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TaqMan assays for the genetic identification of delta smelt (*Hypomesus transpacificus*) and wakasagi smelt (*Hypomesus nipponensis*)

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Abstract

We have developed species-specific TaqMan assays for two California fish species, the threatened delta smelt (*Hypomesus transpacificus*) and the introduced wakasagi smelt (*Hypomesus nipponensis*). The assays are capable of correctly identifying each species with 100% accuracy, with no cross-species amplification. We anticipate these assays will prove useful for future scientific studies requiring genetic species identification (e.g. predation of smelt) or monitoring (e.g. detection of delta smelt near water diversions).

Keywords: mitochondrial DNA, Sacramento—San Joaquin Delta, smelt, species identification

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The recent precipitous decline of delta smelt (*Hypomesus transpacificus*) in the San Francisco Estuary watershed has caught the attention of scientists, environmentalists, policy-makers and the general public (Service 2007). A congener of the delta smelt that has been introduced into the Sacramento—San Joaquin Delta is the wakasagi smelt (*Hypomesus nipponensis*), which is native to Japan. While smelt adults have a species-specific number of chromatophores (small dark spots), larvae and young juveniles of the two species are extremely difficult to distinguish (Moyle 2002). Correct smelt identification is of critical importance because the two smelt species overlap in range, but the delta smelt is listed as threatened under the Endangered Species Act while the wakasagi smelt is an unprotected non-native species. We have developed two TaqMan assays capable of genetically identifying delta and wakasagi smelt. One potential application of this assay is to determine the extent of predation on these two smelt species (i.e. diet analysis of predators). Another future application of this assay could be evaluation of water samples for the presence of smelt DNA at Central Valley Project and State Water Project pumps, the primary water supply for millions of Californians. These pumps export drinking and irrigation water to municipal and agricultural water users in California and Central Valley farmers, which face reduced water supply when the delta smelt population is at risk of entrainment.

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Sample collection details are shown in Table 1. Locations were selected to encompass the distributional ranges of both species to assess potential intra- and inter-species genetic variation. Genomic DNA was extracted from a fin clip of each sample using the Qiagen DNeasy tissue kit.

To design species-specific TaqMan probes, a 485-bp segment of the mitochondrial cytochrome b gene (cyt-b) was sequenced for six delta smelt and four wakasagi smelt individuals using conserved animal primers, H15149 and L14724 (Kocher *et al.* 1989; Irwin *et al.* 1991).

Table 1 Samples of delta smelt and wakasagi smelt for TaqMan design and assay validation

	Location	No. of Samples	Genbank ID
TaqMan design			
H. transpacificus	Montezuma Slough; Sacramento River; San Joaquin River	6	HQ667171
H. nipponensis	Yolo Bypass	4	HQ667170
TaqMan validation			
H. transpacificus	Cache Slough	3	
	Honker Bay	2	
	Montezuma Slough	8	
	Sacramento River	31	
H. nipponensis	Sacramento River	6	
	Yolo Bypass	39	

Table 2 Cyt-b primer and probe sequences used in TaqMan assay to identify delta smelt and wakasagi smelt

Species	Primer/Probe	Sequence (5′-3′)	Reporter	Quencher
H. transpacificus	CytB-Htr-F	AATGGCCAACCTTCGGAAA		
	CytB-Htr-R	GARATATTRGAGGGTGCAGG		
	CytB-Htr-P	CCCATCCCCTCCTGAAAATTACCAACG	6FAM	BHQ
H. nipponensis	CytB-Hni-F	GGCCCGTAAGGATTTGGATAA		
	CytB-Hni-R	CCCTCCAATATTTCAATCTGATGA		
	CytB-Hni-P	AAGACACAGCCCAAGAAGGGATCCAAA	ROX	BHQ

Sequences were aligned using Sequencher ver. 4.8 (Gene Codes). Primer Express® Oligo Design software (Applied Biosystems) was used for primer and probe design (Table 2). According to Primer Express®, the highest scoring region for the delta smelt primers and probe was not optimal for the wakasagi smelt, and vice versa, so primers and probes for the two species were designed using distinct regions of the cyt-b gene.

The two species-specific assays were conducted independently of each other. For samples used to validate each species-specific assay, PCR was performed in a 5 μl total volume containing: 1 μl DNA template, 1× QuantiTect Multiplex PCR NoROX kit (Qiagen), 1.8 μM final concentration of both forward and reverse primers, 0.06 μM (delta smelt) or 0.18 μM (wakasagi smelt) final concentration for the probe. For both assays, thermal cycling occurred with 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 and 1-min annealing/extension at 63 °C. Ct values were quantified using Opticon Monitor software (ver 3.1; Bio-Rad). Each assay was tested for its ability to amplify samples of the probe's target species and not amplify samples of the non-target smelt and 21 other potentially co-occurring fish species. These other tested species (N = 2–5 individuals per species) included: American shad (*Alosa sapidissima)*, bigscale logperch (*Percina macrolepida*), black crappie (*Pomoxis nigromaculatus*), bluegill (*Lepomis macrochirus*), carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*, longfin smelt (*Spirinchus thaleichthys*), Mississippi silverside (*Menidia beryllina*), mosquitofish (*Gambusia affinis*), Pacific herring (*Clupea pallasii*), prickly sculpin (*Cottus asper*), pumpkinseed (*Lepomis gibbosus*), Sacramento sucker (*Catostomus occidentalis*), Shimofuri goby (*Tridentiger bifasciatus*), Sacramento splittail (*Pogonichthys macrolepidotus*), striped bass (*Morone saxatilis*), threadfin shad (*Dorosoma petenense*), white catfish (*Ameiurus catus)*, white crappie (*Pomoxis annularis*) and yellowfin goby (*Acanthogobius flavimanus*). Eight no template controls were included per plate, and the threshold was set above background fluorescence for each reporter dye. Samples were considered positive with a Ct value ≤35.

All 42 delta smelt samples amplified with the 6FAM dye and did not amplify with the ROX dye. With the threshold set to 0.010, the average Ct value with the 6 FAM dye for the delta smelt samples was 19. All 45 wakasagi smelt samples amplified with the ROX dye and did not amplify with the 6FAM dye. With the threshold set to 0.004, the average Ct value with the ROX dye for the wakasagi smelt samples was 18. None of the potentially co-occurring non-target fish amplified for either assay. Therefore, 100% accuracy was achieved using these TaqMan assays to genetically distinguish delta smelt and wakasagi smelt. We have no reason to believe that other dyes cannot be used in place of 6FAM or ROX (e.g. VIC) for either of these assays.

Like all assays using mitochondrial genes, these assays will not reliably estimate hybridization levels between delta and wakasagi smelt. However, hybridization between these smelt species is not believed to be common (~1% occurrence, Kathleen Fisch, personal communication) and introgression has not been detected to date. Therefore, these assays will be of great benefit for future studies of delta and wakasagi smelt, particularly when visual identification is difficult.

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