



IEP NEWSLETTER

VOLUME 39, NUMBER 1, 2020

Contents

- 2019 Status and Trends Report for Pelagic Fishes of the Upper San Francisco Estuary [3](#)
- Exploring Secondary Field Identification of Delta Smelt and Wakasagi Using Image Software [16](#)

OF INTEREST TO MANAGERS

Annika Keeley (DSC), Annika.Keeley@deltacouncil.ca.gov

Of Interest to Managers

This issue of the Interagency Ecological Program (IEP) features a contributed paper and a status and trends report.

1. Timothy Malinich (CDFW) and colleagues summarize the status and trends of pelagic fishes in the upper San Francisco Estuary using data from the IEP long-term fish monitoring surveys. The report describes where and when the five different surveys have been conducted and explains how abundance indices are being calculated. For seven species, native and non-native, the authors summarize 2019 catch numbers by region and put them into context by comparing them with the long-term survey results. For most of the seven pelagic fish species, annual abundance indices from all 2019 surveys increased modestly compared to the previous year. Surveys that targeted littoral or demersal habitats revealed notable increases in Splittail and Longfin Smelt indices. Non-native species generally showed minor increases in abundance.

2. Jeff Jenkins (DWR) and colleagues studied whether high-resolution imagery of morphological traits could be used as a complimentary tool to differentiate Delta Smelt and Wakasagi. Based on genetic verification, currently used chromatophore counts and patterns are not reliable. The authors tested several morphometric ratios and found that the body width: fork length ratio differed significantly between the two species; hybrids fell in the middle.

Did you know that highlights about current IEP science can be found on the IEP webpage along with IEP Project Work Team and other IEP-related public meetings? To view these features see the links below:

<https://water.ca.gov/Programs/Environmental-Services/Interagency-Ecological-Program>

STATUS AND TRENDS

2019 Status and Trends Report for Pelagic Fishes of the Upper San Francisco Estuary

Timothy D Malinich (CDFW), timothy.malinich@wildlife.ca.gov; James White (CDFW), james.white@wildlife.ca.gov; Emily Campbell (CDFW), emily.campbell@wildlife.ca.gov; Cory Graham (USFWS), cory_graham@fws.gov; Adam Chorazyczewski (CDFW), adam.chorazyczewski@wildlife.ca.gov; Trishelle Tempel (CDFW), trishelle.tempel@wildlife.ca.gov; Steven B. Slater (CDFW), steve.slater@wildlife.ca.gov; and Kathy Hieb (CDFW), kathy.hieb@wildlife.ca.gov

Introduction

The 2019 Pelagic Fishes Status and Trends Report presents relative abundance trends for pelagic fishes using data from five of the Interagency Ecological Program's (IEP) long-term fish monitoring surveys: 1) 20-mm Survey, 2) Summer Trawl Survey (STN), 3) Fall Midwater Trawl (FMWT), 4) the San Francisco Bay Study (SFBS) and 5) US Fish and Wildlife Service (USFWS) Beach Seine Survey (Honey et al. 2004). Abundance indices, as well as long-term trends in abundance and distributional information, are presented for seven species: American Shad (*Alosa sapidissima*), Threadfin Shad (*Dorosoma petenense*), Delta Smelt (*Hypomesus transpacificus*), Longfin Smelt (*Spirinchus thaleichthys*), Wakasagi (*Hypomesus nipponensis*), Splittail (*Pogonichthys macrolepidotus*), and age-0 Striped Bass (*Morone saxatilis*). Unlike previous Status and Trends Reports, this report also includes abundance data collected by the San Francisco Bay Study otter trawl for a subset of species (Longfin Smelt, age-0 Striped Bass, and Splittail). Many of the focal species, particularly natives, have undergone significant

population declines since the start of these long-term surveys. However, this year several surveys report small increases in indices for several fish species, including American Shad, Threadfin Shad, Splittail, and age-0 Striped Bass.

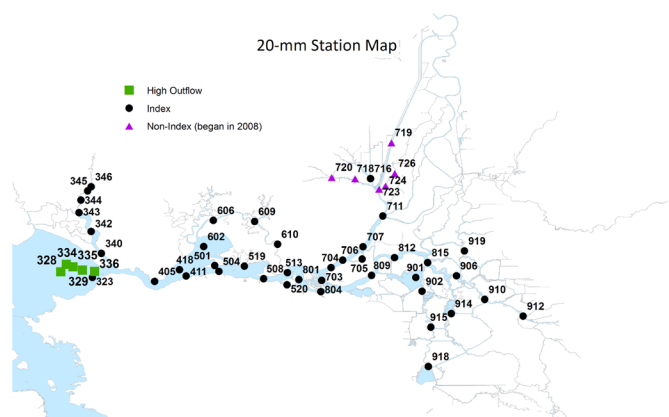
Methods

Sampling Background

20-mm Survey

The California Department of Fish and Wildlife (CDFW) 20-mm Survey monitors distribution and relative abundance of larval and juvenile Delta Smelt throughout its historical spring range. This includes the entire Delta and downstream to eastern San Pablo Bay and the lower Napa River. The survey name refers to the size of Delta Smelt that the survey gear targets, which corresponds to the size at which Delta Smelt are readily identifiable and counted at the State Water Project and Central Valley Project fish salvage facilities. Since 1995, CDFW has conducted the 20-mm Survey on alternate weeks from early March through early July, completing nine surveys per year since 2005. Three tows are conducted at each of the 47 stations (Figure 1) using a fixed-mouth, 1,600 μ m mesh net (Dege

Figure 1. Map of the 20-mm Survey stations. Index stations (n=41) have been sampled since survey inception in 1995. Data collected at index stations were used to calculate survey and annual abundance indices. Non-index stations (n=6 within Cache Slough, Sacramento Deep Water Channel, and Miner Slough) were added to the survey in 2008 to better assess the distribution of Delta Smelt and other pelagic fishes. Five non-index stations (green squares) within San Pablo Bay are only sampled during high flow years.

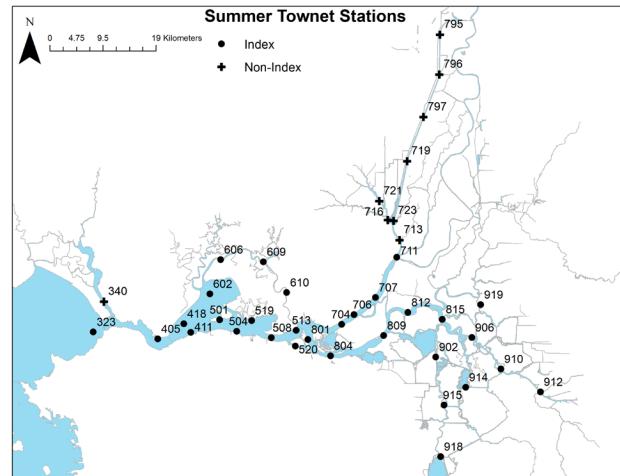


and Brown 2004). The survey added five Napa River stations in 1996. In 2008, two stations each were added in Lindsey Slough, Miner Slough, and the Sacramento River Deep Water Ship Channel (SDWSC). A <60 mm fork length (FL) criterion is used to select length data for age-0 Delta Smelt, which are then averaged by survey for all stations sampled to determine when mean FL reaches or surpasses 20 mm. The two surveys before and after the mean 20 mm FL is reached are used to calculate the annual abundance index. From this subset of surveys, Delta Smelt catch per unit effort (CPUE) is calculated for each of the 41 index stations (Figure 1). CPUE for each tow is calculated by dividing catch by the volume (m³) filtered during the sample and multiplying by 10,000 to obtain a whole number. CPUE is then averaged across tows for each index station. The resulting mean station CPUE values are incremented by one and then log₁₀ transformed (i.e., log₁₀(x+1)). These transformed values are averaged within each survey and then the mean values are back transformed (i.e., 10^x), to return them to their original scale. Finally, one is subtracted from each value and these values are summed across the four surveys to obtain the 20-mm Survey annual abundance index.

Summer Towntet Survey

The Summer Towntet Survey (STN) began in 1959 and its data have been used to calculate age-0 Striped Bass indices for all years since, except 1966, 1983, 1995, and 2002. Delta Smelt indices have also been calculated for the period of record, except for 1966 through 1968. Historically, STN conducted between two and five surveys annually, depending on when the mean FL of age-0 Striped Bass exceeded 38.1 mm, at which time the index could be determined, and sampling terminated for the year. In 2003, CDFW standardized sampling to six surveys per year, beginning in early June and continuing every other week into August (Hieb et al. 2005). STN samples 32 historic stations, one of which is located in the Napa River and is excluded from index calculations due to historically infrequent sampling. Index stations are distributed from eastern San Pablo Bay to Rio Vista on the Sacramento River and to Stockton on the San Joaquin River (Figure 2). In 2011, STN added eight supplemental stations in the Cache Slough and SDWSC regions to increase spatial coverage and better describe Delta Smelt range

Figure 2. Map of the Summer Towntet Survey stations. Index stations have been sampled since survey inception in 1959 and their data were used for calculating survey and annual abundance indices. The Napa River non-index station was sampled in 1959 and then consistently sampled each year starting 1978. The Sacramento Deep Water Channel (n=5), and Cache Slough stations (n=3) were added as non-index stations beginning in 2009 and 2011, respectively, to better assess the distribution of Delta Smelt and other pelagic fishes.



and habitat. A minimum of two tows are completed at historic stations, and a third tow is conducted if any species of fish are caught during either of the first two tows. Three tows are conducted at the San Pablo Bay station (STN 323) regardless of catch due to the large volume of water represented by this station. Two tows are completed at supplemental stations in the Cache Slough-SDWSC region unless ten or more Delta Smelt are captured during the first tow at a station. In these instances, a second tow is not completed.

Catch per tow data from the 31 STN index stations are used to calculate annual abundance indices for age-0 Striped Bass and Delta Smelt. First, the catch of a species is summed across tows at each station. Then, the sum is multiplied by a volume-weighting factor (i.e., the estimated volume [thousand acre-feet] represented by each station; see Chadwick, 1964). These products are then summed across all 31 index stations within a survey, and then divided by 1000, to produce the survey abundance index. The annual abundance index for age-0 Striped Bass is interpolated using the abundance indices from the two surveys that bound the date when mean FL reached 38.1 mm (Chadwick 1964; Turner and Chadwick 1972). STN did not consistently measure

Delta Smelt FL until 1973, so no length criterion is used for the Delta Smelt index calculation. Instead, the annual index for Delta Smelt is the average of the first two survey indices of each year; however, in 1996 the first survey was cut short due to equipment malfunction, so the index was calculated as the average of the indices for the second and third surveys.

