more precise and accurate the conclusions. Even with a predator control, PCR inhibitors may have a more substantial effect on particular genetic assays and false negatives due to technical difficulties are quite possible. Future use of PCR enhancers, such as bovine serum albumin, could potentially help improve detection rates.

In our study, we detected delta smelt in the gut contents of only 2.3% of 651 Mississippi silversides analyzed from the wild. All 15 Mississippi silverside guts with detectable delta smelt were caught by the Spring Kodiak trawl sampling, with 41% of the Mississippi silversides collected with this method having delta smelt DNA in their guts versus 0% with beach seining. These two sampling methods target distinct habitats, with beach seining sampling nearshore fish assemblages while the Spring Kodiak trawl collects fish in the upper channel. Several factors could influence predation of delta smelt by Mississippi silversides collected with these two methods including (1) uneven distribution of prey, (2) spatial differences in Mississippi silverside feeding behavior, (3) temporal differences in sampling (i.e., Mississippi silverside were more likely to be preying on delta smelt during the trawl sampling and not in the subsequent weeks when beach seine sampling occurred), (4) random fluctuations in predation, and (5) unexamined environmental or habitat variables. Of these, the uneven distribution of delta smelt larvae may be the most plausible. Other studies in the Deep Water Ship Channel have found that delta smelt larvae are most abundant in the upper water column of the midchannel, though they still occur in nearshore, shallow-water habitats as well (L. Grimaldo, U.S. Bureau of Reclamation, personal communication). Thus, prey distribution is insufficient to fully explain the lack of detectable delta smelt predation by Mississippi silversides in shallow water habitats. In another California water body, the daily inshore and offshore movement of inland silversides *Menidia beryllina* in relation to prey distribution has been documented (Wurtsbaugh and Li 1985). With this diel migration in mind, we believed that primarily targeting nearshore Mississippi silversides via beach seining throughout the day would capture channel-wide predation, but this assumption may not be true and should be examined in a future study. Given that all guts positive for delta smelt were collected during a few trawl tows on a single day, it seems likely that a certain amount of pure chance enabled us to detect delta smelt predation by Mississippi silversides.

Examining the effects of other nonrandom factors on delta smelt predation was outside the scope of the limited predator sampling conducted for this study. Future work should focus on elucidating the spatial, temporal, and habitat changes that may influence predation pressures. Understanding how these factors influence predation on delta smelt will be highly informative for implementing effective habitat restoration design. More intensive sampling efforts, coupled with predator and prey abundance estimates, as well as bioenergetics modeling may enable better estimations of the effect predation is having on overall delta smelt productivity.

Delta smelt predation was not positively identified in the guts of the six opportunistically collected non–Mississippi silverside fish species. Although below the limit of reliable amplification, the positive identification of delta smelt DNA in 4 out of 10 replicates for one of the two Chinook salmon should be noted and follow-up sampling would be useful. The small sample sizes (1–13 individuals collected/species) for all six species makes it impossible to draw conclusions regarding delta smelt predation by these predators. Future research should expand on sampling of these and other potential predators to gain a better understanding of the role predation by a complete suite of species and their alternative life stages may have on the abundance of delta smelt at all life stages.

The introduction of nonnative species and other ecosystem alterations undoubtedly have long-lasting consequences for trophic interactions. The results presented herein represents the first predation study conducted in the SFE to employ DNA-based methodology for prey detection and we hope that the encouraging results will promote further use of genetic detection to gain insight into the food web dynamics in this and other highly invaded and constantly evolving ecosystems.

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REFERENCES

Albaina, A., C. J. Fox, N. Taylor, E. Hunter, M. Maillard, and M. I. Taylor. 2010. A TaqMan real-time PCR based assay targeting plaice (*Pleuronectes platessa* L.) DNA to detect predation by the brown shrimp (*Crangon crangon* L.) and the shore crab (*Carcinus maenas* L.): assay development and validation. Journal of Experimental Marine Biology and Ecology 391:178–189.

Baerwald, M. R., G. Schumer, B. M. Schreier, and B. May. 2011. TaqMan assays for the genetic identification of delta smelt (*Hypomesus transpacificus*) and wakasagi smelt (*Hypomesus nipponensis*). Molecular Ecology Resources 11:784–785.

