# Monitoring Responses of the Delta Smelt Population to Multiple Restoration Actions in the San Francisco Estuary

**FINAL REPORT**

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# Maternal and Environmental Influences on Egg and Larval Size, Viability, and Survival of the Endangered Delta Smelt, *Hypomesus transpacificus*.

## INTRODUCTION

Variability among females in life history traits has important implications for managing fisheries and conserving threatened species. This inherent variation and interactions with environmental conditions can profoundly influence the phenotypes and probability of survival for progeny, and may contribute to wide fluctuations in abundance and the population dynamics of a variety of organisms, including fish (Bernardo 1996, Green 2008). Maternal effects associated with variation in size and age of females impart relatively small differences in timing, number, and size of spawned clutches, egg size, development time, larval hatch size and yolk volume, as well as growth rate, resistance to food shortages, and survival (Chambers and Leggett 1996, Marteeinsdottir and Steinarsson 1998, Benoit and Pepin 1999). In turn, this variation can dramatically influence the patterns and strengths of selective mortality on larvae and juveniles, which underlie annual year-class success, population resilience, and dynamics (Houde 1987, Sogard 1997, Green and McCormick 2005, Wright and Gibb 2005).

Although selective mortality is commonly associated with higher survival of larvae and juveniles of larger size and/or faster growth (Houde 1987, Sogard 1997), the advantage of such traits can vary widely as environmental conditions change (Roff 1992). Life history theory predicts a shift toward fewer, larger, and slower growing offspring when environmental conditions are harsh (Smith and Fretwell 1974, Roff 1992, Johnston and Leggett 2002). As a result, many species exhibit bet-hedging strategies in which mothers provision reproductive investment to offspring during the spawning season to increase the likelihood that the numbers and characteristics of larvae produced encounters favorable conditions as the environment changes (Cargnelli and Gross 1996, Conover et al. 2003). Understanding the extent and importance of such variability is especially critical when human activities, e.g. fisheries, selectively remove individuals favored by the prevailing natural selective forces, thus imposing unnatural selection on populations (Darimont et al. 2009).

Here, we report on a series of studies examining the potential for maternal influences on offspring variability for the endangered delta smelt (*Hypomesus transpacificus*). This small (60-90mm), primarily annual, pelagic fish is endemic to the northern San Francisco Estuary (SFE) and currently protected under the State and Federal Endangered Species Acts. Given that delta smelt occur exclusively within the northern SFE, a central hub in California's freshwater supply system, the species has become a major concern of environmental and water management in the

western U.S. Currently, restrictions on operations by the major state and federal water export facilities to protect delta smelt interfere with freshwater allocations throughout California (Sommer et al. 2007). However, relative to other species, key gaps remain in the knowledge of delta smelt life history and factors regulating their abundance, which has limited the effectiveness of restoration efforts (Bennett 2005). In particular, little is known about the ecology of delta smelt spawning, reproductive success, and the mechanisms of selective mortality that influence annual year-class success and abundance (Bennett 2005, Bennett et al. 2008). A better understanding of the relative importance and interactive nature of processes influencing individual variability can help to distinguish among the various factors limiting abundance, and especially the highly controversial role of entrainment mortality in causing the decline of delta smelt (Bennett and Moyle 1996).

## METHODS

### Egg & Clutch Sizes

We used field specimens from the IEP Spring Kodiak Trawl Survey (SKT) in 2003-2005. This program monitors the distribution of fish as they transition from maturing to spent condition in February to May since 2002.  All fish collected are given an individual code, measured, and identified to reproductive state using a qualitative grading-scheme developed by Mager et al. (2004). Then each fish was decapitated and the heads preserved in ETOH, with the remaining carcass fixed in a buffered formaldehyde solution. The results of each annual survey are then summarized and presented online (Sousa, 2002; CDFG, http://www.delta.dfg.ca.gov).

*Fecundity* - First, we examined the ovaries from 23 ripe (scoring 4 or 5) females collected on April 2 and April 28, 2003. Ovaries were examined, wet-weighed, and the eggs enumerated and graded into two categories, normal and small to develop a size-fecundity relationship for wild-caught specimens. Then, ovaries from an additional 106 of fish from March 18 and April 2, 2003 were subsequently weighed, and relative amount of lipid stores were graded visually on a scale of 0-4, with 0= low and 4= high lipid content in the body tissues. All body parts were then collectively weighed to get an approximate body weight. The wild-caught size-fecundity relationship was then used to estimate fecundity in the larger sample of ovaries, and compared with the lipid content index, as well as a simple condition factor, K = weight (gm) / fork length (mm). Finally, apparently eggs apparently maturing and immature in ovaries from five fish collected in 2003 were assessed for viability using histopathology.

*Egg Size* – Egg volumes were evaluated from 8 females from the 2003, and 4 from the 2005 SKT. We used oocytes, which are easier to determine developmental stage than mature eggs. Initially, oocytes from multiple females were assessed to find developing embryos at the same developmental stage. Female sizes ranged from 66-78mm FL in the 2003 samples, and 70-81mm FL from the 2005 SKT.  used oocytes (n=46-73) in the late vitellogenic stage. We also selected 2

relatively large females (80-81mm FL, collected 3/07/2005) and 2 small (70mm FL, collected 1/26/2005). Then, 46-73 oocytes (2003), and 40 (2005) from each female were randomly selected and the perimeter measured using digitized images; the diameter was then calculated by dividing that measure by 3.14 (*pi*).

### *Female Reproductive Potential & Maternal Effects*
Laboratory studies (i.e., bioassays) were conducted in each year 2007-2009 to investigate variation in (1) female reproductive potential and success (2007-2008), as well as (2) the expression of maternal effects during the early development of larvae (2008-2009). Each year's study design was generally similar, and relied on selecting the widest size range of 12 ripe female delta smelt available from the Fish Conservation and Culture Laboratory (FCCL), UC Davis, located near Byron, CA.

*2007* - The size range of mature females available from the FCCL was unfortunately quite narrow ranging only from 60-67mm FL. Nevertheless, strip-spawning of mature females occurred on 3/16 in collaboration with the FCCL, and the fertilized eggs were then transported to UCD and incubated in two treatments at $11^{o}$C (6 females) and $16^{o}$C (6 females).

Hatching occurred in the warm treatment ($16^{o}$C) from 3/24-3/26, and subsequently in the cool treatment from 4/1-4/3. Larval first-feeding with cultured rotifers, occurred in the warm treatment on 3/29, and on 4/9 in the cool treatment. The study was terminated in warm treatment from about 4/3-4/5, and then in the cool treatment on 4/9-4/11, because few larvae remained for daily sub-sampling, especially in the warm treatment. Throughout the study, daily egg and larval mortality rates were recorded.

*2008* – In contrast to the previous year, the size range of mature females (n=12) available from the FCCL ranged from 61-85mm FL. Strip spawning and fertilization occurred on 3/12/2008 with milt pooled from 12 males ranging in length from 61-76mm, and in body weight from 1.8-3.6g.

Following spawning, several characteristics were measured from each female, including body and liver weight, Fulton's body condition index (CI), hepatosomatic index (HIS), gonadosomatic index (GSI), fecundity, egg volume, as well as percent fertility and hatchability. Fertilized eggs were then transported to UCD and incubated at 15-16$^{o}$C until they hatched 11-13 days later, from 3/21-3/30/2008. On day 1ph, 50 larvae in each of three replicates were pooled into two size treatment groups from "small" (61-67mm) and "large" (78-85mm) females. Also, on day 1ph and on day 7ph, 50 larvae from each group were analyzed for RNA/DNA ratios using the ethidium bromide fluorometric method (Wagner et al. 1998). Following that, beginning on day 7ph, larvae in each treatment were subjected to increasing temperature from $16^{o}$C to $24^{o}$C at a rate of $15^{o}$C every 15 minutes. Temperatures were then maintained at $24^{o}$C for 4 hrs, and then the larvae in

each size treatment were analyzed for expression of heat shock proteins (HSP70) using the western blot technique. Egg and larval mortality were also recorded throughout the study.

*2009* - At FCCL on 2/19/2009, 12 females ranging from 50-71mm FL and weighing from 1.06-3.78g were strip-spawned and the eggs then fertilized with the milt from 12 males pooled as in previous years. The fertilized eggs were then transported to UCD and incubated in two temperature treatments, $16^{o}$C and $20^{o}$C. Hatching occurred on 2/27/2009 at $20^{o}$C and on 3/02/2009 at $16^{o}$C. Egg and larval mortality were recorded daily until day 11ph.