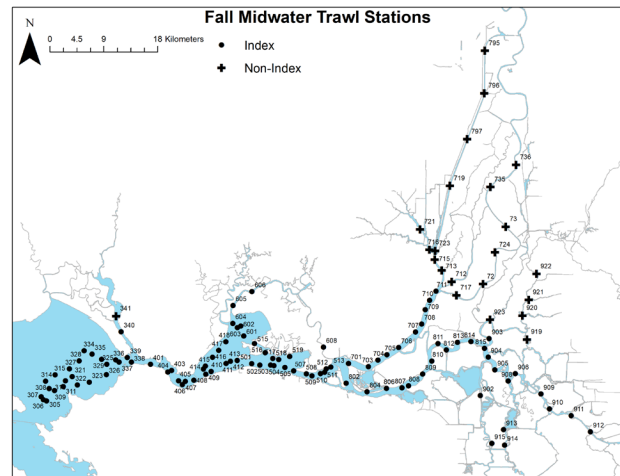
Fall Midwater Trawl

The Fall Midwater Trawl (FMWT) survey began in 1967 and has been conducted in all years except 1974 and 1979. CDFW established the FMWT survey to examine the relative abundance and distribution of pelagic fish species in the upper estuary, focusing on age-0 Striped Bass (Stevens 1977). Later, FMWT developed abundance and distribution information for other upper-estuary pelagic fishes, including American Shad, Threadfin Shad, Delta Smelt, Longfin Smelt, and Splittail. The FMWT survey currently conducts single tows at 122 stations monthly from September through December. Trawl sampling ranges from western San Pablo Bay to Hood on the Sacramento River, and from Sherman Lake to Stockton on the San Joaquin River (Figure 3). The annual abundance index calculation uses catch per tow data from 100 of 122 stations (Stevens 1977). The remaining 22 stations were added in 1990, 1991, 2009, and 2010 to improve understanding of Delta Smelt distribution and habitat use. To calculate survey abundance indices, the 100 index stations are grouped into 17 regions. Monthly indices are calculated by averaging index-station catch-per-tow in each region, multiplying these regional means by their respective weighting factors (Chadwick 1964), and summing these products. Annual abundance indices are the sum of the four (September – December) monthly indices.

San Francisco Bay Study

The San Francisco Bay Study (SFBS) began in 1980 to determine the effects of freshwater outflow on the abundance and distribution of fishes and mobile crustaceans throughout the San Francisco Estuary. Sampling ranges from south of the Dumbarton Bridge in South San Francisco Bay (South Bay), to just west of Alcatraz Island in Central San Francisco Bay (Central Bay), throughout San Pablo and Suisun bays, north to the confluence of Steamboat and Cache Sloughs

Figure 3. Map of the Fall Midwater Trawl Survey stations. Index stations have been sampled since survey inception in 1967 and their data were used for calculating survey and annual abundance indices. Non-index stations were added to better assess the distribution of Delta Smelt and other pelagic fishes. These stations were added in the following order; Lower Sacramento in 1990 (n=4), Upper Sacramento and Mokelumne River (n=11), the Sacramento Deep Water Channel (n=4) in 2009, and Cache Slough (n=1) in 2010.

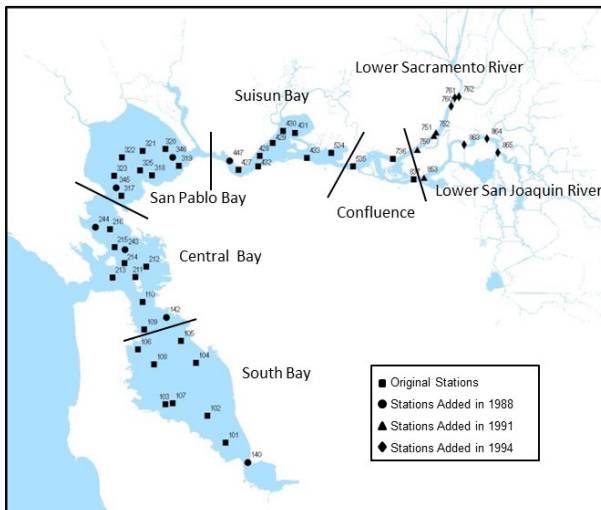


on the Sacramento River, and east to Old River Flats on the San Joaquin River (Figure 4). Every station is sampled with two tows: one against the current with an otter trawl (OT) to sample the demersal community, and one with the current using a midwater trawl (MWT) to sample pelagic species. There are data gaps in this long-term sampling; most significantly, there was limited midwater trawl sampling in 1994, no winter sampling (November through January) from 1989 to 1997 to reduce survey costs, limited sampling at stations in and near the confluence of the Sacramento and San Joaquin rivers in 2007 and 2008 to reduce Delta Smelt take, and finally most recently in 2016 due to repeated vessel breakdowns. See Hieb et al. (2019) for fish and abundance trend information through 2016. Of the 52 stations the Bay Study currently samples, 35 core stations (i.e., original stations; Figure 4) have been consistently sampled since 1980 and are used to calculate the annual abundance indices (Baxter et al. 1999). Annual abundance indices are calculated as the average of monthly indices over the period for which the life stage was most abundant (May through October), and only include data from Bay Study's 35 index (core) stations. Monthly indices are calculated as the product of mean CPUE at all index stations within each of five

geographical regions and that region's water volume weighting factor (for the MWT) or the region's areal weighting factor (for the OT), and then these products are summed across all 5 regions. Additional information about study methods, including index calculation, can be found in IEP Technical Report 63 (Baxter et al. 1999).

Figure 4. Map of the San Francisco Bay Study (SFBS) stations. Stations are assigned to 1 of 7 regions: South Bay, Central Bay, San Pablo Bay, Suisun Bay, Confluence, Lower Sacramento River and Lower San Joaquin River. There are 35 original stations, sampled since 1980. Additional stations were added in 1988 (n=7 within the South Bay, Central, San Pablo Bay and Suisun Bay), 1991 (n=4 within the Lower San Joaquin River) and 1994 (n=6 within the Lower Sacramento River).

San Francisco Bay Study Boat (Open Water) Stations



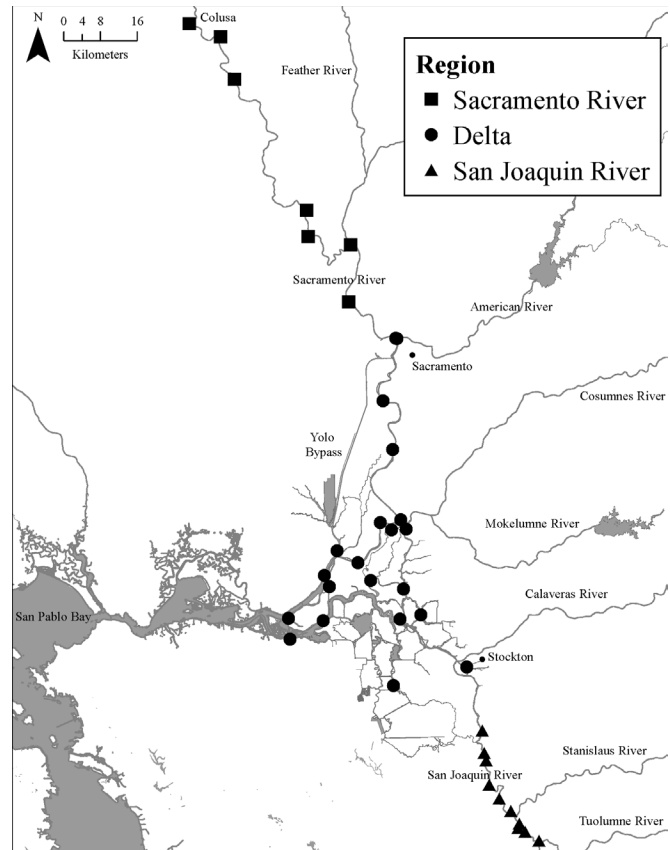
USFWS Beach Seine

Since 1994, USFWS has conducted weekly beach seine sampling year-round at approximately 40 stations in the Delta and in the lower Sacramento and San Joaquin rivers (Brandes and McLain 2001). Data from 33 stations were used to calculate the annual age-0 Splittail abundance index. These stations ranged from Sherman Lake to Ord Bend on the Sacramento River, and to just downstream of the Tuolumne River confluence with the San Joaquin River (Figure 5). Hereafter, we refer to the confluence of the Sacramento and San Joaquin rivers at Sherman Lake as ‘the

Confluence’, and the Tuolumne River confluence with the San Joaquin River as ‘the Tuolumne confluence’. To calculate the juvenile Splittail index, all Splittail <25 mm FL (measured individuals and proportions resulting from plus counts) and ≥ 85 mm in May and ≥ 105 mm in June (cutoffs for age-1) were removed from calculations, leaving only age-0 individuals. The 33 index stations are grouped into 10 regions. The annual index was calculated as the mean catch per m³ for seine hauls conducted first at each station and month for the months May and June, and then across months for each sub-region. Finally, the mean catch per m³ for each year and sub-region was summed across regions to produce the annual age-0 Splittail index.

The long-term monitoring surveys summarized above provide a look into the past 60 years of fish

Figure 5. Map of the USFWS beach seine survey stations. Data from 1994 through present and from the Sacramento River, the San Joaquin River and the Delta were used for age-0 Splittail annual abundance indices.



abundance and index patterns. FMWT data were used to describe abundance trends and distribution patterns of all six fish species listed in the introduction. This year, the SFBS otter trawl data are used to describe trends for age-0 Longfin Smelt, age-0 Splittail, and age-0 Striped Bass. STN describes trends for Delta Smelt and Striped Bass. Two studies provided single species information: the 20-mm Survey for the abundance and distribution of larval and juvenile Delta Smelt, and USFWS beach seine data for age-0 Splittail abundance and distribution. Because recent abundance indices are much lower than earlier years, inset graphs of the most recent 5 years were added to abundance graphics for greater clarity.