Baeta, A., N. Niquil, J. C. Marques, and J. Patrício. 2011. Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine benthic food webs. Ecological Modelling 222:1209–1221.

Bailey, K. M., and E. D. Houde. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Advances in Marine Biology 25:1–83.

Baxter, R., R. Breuer, L. Brown, L. Conrad, F. Feyrer, S. Fong, K. Gehrts, L. Grimaldo, B. Herbold, P. Hrodey, A. Mueller-Solger, T. Sommer, and K. Souza. 2010. 2010 pelagic organism decline work plan and synthesis of results. Interagency Ecological Program for the San Francisco Estuary, California Department of Water Resources, Sacramento. Available: www.water.ca.gov/iep/docs/FinalPOD2010Workplan12610.pdf. (May 2012).

Bennett, W. A. 2005. Critical assessment of the delta smelt population in the San Francisco Estuary, California. San Francisco Estuary and Watershed Sciencee [online serial] 3(2).

Bennett, W. A., and P. B. Moyle. 1996. Where have all of the fishes gone? interactive factors producing fish declines in the Sacramento–San Joaquin estuary. Pages 519–542 *in* J. T. Hollibaugh, editor. San Francisco Bay: the ecosystem. American Association for the Advancement of Science, San Francisco.

Burns, M., and H. Valdivia. 2008. Modelling the limit of detection in real-time quantitative PCR. European Food Research and Technology 226:1513–1524.

Caraguel, C. G. B., H. Stryhn, N. Gagn, I. R. Dohoo, and K. L. Hammell. 2011. Selection of a cutoff value for real-time polymerase chain reaction results to fit a diagnostic purpose: analytical and epidemiologic approaches. Journal of Veterinary Diagnostic Investigation 23:2–15.

Carreon-Martinez, L., T. B. Johnson, S. A. Ludsin, and D. D. Heath. 2011. Utilization of stomach content DNA to determine diet diversity in piscivorous fishes. Journal of Fish Biology 78:1170–1182.

Cohen, A. N., and J. T. Carlton. 1998. Accelerating invasion rate in a highly invaded estuary. Science 279:555–558.

Cushing, D. H. 1990. Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Advances in Marine Biology 26:249–293.

Dunshea, G. 2009. DNA-based diet analysis for any predator. PLoS (Public Library of Science) Onee [online serial] 4(4):e5252.

Ehrenfeld, J. G. 2010. Ecosystem consequences of biological invasions. Annual Review of Ecology, Evolution, and Systematics 41:59–80.

Feyrer, F., B. Herbold, S. A. Matern, and P. B. Moyle. 2003. Dietary shifts in a stressed fish assemblage: consequences of a bivalve invasion in the San Francisco Estuary. Environmental Biology of Fishes 67:277–288.

Fisch, K., T. Rettinghouse, L. Ellison, G. Tigan, J. Lindberg, and B. May. 2009. Delta smelt refugial population development and genetic management: 2009 season summary. Interagency Ecological Program Newsletter 22:3–9.

Glibert, P. M. 2010. Long-term changes in nutrient loading and stoichiometry and their relationships with changes in the food web and dominant pelagic fish species in the San Francisco Estuary, California. Reviews in Fisheries Science 18:211–232.

Grimaldo, L. F., T. Sommer, N. Van Ark, G. Jones, E. Holland, P. B. Moyle, B. Herbold, and P. Smith. 2009. Factors affecting fish entrainment into massive water diversions in a tidal freshwater estuary: can fish losses be managed? North American Journal of Fisheries Management 29:1253–1270.

Harper, G. L., R. A. King, C. S. Dodd, J. D. Harwood, D. M. Glen, M. W. Bruford, and W. O. C. Symondson. 2005. Rapid screening of invertebrate predators for multiple prey DNA targets. Molecular Ecology 14:819–827.

Hoegh-Guldberg, O., and J. F. Bruno. 2010. The impact of climate change on the world's marine ecosystems. Science 328:1523–1528.

Irwin, D. M., T. D. Kocher, and A. C. Wilson. 1991. Evolution of the cytochrome *b* gene of mammals. Journal of Molecular Evolution 32:128–144.