Four experiments were then conducted using day 1ph and day 4ph larvae at each temperature (16 and $20^{o}$C). In each study, 45 larvae (n=15 in each of 3 replicates) were pooled into two size treatment groups, from "small" (50-54mm) and "large" (55-71mm) females (as in 2008), and then deprived of food and exposed to the pesticide, permethrin, in the following manner. In the first experiment, 1dph larvae were initially exposed to permethrin for 4 days, and then deprived of food for another 4 days at $20^{o}$C. In the second study, 4 dph larvae were first deprived of food for 4 days followed by exposure to permethrin for 4 days at $20^{o}$C. The third and fourth study essentially duplicated the first two, except they were run at $16^{o}$C.
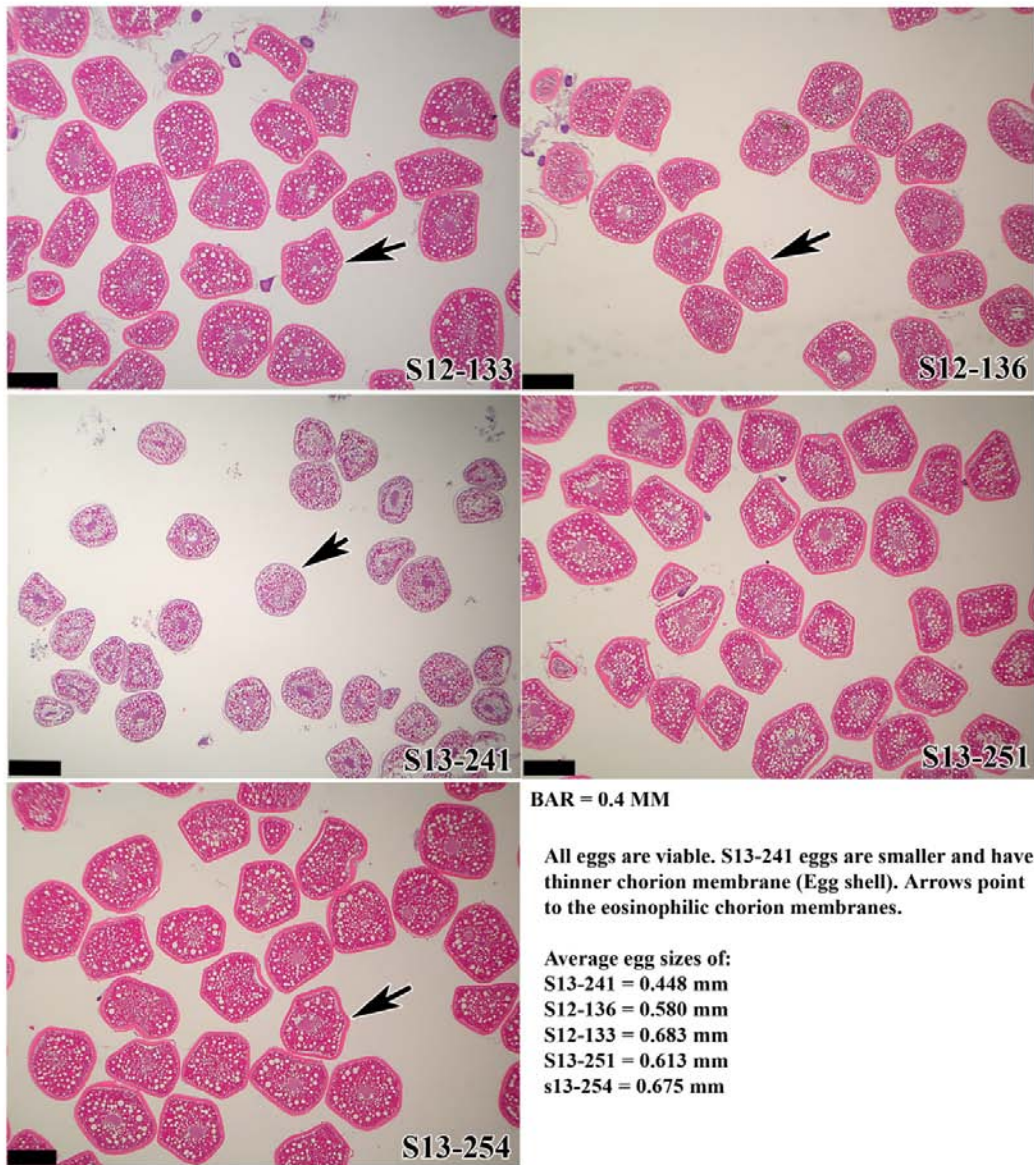
**RESULTS**

***Egg & Clutch Sizes***
*Fecundity* – Wild delta smelt exhibited a significant size-fecundity relationship (Figure 1A). This relationship developed by enumerating mature eggs from 23 fish caught in 2003 is similar to that exhibited by females held in aquaculture by B. Baskerville-Bridges in 2002 (Figure 1A, also see Bennett 2005). Overall, the numbers of immature eggs per female were highly variable but also significantly associated with female size (Figure 1B). Similarly, clutch weights were also associated with the numbers of maturing eggs (Figure 1C) as well as female size (Figure 1D). Histological evaluations indicated that both maturing and immature eggs were viable, however, in addition to the obvious size difference, immature eggs had much thinner chorion membranes (or, egg shells) (Figure 2). It is likely that these differences reflect a second batch of eggs to be spawned at a later time during the spawning season, as observed in aquaculture by the FCCL. Mature females used in subsequent studies from 2007-2008 (from the FCCL) also exhibited a significant relationship between egg number and female size (Figure 3).

*Egg Size* – Oocyte diameters increased with female size in samples collected in 2003 and 2005 (Figure 4). Pair-wise comparisons indicated that oocyte sizes were significantly larger in 78mm females relative to either 66mm ($t = -9.63$, $df = 120$, $P = 0$) or 70mm females ($t = -6.79$, $df = 119$, $P=0$) from 2003. Similarly, for 2005 samples, oocytes were significantly larger in 80mm versus 70mm females ($t = -9.85$, $df = 68$, $P = 0$).
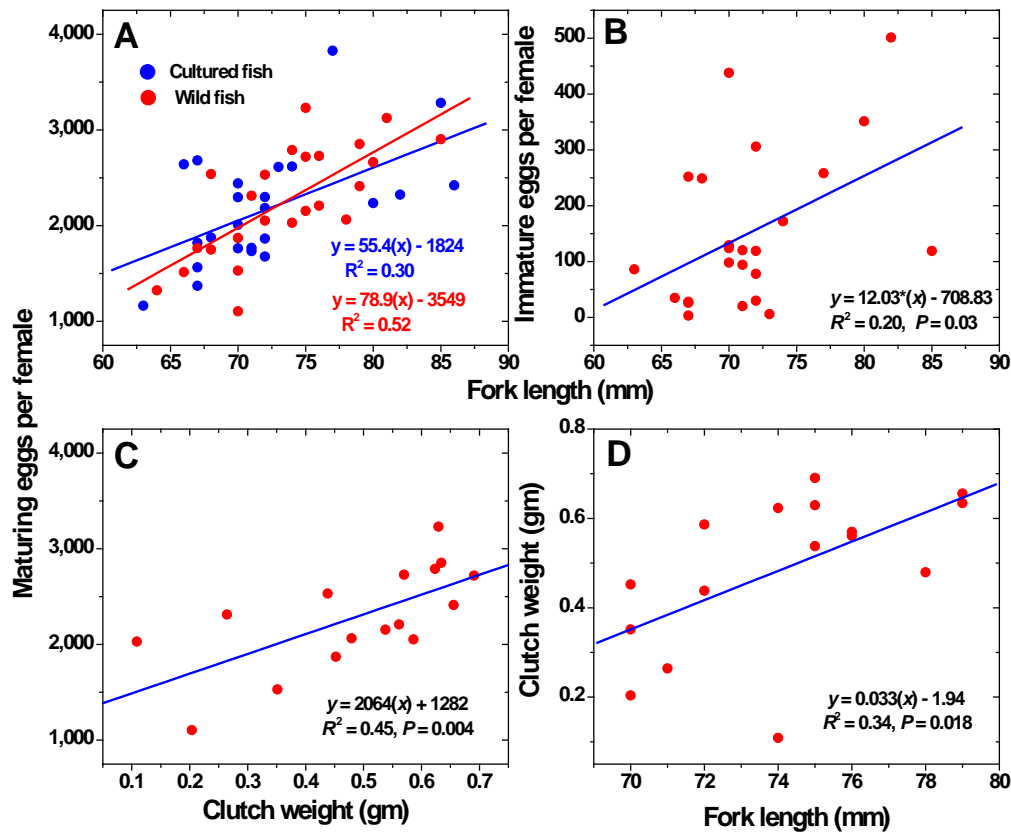
**Figure 1**. Delta smelt fork length and fecundity relationships. (A) Comparison
of  size-fecundity  relationships for wild females caught in 2003 with those from aquaculture in 2002 (see Bennett
2005). (B) Numbers of immature eggs with fork length of wild females. (C) Maturing egg numbers with weight of
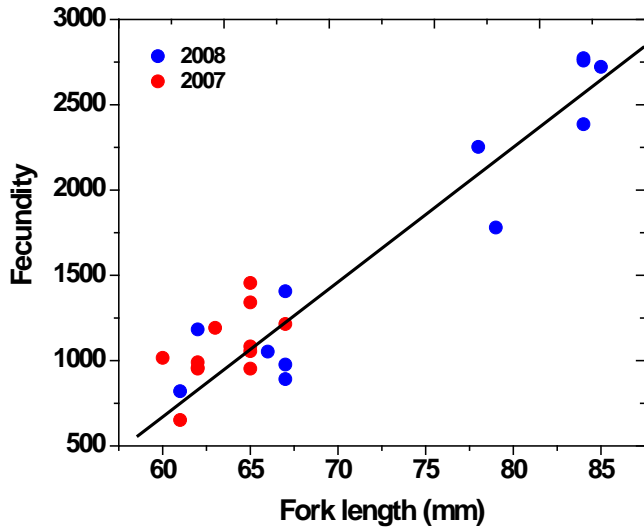ovaries (clutches). (D) Clutch weight with fork length of wild females.