Results

American Shad

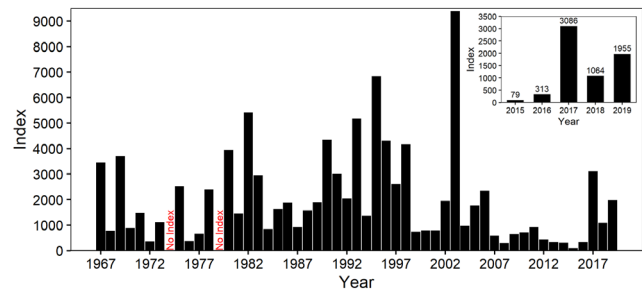
American Shad was introduced into the Sacramento River in 1871 (Dill and Cordone 1997). This anadromous species spawns in the Sacramento, Feather, Yuba and American rivers from April through June. Juveniles can be found in freshwater areas within the Delta from late May through summer and into fall. From summer through fall, juveniles migrate to the ocean where they mature. Males reach maturity at 3 to 4 years whereas females mature slightly later at 4 to 5 years (Moyle 2002). A large proportion of the spawning population in the Delta succumbs to natural mortality shortly after spawning; however, spent females have been observed downstream of spawning sites indicating some post-spawning survival (Stevens 1966). Surveys conducted in the Susquehanna River in the Northeastern United States, suggest that post-spawning mortality is higher among females than males (Walburg and Nichols 1967).

The 2019 FMWT index for American Shad was 1955, an 84% increase from the 2018 FMWT index value (Figure 6). The index value peaked at 9360 in 2003 and has fluctuated greatly between 2015 and 2019.

In the 2019 FMWT sampling season, 1465 American Shad were collected at index stations throughout the upper estuary and Delta. In September, American Shad were collected at index stations in San Pablo Bay (n=33),

Carquinez Strait (n=15), Suisun Bay (n=252), the lower Sacramento River (n=60), and the lower San

Figure 6. Bar plot of the annual abundance indices for American Shad from the Fall Midwater Trawl Survey, 1967-2019 (all sizes). Inset graphics show the most recent 5 years in more detail.



Joaquin River (n=78). American Shad were collected at non-index stations in the SDWSC (n=10), the Mokelumne River (n=3), Napa River (n=3), Cache Slough (n=1), and Little Potato Slough (n=1). In October, they were collected at index stations in San Pablo Bay (n=6), Carquinez Strait (n=3), Suisun Bay (n=188), the lower Sacramento River (n=16), and the lower San Joaquin River (n=4). American Shad were collected at non-index stations in the SDWSC (n=31) and Steamboat Slough (n=8). November catches were from index stations in San Pablo Bay (n=7), Carquinez Strait (n=21), Suisun Bay (n=261), the lower Sacramento River (n=181), and the lower San Joaquin River (n=26). American Shad were collected at non-index stations in the SDWSC (n=9). In December, American Shad were collected at index stations in San Pablo Bay (n=48), Carquinez Strait (n=55), Suisun Bay (n=146), the eastern Delta (n=27), the lower Sacramento River (n=12), and the lower San Joaquin River (n=26). American Shad were collected at non-index stations in the Cache Slough (n=23) and the Napa River (n=1).

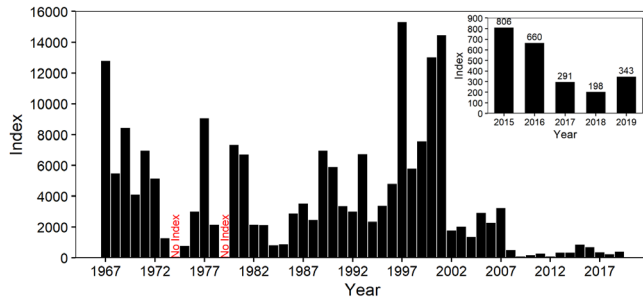
Threadfin Shad

The Threadfin Shad was introduced to California reservoirs in the late 1950s and quickly spread downstream into the Sacramento and San Joaquin rivers (Dill and Cordone 1997). It has become established throughout the Delta and is most common in slow moving, fresh to oligohaline water found in dead-end sloughs (Wang 1986). Threadfin Shad are planktivorous throughout their life history (Holanov and Tash 1978). Spawning occurs from late spring through

summer, peaking from May to July (Wang 1986). Individuals can reach maturity in their first year and live up to four years (Moyle 2002).

The FMWT Threadfin Shad index for 2019 was 343, representing a 73% increase from the previous year (Figure 7). This is the first increase in the Threadfin Shad Index since 2015. The abundance indices were highest during the late 1990s and early 2000s, with the two highest indices occurring in 1997 (15,267) and 2001 (14,401).

Figure 7. Bar plot of the annual abundance indices for Threadfin Shad from the Fall Midwater Trawl Survey, 1967-2019 (all sizes). Inset graphics show most recent 5 years in more detail.

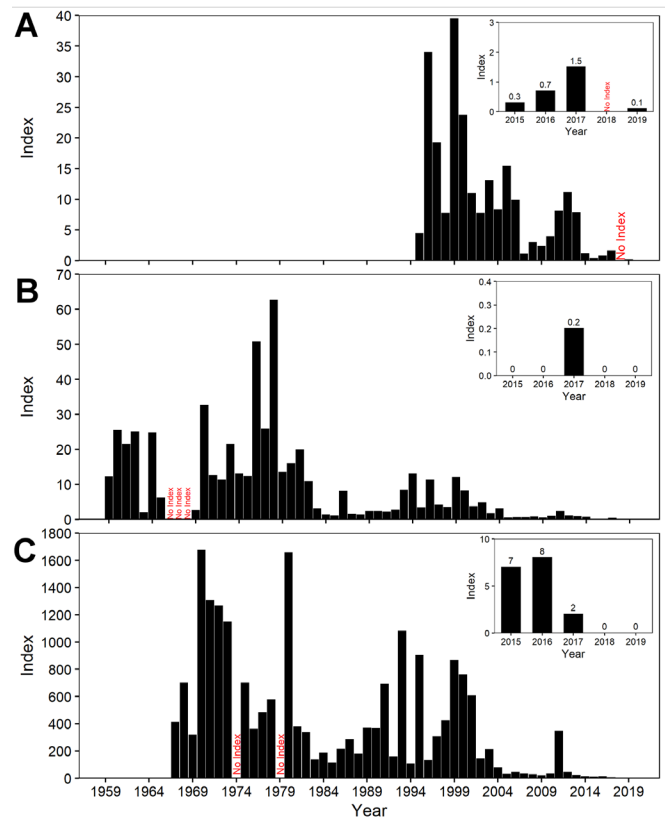


During FMWT, 279 Threadfin Shad were collected at index stations and 1281 Threadfin Shad were collected from non-index stations. In September, Threadfin Shad were collected at index stations in San Pablo Bay (n=4), Suisun Bay (n=12), the lower Sacramento River (n=2), and the Eastern Delta (n=4). The majority of Threadfin Shad were collected at non-index stations in the SDWSC (n=95). In October, fish were collected at index stations in Suisun Bay (n=10), and the lower San Joaquin River (n=6). Threadfin Shad were collected at non-index stations in the SDWSC (n=1046) and Steamboat Slough (n=2). Catches in November were collected at index stations in Suisun Bay (n=28), lower Sacramento River (n=182), and the lower San Joaquin River (n=7). Threadfin Shad were also caught at non-index stations within the SDWSC (n=128). Catches in December were found at index stations in San Pablo Bay (n=2), Suisun Bay (n=9), the lower Sacramento River (n=5), the lower San Joaquin River (n=1), and the Eastern Delta (n=7). Threadfin Shad were collected at non-index stations in the SDWSC (n=10).

Delta Smelt

Delta Smelt is a small (<90 mm FL) osmerid endemic to the San Francisco Estuary. In the 1980s, Delta Smelt underwent a severe population decline (Figures 8 B-C) and in 1993 was listed as a threatened species by state and federal agencies, and was uplisted to endangered by the State in 2010. It is considered environmentally sensitive due to an annual life cycle, dependent on a spatially-limited oligohaline to freshwater habitat, and low fecundity (1,200 to 2,600 eggs per female on average; [Moyle, Herbold, Stevens, & Miller, 1991]). Low fecundity appears to be offset by the ability of females to produce multiple clutches in a single spawning season (Bennett 2005; Damon et al. 2017).

Figure 8. Bar plots of the annual abundance indices for Delta Smelt from: A) 20-mm Survey (larvae and juveniles; 1995-2019); B) Summer Towner Survey (all sizes 1959-2019); C) Fall Midwater Trawl Survey (sub-adults; 1967-2019). Inset graphics show most recent 5 years in more detail. Note: Differences in the y-axis scales for each graph.



The 20-mm Delta Smelt index for 2019 was 0.1. This was marginally higher than the previous year when Delta Smelt catch was insufficient to calculate an index value (Figure 8A). The 2019 index was calculated using surveys 3–6 (April 8–May 23). During the 2019 20-mm survey, a total of 16 Delta Smelt were collected throughout the Delta, but only 3 fish were caught at index stations contributing to the 20-mm Delta Smelt index. Specifically, Delta Smelt were collected in San Pablo Bay, Napa River, Suisun Bay (Montezuma Slough), the Confluence, Cache Slough (Lindsey Slough), the lower Sacramento River, Miner Slough and the SDWSC.

The STN Delta Smelt index for 2019 was 0. It is the fourth year in which the number of Delta Smelt collected was low enough to result in an index of zero (Figure 8B). The previous zero indices occurred in 2015, 2016 and 2018. The first two surveys of 2019 were conducted during the weeks of June 9 and June 23. During the first survey, no Delta Smelt were collected at index stations. One Delta Smelt was collected at supplemental station 795 in the SDWSC.

The FMWT Delta Smelt index for 2019 was 0 and is tied with 2018 as the lowest in FMWT history (Figure 8C). No Delta Smelt were collected from any station during the survey months of September–December. This year’s catch is consistent with the low catches and limited geographic distribution seen in recent years and may indicate that the population has dipped below the detection threshold for the FMWT gear.