Kimmerer, W. J. 2002. Effects of freshwater flow on abundance of estuarine organisms: physical effects or trophic linkages? Marine Ecology Progress Series 243:39–55.

Kimmerer, W. J., E. Gartside, and J. J. Orsi. 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton of San Francisco Bay. Marine Ecology Progress Series 113:81–93.

King, R. A., D. S. Read, M. Traugott, and W. O. C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Molecular Ecology 17:947–963.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the USA 86:6196–6200.

Köster, F. W., D. Schnack, and C. Mölmann. 2003. Scientific knowledge of biological processes that are potentially useful in fish stock predictions. Scientia Marina 67(Supplement 1):101–127.

Leggett, W. C., and E. Deblois. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Netherlands Journal of Sea Research 32:119–134.

Legler, N. D., T. B. Johnson, D. D. Heath, and S. A. Ludsin. 2010. Water temperature and prey size effects on the rate of digestion of larval and early juvenile fish. Transactions of the American Fisheries Society 139:868–875.

Mac Nally, R., J. R. Thomson, W. J. Kimmerer, F. Feyrer, K. B. Newman, A. Sih, W. A. Bennett, L. Brown, E. Fleishman, S. D. Culberson, and G. Castillo. 2010. Analysis of pelagic species decline in the upper San Francisco Estuary using multivariate autoregressive modeling (MAR). Ecological Applications 20:1417–1430.

Mills, C. E., and F. Sommer. 1995. Invertebrate introductions in marine habitats: two species of hydromedusae (Cnidaria) native to the Black Sea, *Maeotias inexspectata* and *Blackfordia virginica,* invade San Francisco Bay. Marine Biology 122:279–288.

Nichols, F. H., J. E. Cloern, S. N. Luoma, and D. H. Peterson. 1986. The modification of an estuary. Science 231:567–573.

Nobriga, M. L., and F. Feyrer. 2008. Diet composition in San Francisco Estuary striped bass: does trophic adaptability have its limits? Environmental Biology of Fishes 83:495–503.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297–330.

Rosel, P. E., and T. D. Kocher. 2002. DNA-based identification of larval cod in stomach contents of predatory fishes. Journal of Experimental Marine Biology and Ecology 267:75–88.

Schooley, J. D., A. P. Karam, B. R. Kesner, P. C. Marsh, C. A. Pacey, and D. J. Thornbrugh. 2008. Detection of larval remains after consumption by fishes. Transactions of the American Fisheries Society 137:1044–1049.

Sheppard, S. K., and J. D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator–prey food-webs. Functional Ecology 19:751–762.

Sommer, T., C. Armor, R. Baxter, R. Breuer, L. Brown, M. Chotkowski, S. Culberson, F. Feyrer, M. Gingras, B. Herbold, W. Kimmerer, A. Mueller-Solger, M. Nobriga, and K. Souza. 2007. The collapse of pelagic fishes in the upper San Francisco Estuary. Fisheries 32:270–277.

Swain, D. P., and A. F. Sinclair. 2000. Pelagic fishes and the cod recruitment dilemma in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences 57:1321–1325.

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Molecular Ecology 11:627–641.

Thomson, J. R., W. J. Kimmerer, L. R. Brown, K. B. Newman, R. Mac Nally, W. A. Bennett, F. Feyrer, and E. Fleishman. 2010. Bayesian change point analysis of abundance trends for pelagic fishes in the upper San Francisco Estuary. Ecological Applications 20:1431–1448.

USFWS (U.S. Fish and Wildlife Service). 1993. Endangered and threatened wildlife and plants: determination of threatened status for the delta smelt. Federal Register 58:42(5 March 1993):12854–12864.

Wang, J. C. S. 1986. Fishes of the Sacramento–San Joaquin Estuary and adjacent waters, California: a guide to the early life histories. Interagency Ecological Program, Technical Report 9, California Department of Water Resources, Sacramento.

Wurtsbaugh, W., and H. Li. 1985. Diel migrations of a zooplanktivorous fish (*Menidia beryllina*) in relation to the distribution of its prey in a large eutrophic lake. Limnology and Oceanography 30:565–576.