BAR = 0.4 MM

All eggs are viable. S13-241 eggs are smaller and have thinner chorion membrane (Egg shell). Arrows point to the eosinophilic chorion membranes.

Average egg sizes of:
S13-241 = 0.448 mm
S12-136 = 0.580 mm
S12-133 = 0.683 mm
S13-251 = 0.613 mm
s13-254 = 0.675 mm

**Figure 2**. Histology of immature and maturing eggs from wild delta smelt. Immature eggs are smaller and have thinner chorion membranes (egg shells) than mature eggs, suggesting a second batch of eggs to be spawned later in the season.

*Female Reproductive Potential & Maternal Effects*

*2007* – The size range of ripe females available for the study was limited, ranging only 6mm (Figure 3). Fork lengths of these females were positively associated with the volume of eggs spawned at 16$^{o}$C, and negatively at 11$^{o}$C with a positive interaction with temperature (Figure 5A). A similar relationship with yolk volume was positive at 16$^{o}$C, and negative at 11$^{o}$C, with a positive interaction with temperature (Figure 5C). Development time of progeny, however, was not related to female size, but was clearly associated with water temperature (Figure 5B). Female size and water temperature also were not associated with oil volume, hatch length, or larval growth rate until first-feeding (Figure 5).

**Figure 3**. Size and fecundity relationship for delta smelt
from aquaculture used in laboratory studies in 2007-2008.

*2008* – Females spawned for the study ranged 24mm in fork length (Figure 3). Several female characteristics were associated with their fork length, including liver weight and gonadosomatic index, as well as the number and percent of eggs hatching (Figure 6). Fork lengths of mothers also were associated various characteristics of their eggs and larval progeny (Figure 7). Overall, female fork length was either negatively, or not, associated with egg and larval characters at hatch, including egg and yolk volume, hatch length, or oil volume (Figure 7A-D). By day 5 post-hatch, however, positive relationships were exhibited with larval length, and oil volume (Figure 7E, G); yolk volumes declined (Figure 7F), yet larval growth rates were not associated with size of mother (Figure 7H).

Overall, evaluations of RNA/DNA ratios and heat shock proteins indicated development rates and the ability to handle heat stress were similar between larvae of small versus large mothers (Figure 8). However, at day 1ph, larvae from large mothers exhibited much higher variability in RNA/DNA ratios, whereas at day 7ph larvae from small mothers were more variable (Figure 8A). Similarly, larvae from large females exhibited a slightly higher ability to handle heat stress, although the difference it was not significant (Figure 8B).

*2009* – Female fork lengths ranged 21mm for the study. Female size, however, was not associated with egg or larval characteristics during development or at hatching. Nevertheless, larval growth during their first week differed markedly at water temperatures 16$^{o}$C and 20$^{o}$C, as
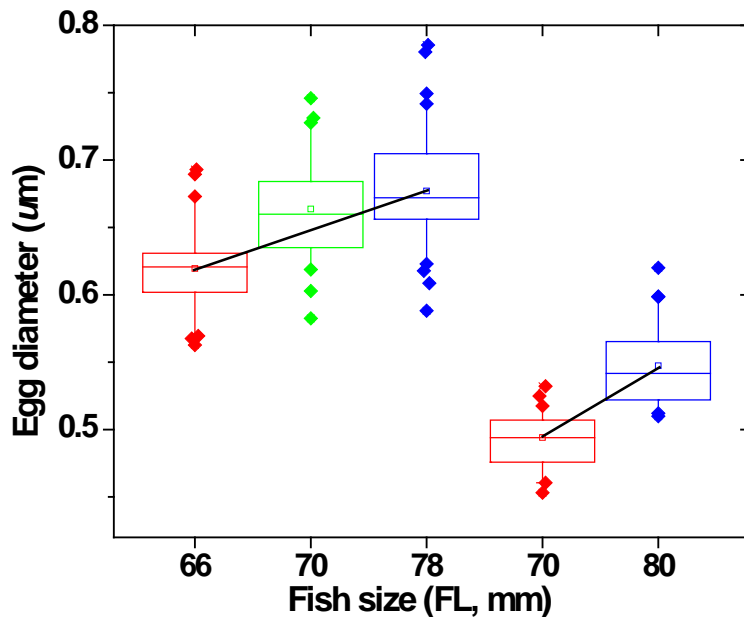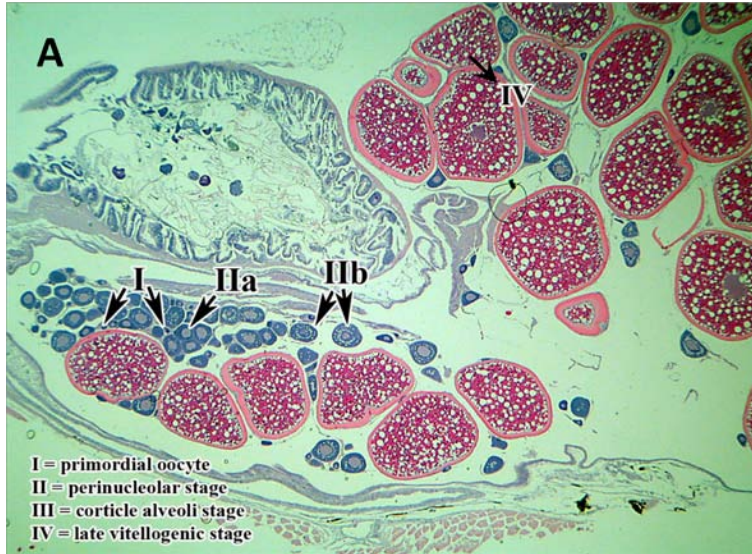
**Figure 4**. Oocyte diameters with fork length of maturing delta smelt caught in 2003 and 2005.

well as between larvae from small versus large mothers (Figure 9A). Overall, larval growth and survival was much better at $16^{o}$C, with no difference between mother size groups detected at $20^{o}$C (Figure 9B). However, larvae from large mothers grew about twice as fast as those from small mothers at $16^{o}$C (Figure 9B).