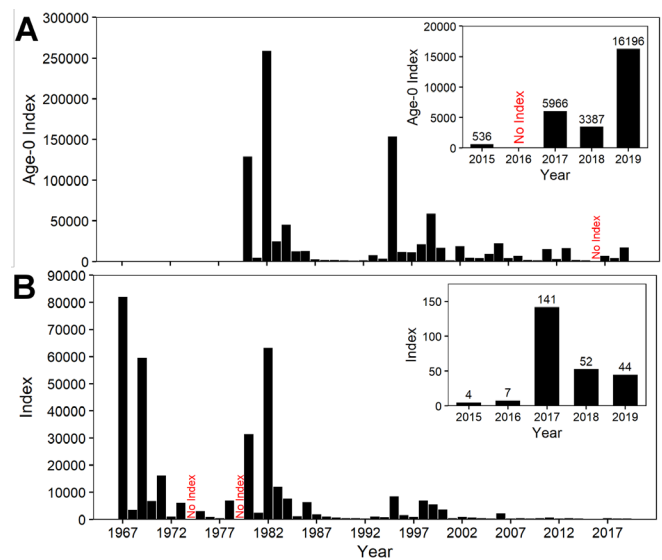
Longfin Smelt

Longfin Smelt is a short-lived, anadromous fish that spawns in freshwater or slightly brackish water in winter and spring. It rears primarily in brackish water with some young-of-the-year and age-1+ fish migrating to the ocean in summer and fall. Adults typically return to the estuary as water temperatures drop in late fall and winter. Most Longfin Smelt reach maturity in their second year, but some individuals may wait longer whereas others appear capable of spawning in their first year. A few individuals may survive to spawn a second time (Wang 1986).

The 2019 SFBS produces 3 indices for Longfin Smelt, one for each age (1–3). However, we only discuss age-0 Longfin Smelt in this report. Furthermore, Longfin Smelt numbers reported here are based only on

otter trawl data and do not reflect midwater trawl data. In 2019, the age-0 Longfin Smelt index was 16,196 (Figure 9A). This is a large increase from recent years and is the highest index recorded since 2006. Age-0 Longfin Smelt were collected in Central Bay (n=302), San Pablo Bay (n=289) and Suisun Bay (n=102).

Figure 9. Bar plots of the annual abundance indices for Longfin Smelt from A) Age-0 Longfin Smelt from the San Francisco Bay Study otter trawl (1980-2019) and B) Fall Midwater Trawl Survey (all sizes; 1967-2019). Inset graphics show most recent 5 years in more detail. Note: Differences in the y-axes scales for each graph.



The 2019 FMWT Longfin Smelt index was 44, the second year of decline following the higher (141) index value in 2017 (Figure 9B). Longfin Smelt abundance was highest in the late 1960s and peaked again in the early 1980s. After a brief increase in the late 1990s, abundance dropped again and has remained relatively low for most recent years.

Thirty-one Longfin Smelt were caught during the 2019 FMWT. Thirty fish were caught at index stations throughout the survey and one at a non-index station. In September, Longfin Smelt were collected at index stations in Carquinez Strait (n=2), and Suisun Bay (n=5). In October, Longfin Smelt were collected at index stations in Suisun Bay (n=2). In November, Longfin Smelt were collected at index stations in Carquinez Strait (n=1), and Suisun Bay (n=2). In December,

Longfin Smelt were collected at index stations in San Pablo Bay (n=3), Carquinez Strait (n=3), Suisun Bay (n=7), the lower Sacramento River (n=4), and the lower San Joaquin River (n=1). One Longfin Smelt was caught at a non-index station, in the Napa River (n=1).

Splittail

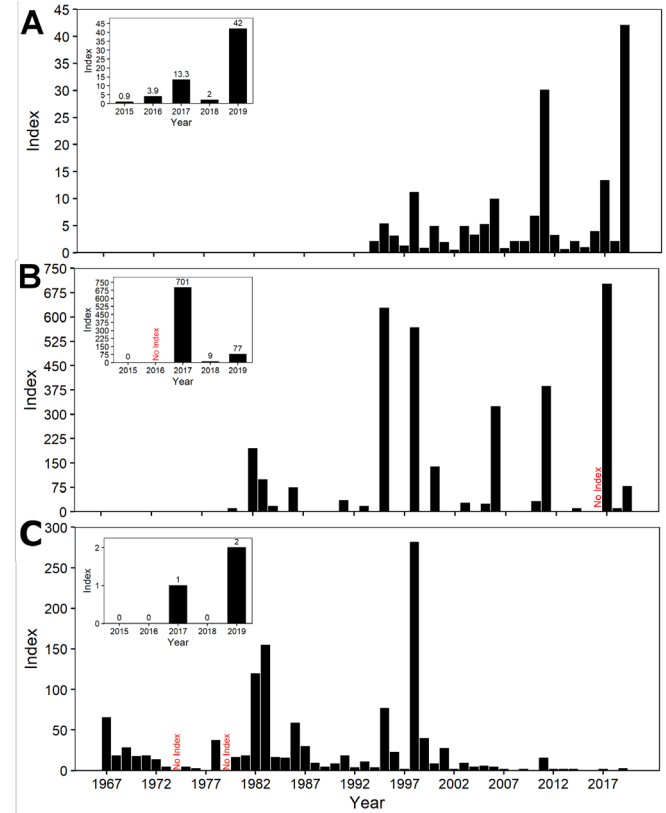
Splittail is a large cyprinid endemic to the San Francisco Estuary and its watersheds. Adults migrate from brackish to freshwater from late fall to early spring as river flows increase. During this time, they forage and eventually spawn on inundated floodplains and river margins (Sommer et al. 1997; Moyle et al. 2004). Spawning migrations occur in the Sacramento, San Joaquin, Cosumnes, Napa, and Petaluma rivers, as well as in Butte Creek and other small tributaries (Moyle et al. 2004; Feyrer et al. 2015). The majority of spawning takes place from March through May, and the resulting larvae and small juveniles disperse downstream in late spring and summer. This outmigration coincides with reduced river flows that decrease available backwater and edge-water habitats. Year-class strength is influenced by timing and duration of floodplain inundation. Moderate to strong cohorts are associated with periods of springtime inundation lasting 30 days or longer (Moyle et al. 2004).

The 2018–2019 USFWS Beach Seine index for age-0 Splittail was 42, the highest value calculated since the start of the survey in 1994 (Figure 10A). Regional abundance was highest in the Delta region (35), followed by the Sacramento River (5) and lowest in the San Joaquin River (2).

The 2019 SFBS Splittail index was 77 and was calculated using CPUE caught between May and October (Figure 10B). This represents an increase from 2018 but is low compared to indices recorded in previous years when the splittail CPUE was high, such as in 2017 (701), 2011 (385) and 2006 (322). Splittail were caught by otter trawl within several regions of the San Francisco Bay-Delta region, including San Pablo Bay (n=5), Suisun Bay (n=4), the Confluence (n=1), Lower Sacramento River (n=22) and Lower San Joaquin River (n=3).

The 2019 FMWT Splittail index for all ages was 0, continuing a trend of very little to no catch of Splittail in FMWT (Figure 10C). Two Splittail were caught within the SDWSC (FL 94 mm and FL 100 mm) a non-index

Figure 10. Bar plots of the annual abundance indices for Splittail from: A) USFWS Beach Seine Survey (juveniles ≥ 25 mm; 1994–2019), B) San Francisco Bay Study otter trawl (1980–2019) and C) Fall Midwater Trawl Survey (all sizes; 1967–2019). Inset graphics show most recent 5 years in more detail. Note: Differences in the y-axis scales for each graph.



station and were therefore not included in the index calculation. The Splittail FMWT index tends to be low or zero except in relatively wet years, such as 2011, when age-0 fish tend to be abundant. FMWT operates in water >2 m deep, whereas Splittail, particularly age-0 fish, appear to primarily inhabit water <2 m deep. Thus, during most years, FMWT data probably does not accurately reflect trends in age-0 Splittail abundance. FMWT generally does detect strong year classes, such as in 1998 and 2011. However, the high index calculated by the USFWS Beach Seine index was not reflected within the FMWT catch in 2019.

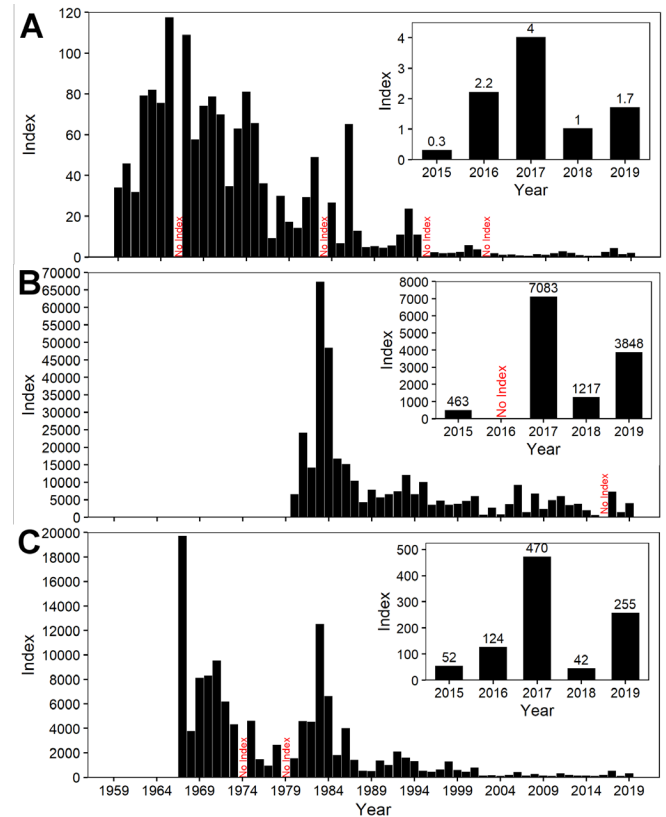
Age-0 Striped Bass

Striped Bass is a long-lived anadromous fish first introduced to the San Francisco Estuary in 1897 (Dill and Cordone 1997). Mature individuals forage in near-shore marine habitats, including coastal bays and estuaries. Many adults migrate to the Delta in fall and early winter, where they remain until swimming upstream to spawn in the spring. Spawning takes place in the water column and both eggs and larvae rely on river and tidal currents to keep them suspended during early development. Larvae are then transported to rearing areas in fresh and brackish waters (Dill and Cordone 1997).

Both STN and FMWT indices showed declines in age-0 Striped Bass abundance in the mid-1970s (Figures 11A & 11C). Abundance dropped further in the late 1980s and again in the 1990s and has not approached historic numbers over the last 15 years. Stevens et al. (1985) hypothesized that four factors were responsible for the low abundance: 1) the adult population was too small to maintain adequate egg production; 2) planktonic food production has decreased to a point that is too low to sustain historic population levels; 3) loss to entrainment in water diversions; and 4) pollution in the form of pesticides, petrochemicals, and other toxic substances. More recently, Sommer et al. (2011) argued that age-0 Striped Bass distribution had shifted almost exclusively to shoal and shoreline areas, which are under-sampled by CDFW trawl surveys. While a shift of this nature would reduce catch and thus reduce abundance indices, Sommer et al. (2011) cautioned against attributing low values solely to a change in habitat use.

The 2019 STN index for age-0 Striped Bass was 1.7, a 58% increase from the previous year (Figure 11A). In 2019, age-0 Striped Bass reached an average FL of 38.1 mm on July 29, between survey 4 (July 22–24) and survey 5 (Aug 5–7). In survey 4, 112 age-0 Striped Bass were collected from index stations, which include Suisun Bay (n=40), the Confluence (n=8), the Sacramento River (n=56), the lower San Joaquin River (n=7), and the South Delta (n=1). No additional age-0 Striped Bass were collected at non-index stations during Survey 4. In survey 5, a total of 47 age-0 Striped Bass were collected from index stations in Suisun Bay (n=32), the Confluence (n=3), the Sacramento River (n=10), and in the lower San Joaquin River (n=2). No additional

Figure 11. Bar plots of the annual abundance indices of age-0 Striped Bass from: A) Summer Townet (all sizes; 1959-2019), B) San Francisco Bay Study otter trawl (1980-2019) and C) Fall Midwater Trawl Survey (all sizes; 1967-2019). Inset graphics show most recent 5 years in more detail. Note: Differences in the y-axis scales for each graph.



age-0 Striped Bass were collected at non-index stations during survey 5.

During the entire 2019 STN season, a total of 895 age-0 Striped Bass were collected from locations ranging from Suisun Bay to the lower Sacramento and San Joaquin rivers, as well as in the SDWSC and the South Delta. Catches were consistently concentrated in the Suisun Bay region (n=679), and to a lesser extent in San Pablo Bay (n=1), Carquinez Strait (n=35), the South Delta (n=20), Old River (n=1), Lower San Joaquin River (n=24), the Confluence (n=12), and the Lower Sacramento River (n=101). Age-0 Striped Bass were also collected from non-index stations in the SDWSC (n=18), the Napa River (n=2), and Cache Slough (n=2). Catch was high in surveys 1–3 (193, 267, 263 respectively) and then gradually decreased each survey to 13 in survey 6.

The 2019 SFBS age-0 Striped Bass index was 3848 and was the second highest index since 2011 (Figure 11B). This index was calculated using CPUE caught between May and October. Age-0 Striped Bass were caught by otter trawl within several regions of the San Francisco Bay-Delta region, including San Pablo Bay (n=24), Suisun Bay (n=258), the Confluence (n=44), Lower Sacramento River (n=84) and Lower San Joaquin River (n=136). The SFBS also collected age-0 Striped Bass in November and December, however these fish were not included in the index calculation. These additional bass were found in San Pablo Bay (n=12), Suisun Bay (n=109), the Confluence (n=2), Lower Sacramento River (n=47) and the Lower San Joaquin River (n=58).

The 2019 FMWT index for age-0 Striped Bass was 255, representing a 6-fold increase from the previous year (Figure 11C). The index was highest at the inception of the survey in 1967, peaked again in 1971, and a third time in 1983. In the late 1980s, age-0 Striped Bass abundance declined and in the early 2000s it dropped again and has remained low since then.

Two hundred and eight age-0 Striped Bass were collected at FMWT index stations spanning from the Carquinez Strait to the lower Sacramento and San Joaquin rivers and the South Delta. In September, age-0 Striped Bass were collected from San Pablo Bay (n=2), Suisun Bay (n=74), the lower Sacramento River (n=1), the lower San Joaquin River (n=1), and the Eastern Delta (n=1). Age-0 Striped Bass were also caught at a non-index station (n=11) in the SDWSC. In October, age-0 Striped Bass were collected in San Pablo Bay (n=2), Carquinez Strait (n=4), Suisun Bay (n=26), the lower Sacramento River (n=1), and the lower San Joaquin River (n=2). One Striped Bass was collected at a non-index station in the SDWSC. In November, age-0 Striped Bass were collected from San Pablo Bay (n=2), Carquinez Strait (n=6), Suisun Bay (n=31), the lower Sacramento River (n=4), and the lower San Joaquin River (n=2). Striped Bass were also collected at non-index stations in the SDWSC (n=2). Finally, in December, age-0 Striped Bass were caught in San Pablo Bay (n=1), Carquinez Strait (n=8), Suisun Bay (n=23), the lower Sacramento River (n=9), the lower San Joaquin River (n=4), and the Eastern Delta (n=4). At non-index stations, two Striped Bass were collected in the SDWSC (n=2).

Wakasagi

Wakasagi (*H. nipponensis*) is native to Japan and was purposely introduced as a forage fish in lakes and reservoirs in 1959 (Dill and Cordone 1997; Moyle 2002) and was first detected in the San Francisco Estuary in 1990. Closely related to the native Delta Smelt (Moyle 2002), Wakasagi are also planktivorous, reach maturity within their first year, and spawn in late winter to spring (Moyle 2002). Wakasagi are able to tolerate higher salinities and a larger range of temperatures than Delta Smelt (Swanson et al. 2000). Despite having a higher salinity tolerance than Delta Smelt, Wakasagi are typically found in freshwater areas in the San Francisco Estuary with the potential to move downstream during wet years. The CDFW does not calculate an index value for Wakasagi therefore we only report the total catch from each CDFW survey below.

In 2019, Wakasagi (n=33) were caught by CDFW's long-term monitoring projects; 20-mm Survey (Table 1), STN (Table 2), and FMWT (Table 3). The majority of Wakasagi were caught as larvae (FL= 6–18 mm) by the 20-mm Survey (April–July). These included San Pablo Bay (n=1), the lower Napa River (n=1), Suisun Bay & Montezuma Slough (n=4), Cache Slough (n=2), the SDWSC (n=3), the lower Sacramento River (n=2), and the upper Sacramento River (n=8). Two Wakasagi were caught by STN, 1 in the SDWSC (June; FL 63 mm) and the other in Suisun Bay (July; FL 71 mm). All 10 Wakasagi caught by FMWT were caught in the SDWSC (Sept–Nov; FL 62–95 mm).

Although the Wakasagi has higher salinity and temperature tolerances than Delta Smelt, like the Delta Smelt it is not found in large abundances within the estuary (Swanson et al. 2000). Wakasagi distribution generally shifts downstream in years with high flow, which could indicate that individuals are being pushed out of their normal home ranges. Wakasagi are capable of hybridizing with Delta Smelt and hybrids of these species have been found in the Yolo Bypass and lower Sacramento River, where both species co-occur (Benjamin et al. 2018). During wet years, the areas where Wakasagi and Delta Smelt co-occur increases, and possibly, so does the chance of hybridization. Increased hybridization of these species could have implications for accurate species identification and management of Delta Smelt.

Table 1. Wakasagi regional catch in the 20-mm Survey from 1995-2019. Stations in the upper Sacramento River region were added to the survey in 2008. Regions with no Wakasagi catch during the 1995 to 2019 period are not shown.

<i>Year</i>	<i>San Pablo Bay</i>	<i>Napa River</i>	<i>Suisun Bay</i>	<i>Confluence</i>	<i>Lower Sac River</i>	<i>Cache Slough</i>	<i>SDWSC</i>	<i>Upper Sac River</i>	<i>South Delta</i>	<i>Lower San Joaquin River</i>
1995	1	0	0	0	1	0	0	no sample	0	0
1996	1	2	6	1	3	0	0	no sample	1	1
1997	0	0	2	1	0	1	0	no sample	0	0
1998	0	0	0	0	0	2	0	no sample	0	0
1999	0	0	1	4	8	0	5	no sample	0	5
2000	0	0	23	5	22	7	4	no sample	2	3
2001	0	0	0	0	0	2	0	no sample	0	0
2002	0	0	0	0	2	0	0	no sample	3	0
2003	0	0	2	0	0	0	1	no sample	0	0
2004	0	0	1	0	4	0	0	no sample	1	0
2005	0	0	2	0	1	0	3	no sample	0	0
2006	1	2	2	1	0	0	0	no sample	0	1
2007	0	0	0	1	0	0	1	no sample	0	0
2008	0	0	0	0	0	0	3	3	0	0
2009	0	0	8	0	14	2	24	45	0	3
2010	0	0	0	0	0	0	2	2	0	1
2011	0	0	5	1	7	1	6	17	0	1
2012	0	0	0	1	4	0	2	14	0	1
2013	0	0	3	5	5	1	12	13	3	0
2014	0	0	0	0	0	0	0	1	0	0
2015	0	0	0	0	0	0	5	0	0	0
2016	0	0	2	1	1	7	5	18	0	0
2017	0	3	6	1	0	0	55	3	0	0
2018	0	0	3	5	3	2	0	1	1	2
2019	1	1	4	0	2	2	3	8	0	0

Conclusion

In 2019, annual abundance indices in all surveys increased modestly for most of the six pelagic fish species. The majority of the 5 surveys observed no change or decreases in the indices of native fishes, however surveys with gear that targeted littoral or demersal habitats (i.e., USFWS beach seine, SFBS otter trawl) observed increases in Splittail and Longfin Smelt indices. Non-native species, across all surveys, generally showed minor increases in abundance. Overall, all fish species examined here had relative abundance levels that are only a fraction of the abundance exhibited through the 1990s and into the early 2000s. The zero to low catches of Delta Smelt indicate that population size may be at or below the detection threshold for most life stages. Given that

abundance indices from these studies have specific management implications, index values of “0” have been and will continue to be problematic.

Table 2. Wakasagi regional catch from the Summer Townt Survey from 1995-2019. Stations in the Sacramento Deep Water Ship Channel (SDWSC) region were added in 2009. Regions with no Wakasagi catch during the 1995 to 2019 period are not shown.