Larval survival in "bioassays" was highly variable in two temperature treatments with different sequences of permethrin exposure and food deprivation. Overall, at $20^{o}$C, mortality from permithrin exposure and food deprivation was indistinguishable from that in the control treatments; mortality was highly variable for 1 dph (yolksac) larvae, and often over 80% for 4 dph (first-feeding) larvae among replicates after 8 days (Figure 10A,B). At $16^{o}$C, 1dph larvae from large females exhibited consistently lower mortality than larvae from small females, with higher and more variable in mortality overall in the stressor treatments (Figure 10C). Mortality for 4dph larvae at $16^{o}$C treatment effects were indistinguishable due to high variability among replicates (Figure 10D).
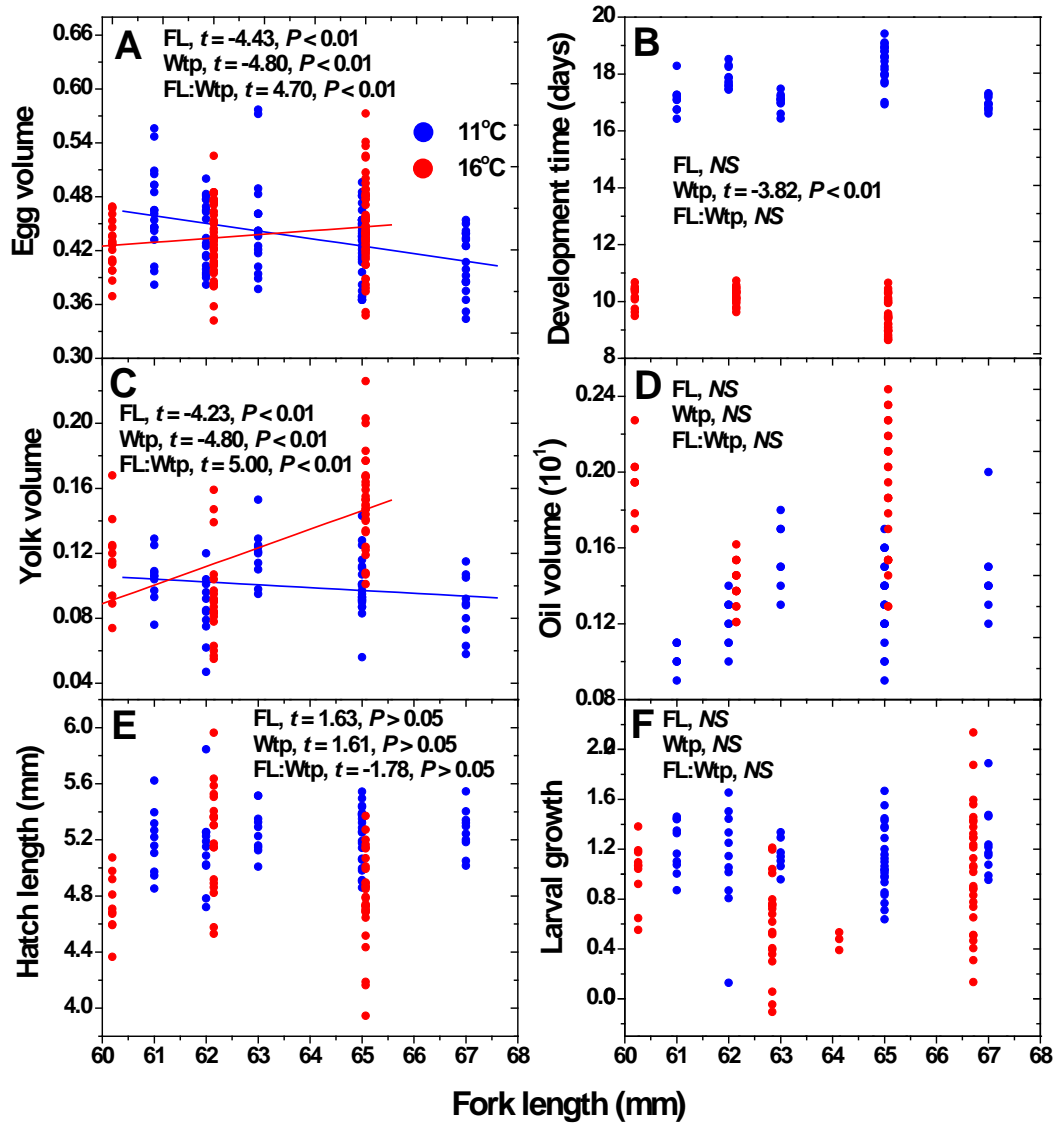
**Figure 5**. Relationships between fork length of females and early life history characteristics of progeny in 2007, including (A) egg volume, (B) larval development time in days, (C) larval yolk and (D) oil volume, (E) hatch length, and (F) larval growth rate until first-feeding .
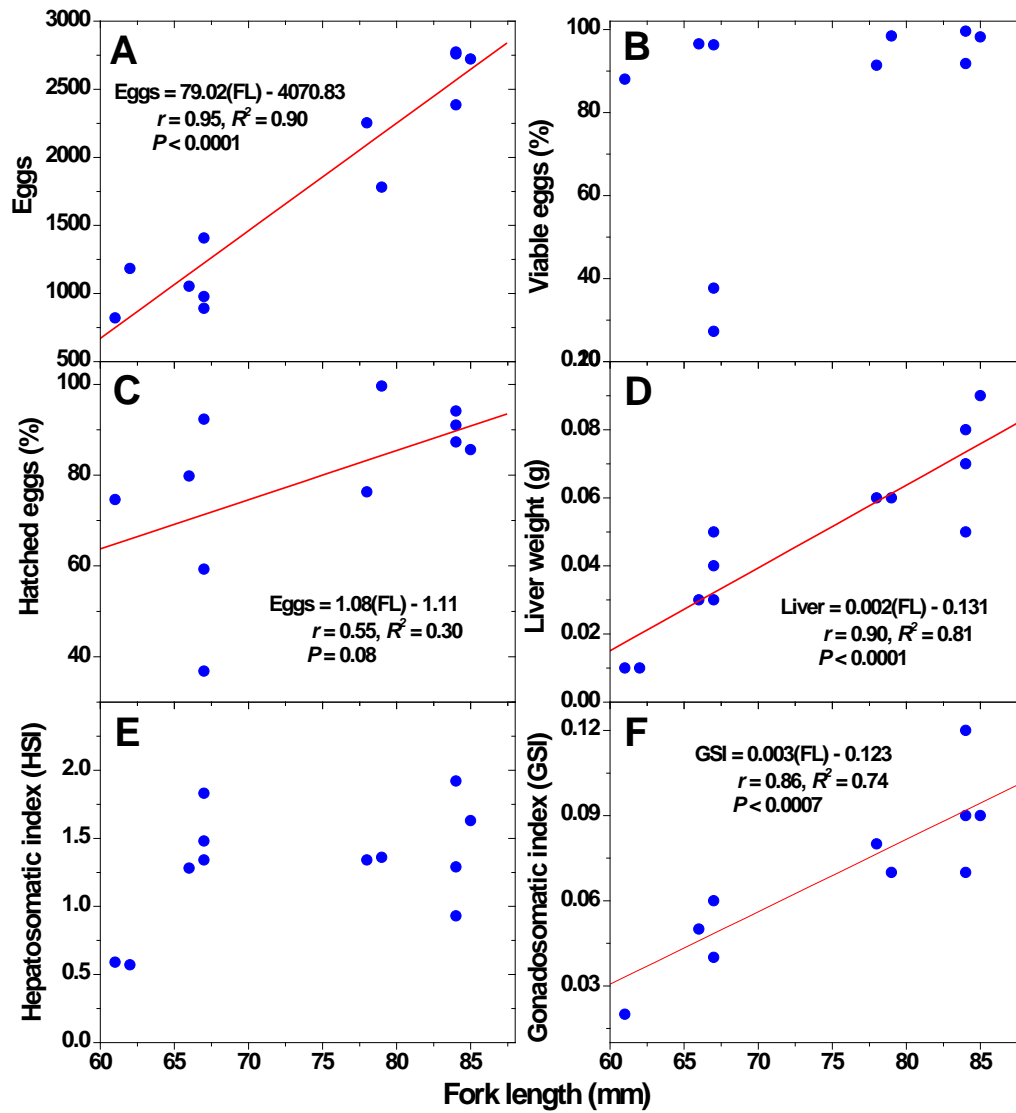
**Figure 6.** Relationships between fork length and various life history characteristics of mature females in 2008, including (A) egg numbers, (B) percent of viable eggs, (C) percent of eggs hatching, (D) liver weight, (E) hepatosomatic index, and (F) gonadosomatic index.
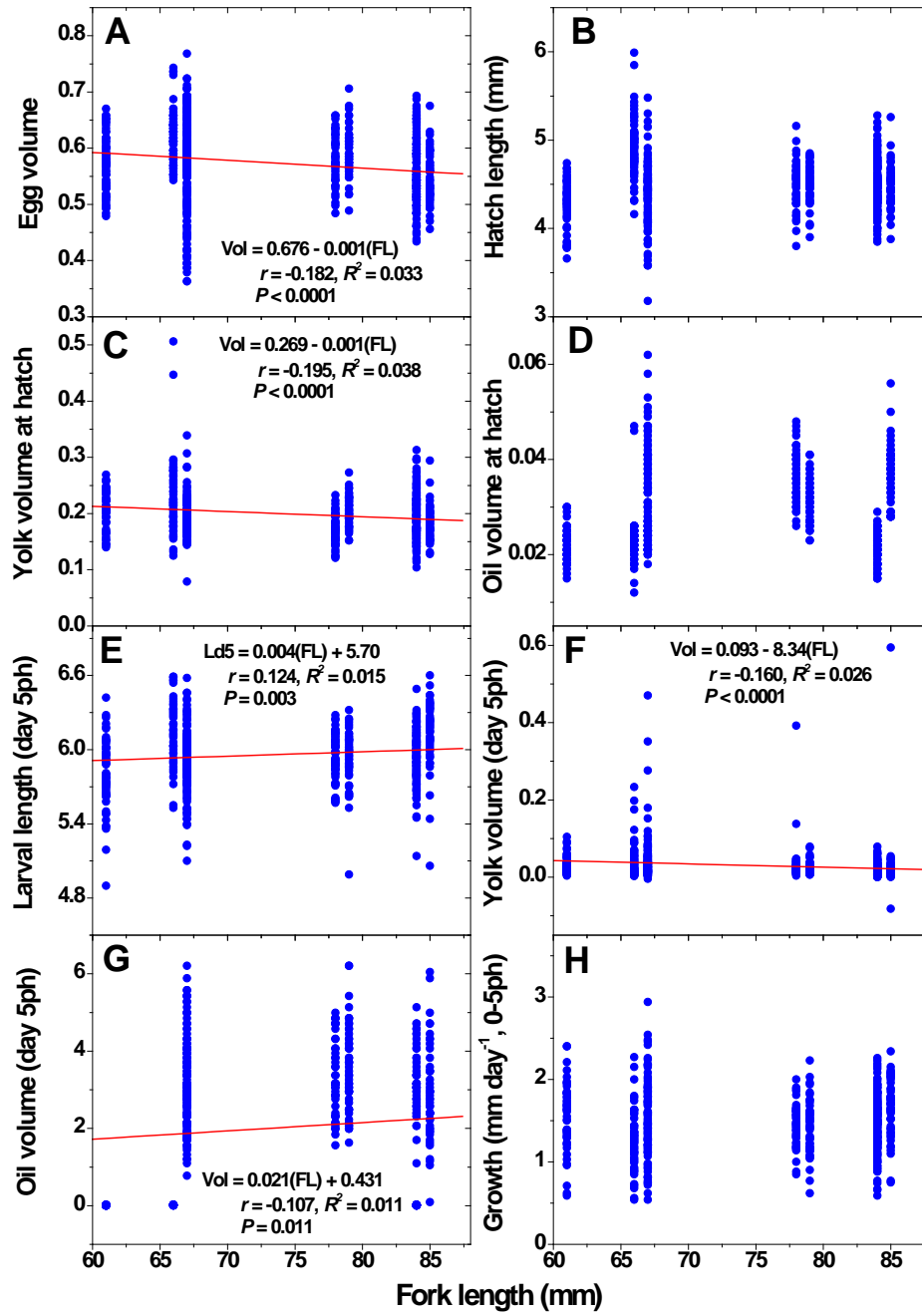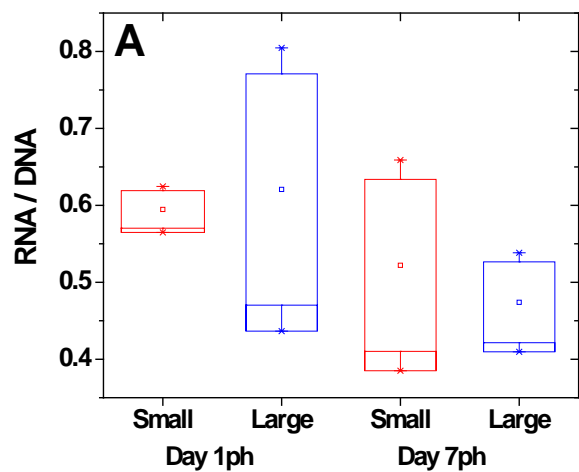
**Figure 7.** Relationships between fork length of females and early life history characteristics of progeny in 2008, including (A) egg volume, (B) larval hatch length, (C) larval yolk volume at hatch (D) oil volume at hatch, (E) larval length, (F) yolk volume, (G) and oil volume at day 5 post-hatch (ph), as well as (H) larval growth rate from day 0-5ph.

**Figure 8.** Evaluations of RNA/DNA ratios at days 1 and 7ph (A) and expression of heat shock proteins (hsp70) at day 7ph (B) for larval delta smelt subjected to increasing water temperatures in 2008.
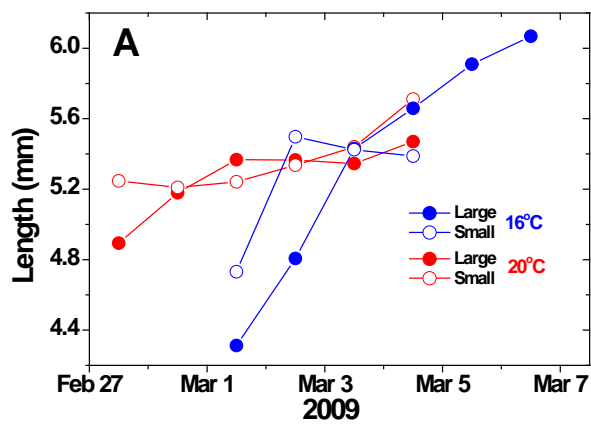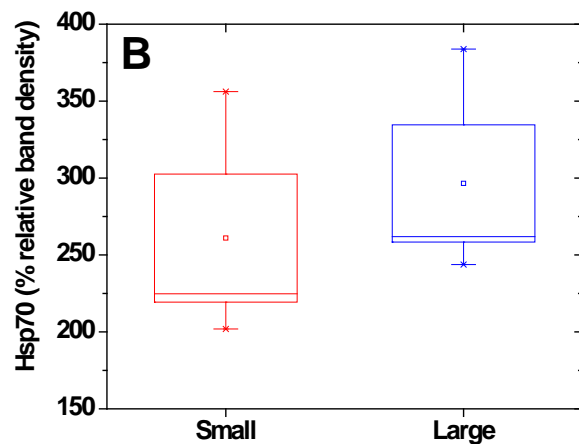


**Figure 9.** Daily growth for larval delta smelt from small (50-54mm) versus large (55-71mm) mothers at 16 and 20°C (A), with comparisons of growth rate (slope from linear regression fit) among size and temperature treatments (B) in 2009.

**Figure 10.** Percent mortality after 8 days of exposure to different sequences of permethrin (4 days) and food deprivation (4 days) for day 1ph and day 4ph delta smelt larvae from small (50-54mm) versus large (55-71mm) mothers at 16 and 20°C, in 2009. Larvae exposed at day 1ph to permethrin, then food-deprived at 20°C (A); larvae food-deprived at day 4ph, then exposed to permithrin at 20°C (B); larvae exposed at day 1ph to permethrin, then food-deprived at 16°C (C); larvae food-deprived at day 4ph, then exposed to permithrin at 16°C (D). Dotted lines with asterisks connect significant comparisons ($p < 0.05$) between relevant treatments.

## DISCUSSION

This work demonstrates that maternal characteristics are associated with, and can influence larval viability and survival of delta smelt. Larger mothers from the wild produce clutches with higher numbers and disproportionately large eggs (oocytes) than those spawned by smaller females. However, studies in the laboratory indicate a strong interaction with temperature, such that a positive relationship with larger females producing larger eggs was observed at 16°C, and a negative association occurred at 11°C. A similar interaction as observed for yolk volumes, with better provisioning at 16°C, and less being provided at 11°C by larger females. Thus, either evidence for maternal effects is masked, or larger females only invest more resources to progeny at temperatures above 11°C. In other fishes, yolk-sacs in larvae produced by larger females contain higher levels of key hormones, nutrients, and antibodies that can enhance larval potential to grow faster and overcome environmental stress than their smaller counterparts (McCormick 1998, 1999, Royle et al. 2003). Although evidence for morphological maternal effects was observed in the egg and yolk-sac larval stages only in the relatively warm (16°C) treatment during 2007, other fishes commonly exhibit similar effects during these early life stages (Benoit and Pepin 1998, Green 2008). Maternal effects appearing in the egg stage alone, however, are

not necessarily the best indicators of offspring success as shown by Ouellet et al., 2001, for Atlantic cod, and generally have weak linkages with recruitment success (Green 2008).

Maternal effects were more clearly and consistently expressed near the end of the yolk-sac and first-feeding stage in 2008. Relationships associating maternal fork length with larval lengths, as well as oil and yolk volumes at day 5ph similarly reflect morphological effects observed at a single time-point, as in 2007. Moreover, RNA/DNA ratios suggest larvae from larger females tended to develop faster in the yolk-sac stage (Day 1ph), although the relationship was not statistically significant due to high variability among replicates; these larvae also tended to have higher levels of heat shock proteins, and thus a better response to stress from increasing water temperatures.

Evidence for maternal effects was also observed for ontological characteristics in 2009, with progeny from larger mothers growing and surviving better over the first week in the $16^oC$ treatment. Larvae from large mothers also exhibited significantly lower mortality from toxic and starvation stress than larvae from smaller mothers at $16^oC$. High mortality overall, and the inability to distinguish among other treatment effects in this study clearly indicates delta smelt larvae do not survive or develop well in water temperatures at or above $20^oC$, as has been noted elsewhere (Bennett 2005).While the expression of maternal effects tends to disappear by the juvenile stage, widespread evidence from a variety of organisms suggests that when observed as ontological characteristics, i.e. processes related to growth and resistance to stress, they are frequently associated with offspring or recruitment success (Wright and Gibb 2005, Green 2008).