Year	Lower Sac				South Delta	Lower San Joaquin River
	Suisun Bay	River	Cache Slough	SDWSC		
1995	0	1	no sample	no sample	0	0
1996	0	1	no sample	no sample	1	0
1997	0	0	no sample	no sample	0	0
1998	2	0	no sample	no sample	0	0
1999	0	0	no sample	no sample	0	0
2000	0	1	no sample	no sample	1	0
2001	0	0	no sample	no sample	0	0
2002	0	0	no sample	no sample	0	0
2003	0	0	no sample	no sample	0	0
2004	0	0	no sample	no sample	0	0
2005	0	0	no sample	no sample	0	0
2006	0	0	no sample	no sample	0	0
2007	0	0	no sample	no sample	0	0
2008	0	0	no sample	no sample	0	0
2009	4	1	no sample	no sample	0	0
2010	0	0	no sample	no sample	0	0
2011	2	0	4	9	0	0
2012	0	0	0	4	0	1
2013	0	2	0	8	0	0
2014	0	0	0	1	0	0
2015	0	0	0	0	0	0
2016	0	0	0	0	0	0
2017	0	0	0	1	0	0
2018	1	0	0	1	0	0
2019	1	0	0	1	0	0

References

- Baxter R, Hieb K, Delebn S, Fleming K, Orsi J, Orsi J. 1999. Report on the 1980-1995 Fish, Shrimp, and Crab Sampling in the San Francisco Estuary, California. Stockton.
- Benjamin A, Saçlam İK, Mahardja B, Hobbs J, Hung TC, Finger AJ. 2018. Use of single nucleotide polymorphisms identifies backcrossing and species misidentifications among three San Francisco estuary osmerids. *Conserv Genet.* 19(3):701–712. doi:10.1007/s10592-018-1048-9. <http://dx.doi.org/10.1007/s10592-018-1048-9>.
- Bennett WA. 2005. Critical Assessment of the Delta Smelt Population in the San Francisco Estuary, California. *San Fr Estuary Watershed Sci.* 3(2). doi:10.15447/sfews.2005v3iss2art1.
- Brandes PL, McLain JS. 2001. Juvenile Chinook Salmon Abundance, Distribution, and Survival in the Sacramento-San Joaquin Estuary. In: *Contributions to the Biology of Central Valley Salmonids*.
- Chadwick HK. 1964. Annual abundance of young striped bass, *Roccus saxatilis*, in the Sacramento-San Joaquin Delta, California. *Calif Fish Game.* 50(2)(September):69–99.
- Damon L, Baxter RD, Fujimura R. 2017. Fecundity and reproductive potential of wild female Delta Smelt in the upper San Francisco Estuary, California. *Calif Fish Game.* 102(4):188–210.
- Dege M, Brown LR. 2004. Effect of outflow on spring and summertime distribution and abundance of larval and juvenile fishes in the upper San Francisco Estuary. *Am Fish Soc Symp.* 2004(39):49–65.
- Dill W a., Cordone a J. 1997. History and Status of Introduced Fishes in California, 1871-1996: Conclusions. *Fisheries.* 22(10):15–18.
- Feyrer F, Hobbs J, Acuna S, Mahardja B, Grimaldo L, Baerwald M, Johnson RC, Teh S. 2015. Metapopulation structure of a semi-anadromous fish in a dynamic environment. *Can J Fish Aquat Sci.* 72(5):709–721. doi:10.1139/cjfas-2014-0433.

Table 3. Wakasagi regional catch from the Fall Midwater Trawl Survey from 1995-2019. Stations in the Sacramento Deep Water Ship Channel (SDWSC) region were added in 2009. Regions with no Wakasagi catch during the 1995 to 2019 period are not shown.

Year	Suisun Bay	Lower Sac River	Cache Slough	SDWSC
1995	0	3	0	no sample
1996	1	0	0	no sample
1997	1	0	0	no sample
1998	0	0	0	no sample
1999	0	0	0	no sample
2000	0	0	3	no sample
2001	0W	1	0	no sample
2002	0	0	0	no sample
2003	0	0	0	no sample
2004	0	0	0	no sample
2005	0	0	0	no sample
2006	0	0	0	no sample
2007	0	0	0	no sample
2008	0	0	0	no sample
2009	1	0	0	8
2010	0	0	1	8
2011	4	0	3	9
2012	0	0	0	1
2013	0	0	0	2
2014	0	0	0	2
2015	0	0	0	5
2016	0	1	0	6
2017	0	0	0	5
2018	0	0	0	9
2019	0	0	0	10

Hieb K, Bautista J, Giannetta J. 2019. Bay Study Fishes Status and Trends Report for the San Francisco Estuary. IEP Newsl. 31(2):3–29.

Hieb K, Bryant ME, Dege M, Greiner T, Souza K, Slater SB. 2005. Fishes in the San Francisco Estuary, 2004 Status and Trends. IEP Newsl. 18(2):19–36.

Holanov SH, Tash JC. 1978. Particulate and filter feeding in threadfin shad, *Dorosoma petenense*, at different light intensities. J Fish Biol. doi:10.1111/j.1095-8649.1978.tb03475.x.

Honey K, Baxter R, Hymanson Z, Sommer T, Gingras M, Cadrett P. 2004. IEP Long-term Fish Monitoring Program Element Review. :302. www.iep.water.ca.gov/.

Moyle PB, Baxter R, Sommer T, Foin T, Matern S. 2004. Biology and Population Dynamics of Sacramento Splittail (*Pogonichthys macrolepidotus*) in the San Francisco Estuary: A Review. San Fr Estuary Watershed Sci. doi:10.15447/sfews.2004v2iss2art3.

Moyle PB, Herbold B, Stevens DE, Miller LW. 1991. Life history and status of delta smelt in the sacramento-san joaquin estuary, california. Trans Am Fish Soc. 121(1):67–77. doi:10.1080/1548-8659(1992)121[0067:LHASOD]2.3.CO;2.

Moyle PB. 2002. Inland Fishes of California. Revised and Expanded Edition. Berkeley: University of California Press.

Sommer T, Baxter R, Herbold B. 1997. Resilience of Splittail in the Sacramento–San Joaquin Estuary. Trans Am Fish Soc. 126(6):961–976. doi:10.1577/1548-8659(1997)126<0961:rosits>2.3.co;2.

Sommer T, Mejia F, Hieb K, Baxter R, Loboschefskey E, Loge F. 2011. Long-term shifts in the lateral distribution of age-0 striped bass in the San Francisco estuary. Trans Am Fish Soc. 140(6):1451–1459. doi:10.1080/00028487.2011.630280.

Stevens D. 1966. Ecological Studies of the Sacramento-San Joaquin Delta. Part II Report No: 136. Distribution and food habits of the American shad (*Alosa sapidissima*) in the Sacramento-San Joaquin Delta.

Stevens DE, Kohlhorst DW, Miller LW, Kelley DW. 1985. The decline of striped bass in the Sacramento-San Joaquin Estuary, California. Transactions of the American Fisheries Society. 114:12-30.

Stevens DE. 1977. Striped bass (*Morone saxatilis*) year class strength San Joaquin estuary, California. Trans Am Fish Soc. 106:34–42.

Swanson C, Reid T, Young PS, Cech JJ. 2000. Comparative Environmental tolerances of threatened delta smelt (*Hypomesus transpacificus*) and introduced wakasagi (*H. nipponensis*) in an altered California estuary. Oecologia. 123:384–390.

Turner JL, Chadwick HK. 1972. Distribution and Abundance of Young-of-the-Year Striped Sacramento . San Joaquin Estuary. Trans Am Fish Soc. 3:442–452.

Walburg CH, Nichols PR. 1967. Biology and management of the American shad and status of the fisheries, Atlantic coast of the United States, 1960. USFWS Spec Sci Rep -- Fish. 550(550):105 p.

Wang JCS. 1986. Fishes of the Sacramento-San Joaquin Estuary and adjacent waters, California: A guide to the early life histories.

CONTRIBUTED PAPERS

Exploring Secondary Field Identification of Delta Smelt and Wakasagi Using Image Software

Jeff Jenkins (CDWR), Jeff.Jenkins@water.ca.gov; Naoaki Ikemiyagi (CDWR), Naoaki.Ikemiyagi@water.ca.gov; Brian Schreier (CDWR), Brian.Schreier@water.ca.gov; and Brittany Davis, Brittany.E.Davis@water.ca.gov

Introduction

The endangered Delta Smelt, *Hypomesus transpacificus*, is a small, pelagic fish endemic to the San Francisco Estuary (SFE). Once widespread and common throughout the upper SFE, Delta Smelt have precipitously declined over the last 20 years (Moyle et al. 2018). The species was State and federally listed as threatened in 1993 (USFWS 1993) and relisted by the State as endangered in 2009 (CDFG 2010). However, despite listing and conservation efforts, the species is increasingly rare, and even targeted monitoring efforts seldomly detect them (Moyle et al. 2016; 2018).

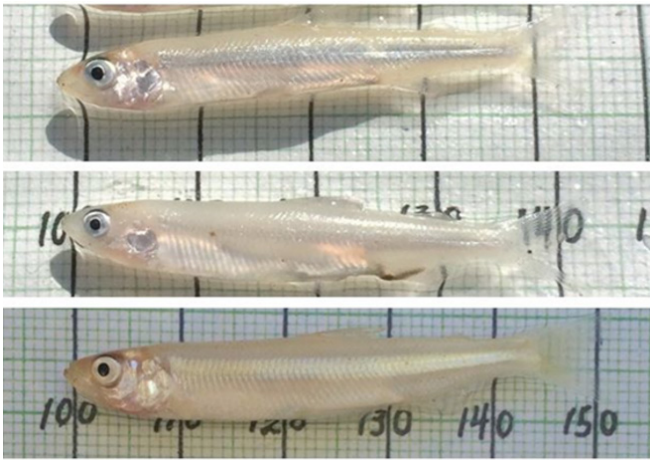
One problem monitoring surveys can encounter in the field is the inability to distinguish Delta Smelt from the non-native congener Wakasagi (*Hypomesus nipponensis*; Japanese Pond Smelt) due to shared

morphological traits, leading to misidentification. Both osmerid species have similar fin ray counts, dorsal pigmentation, and chromatophores on the isthmus (Figure 1; Sweetnam 1995). Additionally, Delta Smelt and Wakasagi can hybridize, further complicating species identification (Figure 1; Trenham et al. 1998; Benjamin et al. 2018). Proper identification is critically important to State and federal agencies tasked with monitoring fish populations in the Delta, as well as to other researchers conducting special studies that may have an impact on Delta Smelt management for three reasons. (1) Scientific sampling permits have strict regulations on the take of Delta Smelt. (2) State and federal water project operations can be affected by the presence of Delta Smelt when individuals are detected in specific Delta regions or entrained at fish salvage facilities. Smelt appearing at the fish salvage facilities causes significant reductions in the volume of water conveyed to Central Valley farms, southern California cities, and the San Francisco Bay Area. (3) New regulatory considerations in Biological Opinions (USFWS 2019) and Incidental Take Permits (CDFW 2020) include the supplementation of the wild Delta Smelt population using cultured fish (USFWS 2019). One concern with this approach is how to accurately distinguish hatchery from wild fish after release, and this problem may be more complex if Wakasagi are found in the same areas. Genetic testing to confirm species identity is reliable (Baerwald et al. 2011; Benjamin et al. 2018) but involves retrieving a physical sample from the fish and processing it in a laboratory – requiring additional handling of individual fish (causing increased stress on the fish), time, and cost for species identification results.