Overall, our studies provide evidence for various traits that are influenced by maternal effects in delta smelt. While many relationships reported here exhibited high variability among replicates, this is characteristic of laboratory studies with young delta smelt that are notoriously fragile, and typically experience high mortality in the lab (Swanson et al. 1996). Studies on growth and survival, particularly in 2009, provide clear evidence for ontological maternal effects which are most likely to be associated with year-class success of delta smelt. Further studies on the interactive effects of environmental conditions, particularly water temperature, and ontological maternal effects can provide insight into how patterns of selective mortality may change within and among years to influence delta smelt abundance. Thus, conservation efforts should recognize the potential value of preserving life history diversity, and specifically the potential for human effects on production of larger females (Darimont et al. 2009) for promoting resilience in the endangered delta smelt population.

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# Growth, Condition, and Selective Mortality in the Declining Delta Smelt

## INTRODUCTION

Fish ecologists are becoming increasingly aware of the necessity to distinguish if mortality is size-selective, or merely indiscriminate among individuals from a cohort of young fishes. The idea that mortality is selective and typically favors larger and/or faster growing individuals arose from widespread observations that mortality declines with body size (Anderson 1988, Sogard 1997). Although now somewhat of a paradigm, it is well-documented that size-selection can favor either the largest, faster growing, or smallest, smallest slower growers in a cohort, particularly in situations in which environmental conditions change dramatically throughout the period of early development (Litvak and Leggett 1992, Roff 1992). Nevertheless, it is critical to determine if mortality is selective, or indiscriminate, because if a morphological trait (e.g., body-size) can be shown to enhance survival of particular individuals, then an understanding of the processes and conditions in which that occurs can provide important clues into what drives year-classes success of populations.

The most reliable approach for determining if size selective mortality has occurred is to conduct longitudinal sampling of a cohort before and after a likely period of elevated mortality. Typically, scales, otoliths, and/or various body condition measures are used to determine the size-distributions and condition of each sample, and their relative similarity. Although assessments of fish size and growth from otoliths and body condition using histology are routinely applied in studies of fishes, they are typically implemented independently and for different reasons by researchers from separate disciplines (Catalan et al. 2006). We have developed a different approach that applies these tools in an integrated fashion to each individual in a sample. Integration of these tools within multiple individuals, combined with an interdisciplinary interpretation of the results, can provide clearer insight into the *processes* (i.e., growth, mortality) as well as *underlying causes* (i.e. poor feeding, chemical exposure) that contribute to *patterns* (i.e., size-selective mortality) observed at the cohort, or population-level. Conceptually, our approach is individually-based, such that it attempts to empirically quantify processes underlying recruitment success. This approach is flexible in that a variety of complementary techniques (i.e. biomarkers) can be included as warranted to provide a more comprehensive assessment of individual fish condition and its implications for fish populations (Bennett et al. 1995, Teh et al. 1997, Bennett 2005, Anderson et al. 2007).
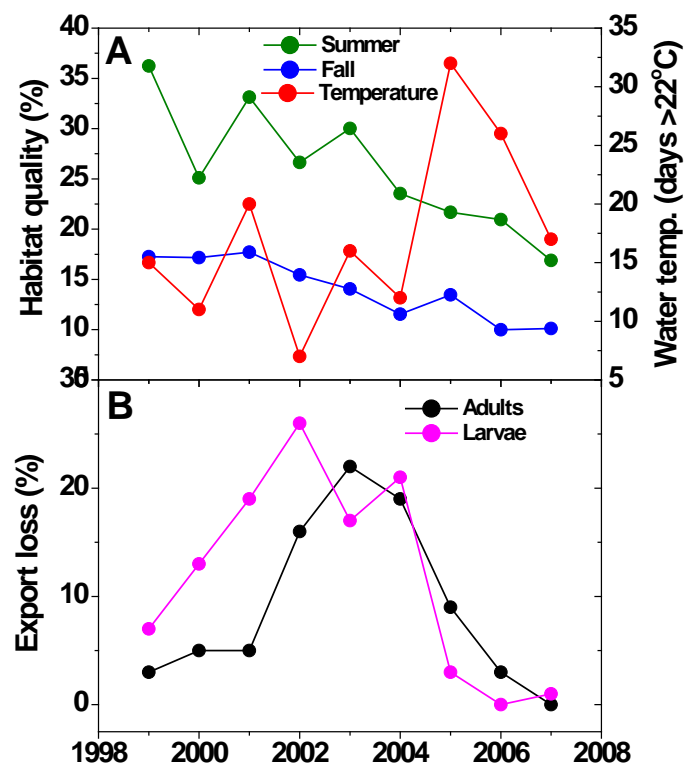
Here we apply our integrated approach to understand the role of multiple, potentially interactive, factors contributing to size selective mortality of juvenile delta smelt, *Hypomesus transpacificus*, in summer (Bennett 2005). Delta smelt is one of the most imperiled and controversial fish

species in the western U.S. This species, endemic to the northern San Francisco Estuary, is currently protected under the California and U.S. Endangered Species Acts following serious declines in abundance in the 1980s and then after 2001. Record-low abundance combined with ongoing entrainment in state (SWP) and federal (CVP) water pumping facilities has heightened tensions between water development and enforcement of the Endangered Species Act to unprecedented levels (Service 2007). Unfortunately, identifying the role of water export operations in the demise of delta smelt is not easy, because they are embedded within a complex array of interacting factors, including the reduced extent and quality of suitable habitat, exotic species, multiple pollutants, numerous in-Delta water diversions, as well as climatic extremes (Bennett and Moyle 1996, Bennett 2005, Feyrer et al. 2007, Sommer et al. 2007, Nobriga et al. 2008). Entrainment in the export facilities, however, continues to reign as the most conspicuous and controversial (Kimmerer 2008). However, it is important to note that these issues are not new. Resource managers have struggled for several decades with the same basic question; how much and how often do water exports, relative to chemical pollutants, and food web changes affect fish populations in the San Francisco Estuary and Delta (Stevens et al. 1985, Bennett and Moyle 1996, Sommer et al. 2007).

Our goal was to design and implement an efficient and effective program for providing baseline quantitative information on the processes potentially regulating the delta smelt population using individual fish caught during routine monitoring by the Interagency Ecological Program (IEP). Our primary objective was to estimate the relative contribution of feeding success and chemical pollutants in determining patterns of growth and mortality of delta smelt throughout most of its, primarily annual, life cycle. This involved analyzing fish otoliths to estimate hatch-dates, growth rates, and likely spawning or rearing habitats (i.e. examining otolith micro-structure), as well as histopathology of key fish organs to assess the mechanisms influencing growth and mortality (i.e. diagnose poor feeding success and chemical exposure).

**METHODS**

*Environmental Data.* A various data sources were compiled to associate environmental conditions with information on fish growth and condition. Data on water temperatures were compiled from four continuous monitoring stations located at Martinez, Pittsburg, Antioch, and Burns Cutoff from the IEP HEC-DSS database (http://wwwiep.water.ca.gov/dss/). Daily water temperatures at each station were averaged over the four locations to estimate daily water temperature in Suisun Bay and the Delta (Figure 1). These daily water temperatures were used to estimate the number of spawning days during spring between $12^{o}C$ and $20^{o}C$ (Bennett 2005), and the number of days of extreme water temperatures ($>22^{o}C$) during summer; this data was also used in estimating larval hatch-sizes and spawning-dates (as described below). Habitat quality during summer and fall was estimated using generalized additive modeling of fish presence/absence with ancillary environmental parameters and collected at the time of fish

sampling in the Summer Tow Net Survey and Fall Mid Water Trawl survey, similar to Freyer et al. (2007) and Nobriga et al. (2008). Finally, estimates of delta smelt losses to entrainment in the federal and state water export facilities were obtained from Kimmerer (2008).

*Field Sampling.* All delta smelt were sampled in routine fish monitoring by the IEP from 1999-2001 and 2005-2007. Fish samples were primarily from the Summer Tow Net Survey (TNS) and Fall Mid-water Trawl Survey (MWT), with others included from the 20mm Survey, Chipps Island Survey, and Central Valley Project Salvage operations (Table 1).

**Figure 1.** Habitat quality in summer (green) and fall (blue) with number of days with water temperatures greater than 22°C (red) in Suisun Bay (A). Estimated total loss of larval delta smelt to the federal and state water export facilities (from: Kimmerer 2008, B).

**Table 1.** Number of specimens evaluated for growth, histology (1st number), and gut contents (2nd number) of life stages of delta smelt from 1999-2007.

| Year | Life stage (season) | | | | |
| | Pro-larvae (spring) | Pro-larvae salvaged* (spring) | Juveniles (summer) | Pre-adult (fall) | Total |
| --- | --- | --- | --- | --- | --- |
| 1999 | 100(0) | | 142(89) | 108(13) | 376 |
| 2000 | 10(9) | 113(0) | 10(9) | 93(30) | 226 |
| 2001 | | 111(0) | 203(41) | 76(47) | 398 |
| 2005 | | | 102(44, 42) | 46(45, 38) | 148 |
| 2006 | | | 60(50, 34) | 29(26, 15) | 98 |
| 2007 | | | 49(30) | 28(25) | 77 |
| Total | | | | | 1,323 |

* Collected in fish salvage operations at the federal Central Valley Project

During fish sampling, after deploying and retrieving the sampling nets biologists onboard identified and measured all delta smelt. Then each fish was decapitated, the head and remaining body individually labeled and placed in separate plastic baggies; the head was preserved in 75-100% ETOH to preserve the otoliths, while the body was fixed in a buffered formaldehyde solution designed for histopathology. Ancillary data on water temperature, salinity, and Secchi depth (i.e. turbidity) were also measured at each sampling station. All fish samples except those designated for other POD studies were then transported to UCD.
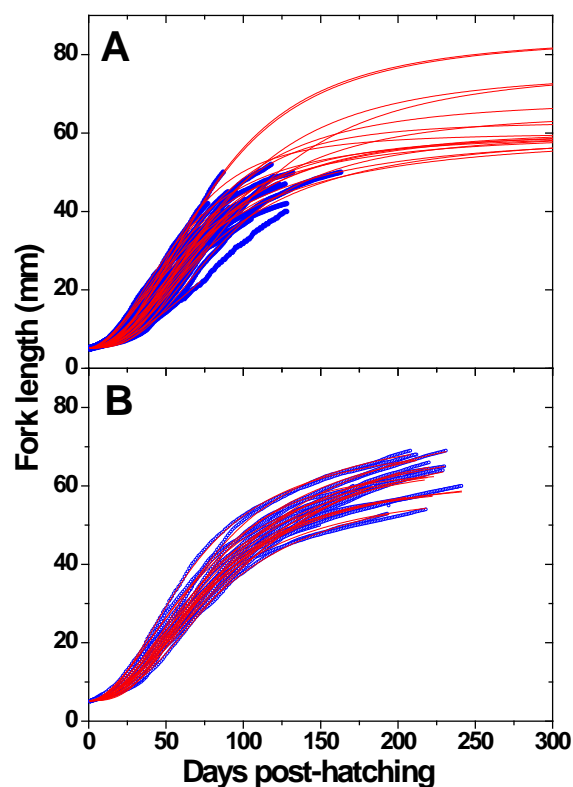
*Otolith Increment Analyses.* The methodology used generally follows that outlined in our previous work applying standard otolith techniques to delta smelt (Hobbs et al. 2007).  Sagittal otoliths were dissected, cleaned and then mounted with the sulcus side down on glass slides using Cyanoacrylic glue. Otoliths were then sanded and polished before being  photographed at 200-400x magnification under a light microscope. Each otolith was then aged by enumerating and measuring daily growth rings from the photographs using imaging software (Image Pro 6.0 ®).  Readings typically started with measuring the first increment from the core, or nucleus, and continued out to the dorsal edge of the otolith (as this was often the clearest trajectory).  All otoliths were similarly measured by at least two, and typically three independent readers. If the age estimates differed by more than 10%, the otolith was re-read, and if subsequent readings did not resolve the difference, the otolith was culled from the study.  Overall, age estimates varied by 3-5% and reflect the mean of 2-4 otolith readings. Age is interpreted as the number of days from hatching (post-hatch).

*Otolith Back-calculations*. Otolith size was converted to fish body size using the standard biological intercept procedure developed by Campana (1990). This method estimates the position on the otolith where the relationship with fish size (i.e., length) becomes linear, thus permitting direct conversion of otolith increment widths into changes in fish size at age (Campana 1990). While this procedure is relatively straightforward it assumes a linear relationship between otolith and fish length (i.e. nonlinear), thus, we initially applied the procedures developed by Vigliola (2000?) to confirm that the linear nature of the relationship before back-calculating sige at age.

*Growth Modeling*. Delta smelt growth was then modeled using size at age estimates for individual fish collected from 1999-2001 and 2005-2007 (N = 1,323 Table 1). For each individual we used a four parameter formulation of the Logistic growth equation,

$$L_t = L_0 + L_0 - L_m / 1 + (t - t_x)^p$$

where $L_0$ = length at hatching, $L_m$ = maximum length (maturity), $t_x$ = age at inflexion point (maturing) which is half way between $L_0$ and $L_m$, and $p$ is the growth factor, or the rate at which

**Figure 2**. Representative logistic growth model fits to estimated daily size at age for delta smelt in 1999 (A) and 2007(B). Total n=1,323.

length approaches $t_x$ (Figure 2). Our previous work has shown this model provides a better fit to delta smelt growth than the traditional von Bertalanffy (Bennett et al. 2008).

Laboratory studies have shown that egg incubation time, hatch-size, and initial feeding success are strongly influenced by water temperatures that typically range widely (about $10^oC$) over the delta smelt spawning season (Baskerville-Bridges et al. 2004). To account for this variation and improve our size at age estimates, we first fitted least-square regression models to the laboratory data of Baskerville-Bridges et al. (2004). Mean hatch-size was 5.2mm ± 0.24 SD, for fish incubated $15^oC$ in that study, which corresponds well with our calculated biological intercept, 5.19 ± 1.8 SD (Figure 3). We then substituted water temperatures averaged over the week prior to hatching into the regression equation to estimate hatch size for each fish (Bennett et al. 2008). Spawn-dates were also estimated using a similar least-square regression model fitted to the data on incubation time under different temperature treatments (Baskerville-Bridges et al. 2004).

*Histopathological Analyses.* Fish bodies were first cut longitudinally into two identical sections, dehydrated in a graded ethanol series, and then embedded in paraffin. Serial longitudinal sections (2-3μm) were mounted on glass slides and stained with hematoxylin and eosin (H&E). This allowed lateral views of the entire body cavity so that gonads, livers, and kidneys could be examined under a light microscope. The tissue slides were examined for abnormalities that were initially scored on a rank scale of 0 to 3 (0 = not present, 1 = mild, 2 = moderate, and 3 = severe). Given that many typical abnormalities develop sequentially and become more difficult to repair (increase in severity) over time, we further weighted our initial scores on a scale of 1 to 5 to reflect the increasing likelihood of mortality or reproductive failure (Figure 3).

*Gut Contents*. Given the regulatory limitations imposed on additional sampling, we devised a method for reversing the histologically prepared specimens so that gut contents could be quantified. Essentially, specimens in paraffin blocks were immersed in a heated paraffin bath to melt the wax, then the fish was removed and rinsed in Citrisolv and ETOH repeatedly in several

steps. After that, the gut was carefully dissected from the specimen, and the gut contents loosened and removed to a vial with ETOH for subsequent identification and enumeration.

## Sequence of Liver & Gonad Alterations
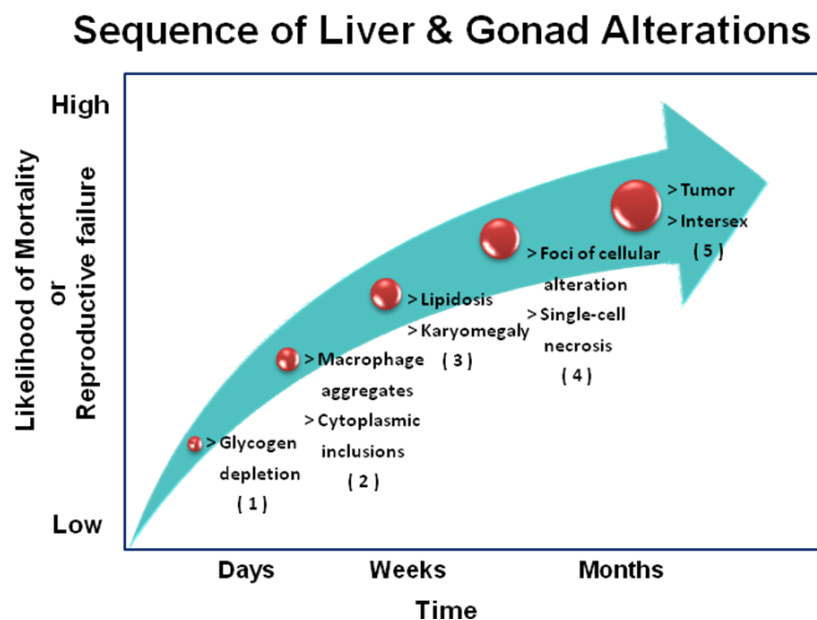


**Figure 3.** Conceptual diagram showing the temporal sequences among the typical cellular abnormalities scored during histological assessment of delta smelt tissues. Likelihood of mortality or reproductive failure increases as altered cells become more difficult to repair over time (i.e. alteration increases in severity). Numbers in parentheses are weightings.