Table 1. Chromatophore markings of genetically confirmed osmerids from the Yolo Bypass Fish Monitoring Program. Isthmus chromatophores (n=218) and the "V" pattern on the caudal fin (n=139) are shown. Hybrid F1 represents an offspring from a Delta Smelt (D) x Wakasagi (W) cross, whereas Hybrid BC is a backcross offspring from a Wakasagi (W) x Hybrid (DW - Delta Smelt x Wakasagi). Several Wakasagi demonstrated atypical chromatophore patterns leading to field misidentification.

Genetic ID	Number of Isthmus Chromatophores						Caudal "V" Pattern	
	0	1	2	3	4	5+	Yes	No
Delta Smelt	47	18	-	1	-	1	13	-
Wakasagi	6	10	8	25	22	74	10	113
F1 (D x W)	1	1	2	-	-	1	1	1
BC (W x DW)	-	-	-	1	-	-	-	1

Figure 1. Morphological comparison of a Wakasagi, Delta Smelt, and hybrid (Wakasagi x Delta Smelt), top to bottom, respectively.



The goal of this study was to determine if high-resolution imagery of additional morphological traits could be used as a complimentary tool for species identification following field determination using chromatophore patterns. We predicted there would be consistent morphometrics that would help to correctly distinguish Delta Smelt and Wakasagi, but that would be the same for hatchery and wild-caught Delta Smelt. Using standardized photos of genetically confirmed fish, a blind analysis of a series of morphometrics was conducted. Aside from increasing accuracy of identification, utilizing imaging for identification could also help reduce unnecessary handling and stress on Delta Smelt.

Methods

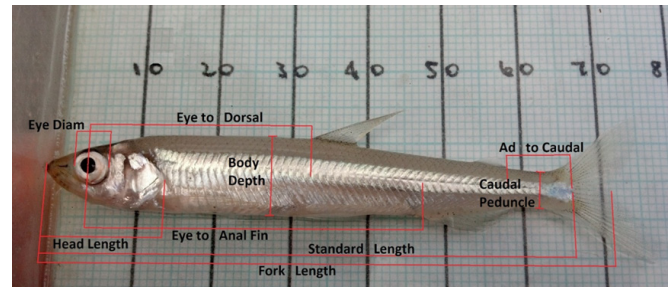
We obtained osmerid photos of fish collected by the Yolo Bypass Fish Monitoring Program during sampling from 2010 to 2015. Each individual was identified genetically by the University of California Davis (UCD) Genomic Variation Laboratory as described in Benjamin et al. (2018). We also acquired osmerid photos taken during Bay-Tributary Surveys conducted by the UCD Hobbs Laboratory, and images of hatchery Delta Smelt from the UCD Fish Culture and Conservation Laboratory (Davis et al. 2019). We only included photos taken directly above the fish laying on a standardized millimeter scale to ensure the calibration of each photo for comparisons. One experimenter cropped any tag or species identification marker from each photo and assigned it a random number. For images with multiple individuals, the same experimenter cropped each fish

into a single image and assigned a random number. The resulting 33 images were then assessed by a second, independent experimenter with no knowledge of the corresponding field or genetic assignment (blind experimenter).

We analyzed individual fish photos using ImageJ software (version 1.52d), an open source image-processing tool capable, for example, of contrast sharpening and manipulation, and measuring distances and angles (<https://imagej.nih.gov/ij/>). The blind experimenter measured nine traditional morphometric traits and 12 morphometric ratios (similar to Keenlyne et al. 1994) that were converted to a percentage (Table 2; Figure 2). We calibrated each photo to a known standard (in millimeters) included in the field photo prior to ImageJ measurements. We then recorded measurements of each morphometric distance three times to reduce error, after which the mean distance and coefficient of variation (CV) were calculated. If the CV was greater than 1, we discarded the values and the measurements were repeated. Next, we used the measurements from each fish in 12 ratio combinations for comparisons (Table 2). After the morphometric data were compiled, we distinguished life stage by assigning adults as 60 mm and greater (n=11) and juveniles between 25 and 59 mm (n=22); however, due to unequal sample sizes between life stages and species, we conducted analyses on all sizes combined.

We used R software (v. 3.5.1) for statistical analyses. First, we analyzed data for parametric assumption of normality and homogeneity of variances of residuals using visual inspections of Quantile-Quantile (Q-Q) plots, followed by Shapiro-Wilks and Levene’s test for confirmation. We initially assessed morphometric

Figure 2. Measurements taken during blind analysis of osmerids’ morphometrics.



ratios for correlations (R package corrplot; Wei et al. 2017) and plotted ratios with a Principle Component Analysis (PCA) having up to 12 repeated morphometrics for each individual. We then only analyzed three morphometric ratios (with low correlation) for species differences, including 1) body width: fork length, 2) eye: head length, and 3) caudal peduncle: fork length. Due to large differences in sample size (wild Delta Smelt = 7, Hatchery Delta Smelt = 7, Hybrid=3, and Wakasagi=19), we conducted an initial multivariate analysis of variance (MANOVA) on wild and hatchery Delta Smelt metrics to

determine if all Delta Smelt could be pooled into a single group (n=14). We conducted a subsequent MANOVA to determine if morphometric ratios differed between Delta Smelt and Wakasagi. To determine which of the three ratios differed between species, we assessed univariate statistics by running a summary function of the MANOVA model (summary.aov), and we adjusted the alpha value for significance to $p < 0.01$ to correct for multiple tests. Hybrids were not included in the statistical analysis but we plotted them for descriptive comparisons of traits between Delta Smelt and Wakasagi.

Table 2. Morphometric measurements of lengths (mm) and ratios (%) examined for each fish.

<i>Morphometric (mm)</i>	<i>Description</i>
Eye Diameter	Edge to edge distance of eye
Head Length	Tip of snout to max edge of operculum
Eye to Dorsal	Center of eye to vertical line where dorsal fin begins on center of bodyline
Eye to Anal fin	Center of eye to vertical line where anal fin begins on center of bodyline
Adipose to Caudal	Rear edge of adipose fin to beginning edge of caudal fin
Caudal Peduncle	Narrowest dorsoventral distance of body at caudal peduncle
Body Width	Widest dorsoventral distance of body near the leading edge of the dorsal fin
Standard Length	Tip of snout to beginning of caudal fin
Fork Length	Tip of snout to inner-most edge of caudal fork
<i>Ratio (%)</i>	<i>Description</i>
Eye:Head	Eye diameter per Head length
Eye:SL	Eye diameter per Standard length
Eye:FL	Eye diameter per Fork length
Eye:Peduncle	Eye diameter per Peduncle
Body Width:SL	Body width per Standard length
Body Width:FL	Body width per Fork length
Head:FL	Head length per Fork length
Eye-Dors:FL	Eye to Dorsal distance per Fork length
Eye-Anal:FL	Eye to Anal fin distance per Fork length
Ad-Caud:FL	Adipose to Caudal distance per Fork length
CaudPed:FL	Caudal Peduncle distance per Fork length
CaudPed:BodyWid	Caudal Peduncle distance per Body width

Results

The PCA of morphometric ratios showed similarities (overlapping vectors) between measures with a common morphometric (i.e. body part), forming three general clusters of the 12 measurements. Only one metric from each cluster (orthogonal vectors with low correlation) was analyzed (described in the methods). A summary of multivariate statistical results is provided in Table 3. Multivariate results for morphometric ratios between Wakasagi and Delta Smelt significantly differed ($p < 0.001$; Table 3); however, only the body width: fork length showed differences between species (Figure 3). Ratios of caudal peduncle: fork length and size of the eye: head length did not show significant differences. Mean (\pm SD) percent body width: fork length of Delta Smelt was 16.2 ± 1.18 , while Wakasagi was 14.3 ± 1.4 , and hybrids fell in the middle at 15.0 ± 0.8 . In general, Delta Smelt were observed having deeper

Figure 3. Body depth versus fork length by species (DSM=Delta Smelt, HYB=Delta Smelt x Wakasagi hybrid, WAG=Wakasagi).

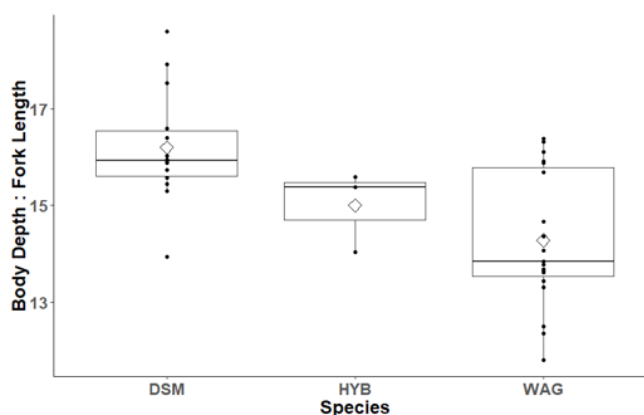


Table 3. Summary of statistical tests for morphometric traits of osmerid species. Multivariate analyses of variance (MANOVA) were conducted for wild and hatchery Delta Smelt, followed by Delta Smelt and Wakasagi for three morphometric ratios (repeated). Pillai's Trace value (ranging from 0-1) is provided in the multivariate output; the associated F test statistic (F), degrees of freedom (Df) and significant p-value (P) are provided for both multivariate and univariate tests. Results were significant at p<0.01.

MANOVA	Df	Pillai's	F	P
Species (wild v. hatchery Delta Smelt)	1,11	0.419	2.17	0.162
Species (Delta Smelt v. Wakasagi)	1,30	0.438	7.282	< 0.001*

Repeated Measures	% Body Width: FL			% Eye: Head Length			% Caudal Peduncle: FL		
	ANOVA	Df	F	P	Df	F	P	Df	F
Species (wild v. hatchery Delta Smelt)	1,11	1.712	0.2174	1,11	1.401	0.262	1,11	6.546	0.027
Species (Delta Smelt v. Wakasagi)	1,30	16.819	<0.001*	1,30	0.001	0.976	1,30	0.084	0.774

bodies than Wakasagi. Our analysis suggests that as Delta Smelt grow, the body depth: fork length ratio increases, however, the differences between Delta Smelt and Wakasagi found in this study still exist across various lengths. Supplemental information and figures including PCA, correlations, and life-stages can be requested from the authors.

Discussion

Considering the tightening restrictions on Delta Smelt take and given that field identification of osmerids can be challenging, we analyzed morphometric parameters of Delta Smelt and Wakasagi to determine if consistencies existed between the two species to improve accuracy of field identification techniques for these fish. Chromatophore count on the isthmus remains the most common method of differentiating these osmerids; however, increased evidence of morphological and phenotypical similarities, possible future release of hatchery Delta Smelt into the Delta, and continued hybridization may erode the reliability of this method (Table 3).

Strategies for the supplementation of wild Delta Smelt populations using hatchery fish are currently being considered by regulatory agencies to assist in species recovery. Releasing hatchery Delta Smelt into the wild comes with many concerns including potential influences on genetic diversity, promulgating domestication affects, and introducing disease or pathogens (Lessard et al. 2018). Additionally, Wang (1995) found that both Delta Smelt and Wakasagi

demonstrate a range of ecophenotypes depending on where they originate. Wakasagi from reservoirs and lakes may have darker pigmentation characteristics and isthmus chromatophores than those collected in the Delta. Conversely, some lab or hatchery-raised Delta Smelt and Wakasagi may lack isthmus pigmentation altogether (Wang 1995), although this may be an artifact of their rearing tanks (Figure 4). Hatchery Delta Smelt may also develop isthmus pigmentation at earlier life stages than wild Delta Smelt (Wang et al. 2005). In addition to the concerns identified by Lessard et al. (2018) and Wang et al. (2005), releasing hatchery Delta Smelt into the wild may result in morphological changes not yet encountered, making field identification increasingly difficult. While phenotypic traits may vary or stray, this study showed that wild and hatchery Delta Smelt are morphologically indistinguishable using the metrics described here.

Recent advances in genetic testing techniques could provide a means for genetic verification of species in the field. Baerwald et al. (2020) found Delta Smelt, Wakasagi, and Longfin Smelt (*Spirinchus thaleichthys*) could be positively identified to species with simple mucus swabs using the CRISPR-based SHERLOCK technology; providing results in as little as 20 to 30 minutes. This method eliminates taking a fin clip from the fish, laboratory processing, and days or weeks waiting for testing results.

The field-based SHERLOCK testing process is being refined but field identification using image software may still have an application for smaller-scale investigations, budget-limited projects, or studies which may only encounter Delta Smelt by coincidence.

Many researchers conduct studies in the SFE, collecting data to estimate population abundances for multiple fish species, including Delta Smelt. Policy decisions based on the correct identification of fish in the field impact water pumping operations, agriculture, habitat restoration, commercial and recreational fisheries, urban development, industry, and the ecosystem's various organisms (Benjamin et al. 2018). Although we had a small sample size of field photos (with genetic identifications), including hybrids and captive-reared Delta Smelt, significant morphological differences were still discovered. We acknowledge results may evolve with a larger sample size, but our data suggest field identification of these species can be additionally reinforced by using imaging protocols described in the current study. Coupled with more

Figure 4. Wild Delta Smelt (top two) versus hatchery-reared Delta Smelt (bottom two).



widespread use of genetic species identification for all osmerids (whether laboratory-based or field-based techniques as described in Baerwald et al. 2020), image analysis can help provide more accurate reporting on, and understanding of osmerids.

References

- Baerwald MR, Schumer G, Schreier BM, May B. 2011. TaqMan assays for the genetic identification of delta smelt (*Hypomesus transpacificus*) and wakasagi smelt (*Hypomesus nipponensis*). *Molecular Ecology Resources* 11-5: 784-785.
- Baerwald MR, Goodbla AM, Nagarajan RP, Gootenberg JS, Abudayyeh, OO, Zhang F, Schreier AD. 2020. Rapid and accurate species identification for ecological studies and monitoring using CRISPR-based SHERLOCK. *Molecular Ecology Resources* doi:10.1111/1755-0998.13186
- Benjamin A., Saglam IK, Mahardja B., Hobbs J, Hung T, Finger A. 2018. Use of single nucleotide polymorphisms identifies backcrossing and species misidentifications among three San Francisco estuary osmerids. *Conservation Genetics* 19: 701-712.
- Castillo GC, Sandford ME, Hung TC, Tigan G, Lindberg JC, Yang WR, Van Nieuwenhuysse EE. 2018. Using natural marks to identify individual cultured adult delta smelt. *North American Journal of Fisheries Management*, 38: 698-705.
- CDFG. 2010. State and Federally listed endangered and threatened animals of California. 771 California Department of Fish and Game, The Natural Resources Agency, North Highlands.
- CDFW. 2020. Incidental Take Permit for Long-Term Operation of the State Water Project in the Sacramento-San Joaquin Delta (2081-2019-066-00). California Department of Fish and Wildlife to the California Department of Water Resources, Sacramento, CA.
- Davis BE, Cocherell DE, Sommer T, Baxter RD, Hung TC, Todgham AE, Fangue NA. 2019. Sensitivities of an endemic, endangered California smelt and two non-native fishes to serial increases in temperature and salinity: implications for shifting community structure with climate change. *Conservation physiology*. 7(1): coy076.
- Keenlyne KD, Henry CJ, Tews A, Clancey P. 1994. Morphometric comparisons of upper Missouri River sturgeons. *Transactions of the American Fisheries Society*. 123(5):779-85.
- Lessard J, Cavallo B, Anders P, Sommer T, Schreier B, Gille D, Schreier A, Finger A, Hung T, Hobbs J, May B, Shultz A, Burgess O, Clarke R. 2018. Considerations for the Use of Captive-Reared Delta Smelt for Species Recovery and Research. *San Francisco Estuary & Watershed Science*. 16-3. Article 3.
- Moyle PB, Hobbs JA, Durand JR. 2018. Delta Smelt and Water politics in California. *Fisheries*. 43-1: 42-50.

- Moyle PB, Brown LR, Durand JR, Hobbs JA. 2016. Delta Smelt: Life history and decline of a once-abundant species in the San Francisco estuary. *San Francisco Estuary and Watershed Science*, 14(2), Article 7.
- Sweetnam D. 1995. Field Identification of Delta Smelt and Wakasagi. Interagency Ecological Program for the Sacramento-San Joaquin Estuary. Spring Newsletter:1-4.
- Trenham PC, Shaffer HB, Moyle PB. 1998. Biochemical identification and assessment of population subdivision in morphologically similar native and invading smelt species (*Hypomesus*) in the Sacramento–San Joaquin estuary, California. *Transactions of the American Fisheries Society*. 127(3): 417-24.
- USFWS. 1993. Final rule: endangered and threatened wildlife and plants; determination of threatened status for the Delta smelt. United States Fish and Wildlife Service. Fed Regist 58: 12854–12864.
- USFWS. 2010. Endangered and threatened wildlife and plants; 12-month finding on a petition to reclassify the Delta Smelt from threatened to endangered throughout its range. United States Fish and Wildlife Service. Federal Register 75: 17667–17680.
- USFWS. 2019. Biological Opinion for the Reinitiation of Consultation of the Coordinated Operations of the Central Valley Project and State Water Project. U.S. Fish and Wildlife Service, Sacramento, California.
- Wang J. 1995. Observation of Possible Larval and Prejuvenile and Wakasagi/Delta Smelt Hybrids in the Delta and Tributary Streams. Interagency Ecological Program for the Sacramento-San Joaquin Estuary. Summer Newsletter:18.
- Wang JCS, Lynch L, Bridges B, Grimaldo L. 2005. Using Morphometric Characteristics to Identify the Early Life Stages of Two Sympatric Osmerids (Delta Smelt and Wakasagi – *Hypomesus transpacificus* and *Hypomesus nipponensis*) in the Sacramento-San Joaquin Delta, California. Bureau of Reclamation, mid-Pacific Regional Office, Tracy Fish Collection Facility Studies Report 30:1-46.
- Wei T, Simko V, Levy M, Xie Y, Jin Y, Zemla J. 2017. Package 'corrplot'. *Statistician*, 56, 316–324.

IEP NEWSLETTER

3500 Industrial Blvd.
West Sacramento, CA 95691



For information about the Interagency Ecological Program, log on to our Web site at <https://water.ca.gov/Programs/Environmental-Services/Interagency-Ecological-Program>. Readers are encouraged to submit brief articles or ideas for articles. Questions and submissions can be sent by e-mail to: IEPNewsletter@water.ca.gov.

IEP NEWSLETTER

Annika Keeley, Delta Stewardship Council, Lead Editor
Sarah Lesmeister, California Department of Water Resources, Managing Editor

The Interagency Ecological Program for the San Francisco Estuary
is a cooperative effort of the following agencies:

*California Department of Water Resources
State Water Resources Control Board
U.S. Bureau of Reclamation
U.S. Army Corps of Engineers*

*California Department of Fish and Wildlife
U.S. Fish and Wildlife Service
U.S. Geological Survey
U.S. Environmental Protection Agency
National Marine Fisheries Service*

BEFORE CITING INFORMATION HEREIN,
CONSIDER THAT ARTICLES HAVE NOT RECEIVED PEER REVIEW.