**Statistical Analyses**

*Growth Selective Mortality.* Potential growth selective mortality was first assessed by comparing fitted model parameters for individuals captured in spring and summer versus fall (e.g., primarily between the Summer Tow Net Survey and Fall Mid Water Trawl). We envisioned that selective mortality is likely to have been more important during summer because environmental conditions appeared least favorable which can limit suitable habitat (Bennett 2005, Bennett et al. 2008, Nobriga et al. 2008). If selective mortality was occurring during the summer it would likely be reflected in a change in the distribution of growth parameters for fish surviving into the fall. For example, an upward shift in the distribution of "$p$", the early growth parameter, would indicate that faster growing individuals had a higher probability of surviving through the summer than those growing more slowly.

Exploratory graphic techniques and principal components analysis (PCA) were then used to examine potential relationships among growth parameters, tissue condition, and gut contents as well as various environmental variables. Differences in the numbers of specimens evaluated for

growth, histology and gut contents, required performing the analysis on three data sets to explore associations among each type of information and several environmental factors (Figure 1). Generalized linear modeling was then used to assess their role as predictors of differences between the summer and fall, i.e. the underlying processes of the summer juvenile bottleneck (Bennett 2005).

*Estimating Natural Selection*. Finally, we estimated the total form of natural selection (linear and nonlinear) on size or growth using the spline-based regression procedure initially developed by Schluter (1988) as modified for cross-sectional data (i.e. successive samples) by Anderson (1995). This approach describes relative survival between sampling periods as the conditional probability, $h(z)$, that a fish of a given size or growth rate is subsequently caught in a future sample of "survivors", given that it was caught in one of the two samples. This is done by coding a sample of fish caught prior to selection as 0, and those in a sample after selection as 1, and then fitting a cubic B-spline function ($u$) of the size or growth trait ($z$) as

$$h(z) = e^u/1\text{-}e^u \ ,$$

choosing the smoothing parameter, $\lambda$, by generalized cross-validation (i.e., $\lambda$ that minimizes the weighted sum of the residuals, Schluter 1988). Following this, relative survival was then calculated using Anderson's (1995) equation:

$$f(z) = n_{(before)}/n_{(after)} * [h(z)/1\text{-}h(z)] \ ,$$

where $n_{(before)}$ and $n_{(after)}$ are the respective sample sizes. All statistical modeling was performed using the S-Plus programming language (Venables and Ripley 2002).

**RESULTS**

*Environmental Conditions.* Estuarine conditions have varied widely since 1999. Delta smelt abundance fell to record-low levels (Chapter 3, this report), with habitat quality also declining in part due to increasing summer water temperatures (Figure 1A). Entrainment losses at the water export facilities were also excessive from 2001-2004 (Figure 1B).

*Growth Modeling.* All Logistic growth models fitted to 1323 delta smelt exhibited coefficients of determination ($r^2$) greater than 0.9 (Figure 2). The annual trend in average size at maturity (*Lm*) was higher overall and declined for fish surviving into fall, as also shown in Chapter 3 (this report), whereas it was generally less variable and declined less for fish sampled in spring and summer (Figure 4A). The growth parameter (*p*) was generally higher for fall fish relative to those in prior months, and increased over time (Figure 4B). The trend in age at the inflexion point (Figure 4C), was less variable and relatively flat over the time period of study.
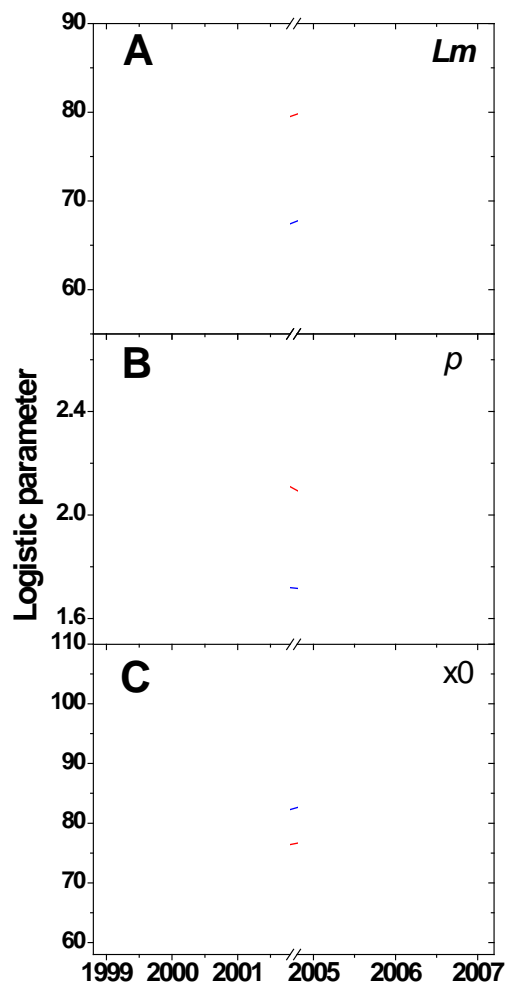
**Figure 4.** Mean size at maturity (*Lm*, A), growth parameter (*p*, B), and age at the inflexion point (x0, C) from fitting Logistic growth models to 1,323 delta smelt.

*Histopathology Analyses.* Poor liver condition (index > 8) remained relatively low (8-10%), whereas glycogen depletion (index > 1) increased from 36% to 67% from 1999-2007 (Figure 5). Interestingly, 3 individuals were diagnosed with ovatestis, or intersex, beginning in 2005, none in 2006, and 3 were again observed in 2007 (Figure 5).

*Gut Content Analyses.* The gut contents of 129 specimens were identified and enumerated from 2005-2006. Over 80% of gut weights were comprised of four species, including in order of importance, Tortanus dextrilobatus (36%), Pseudodiaptomus forbesi (24%), Limnoithona tetraspina (13%), and Acartiella sinensis (9%, Figure 6). In both years examined, gut weights were highly skewed with 65% and 88% containing less than 100$u$gC in 2005 and 2006 respectfully (Figure 7).
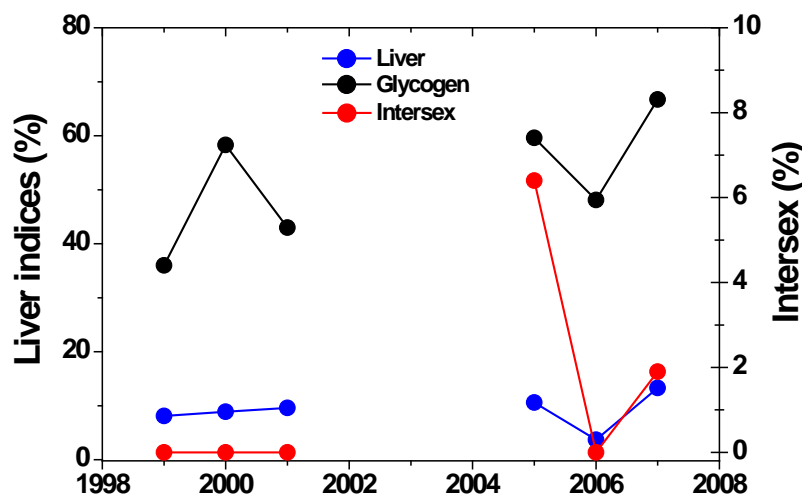


**Figure 5.** Percent of total liver (>8, blue) and glycogen scores (>1), with percent of adult fish with intersex (ovatestis).
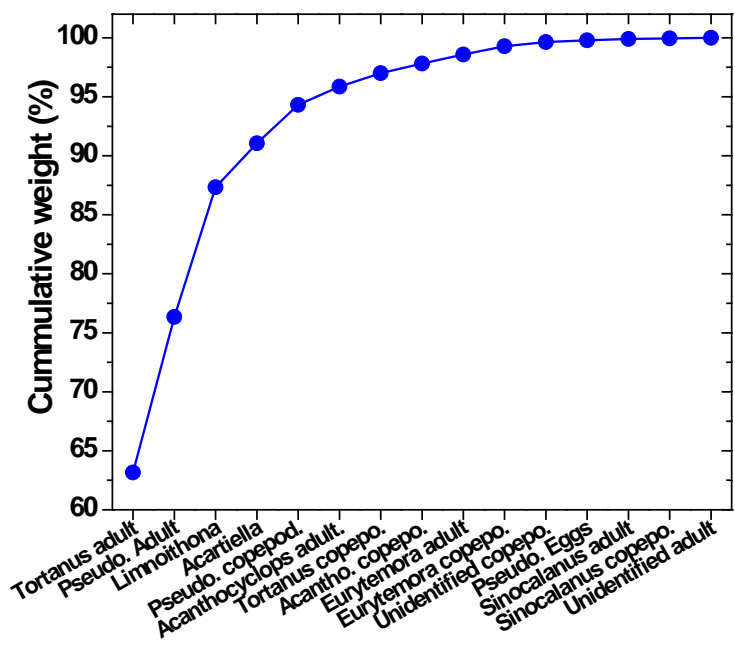
**Figure 6.** Cumulative weight of prey items (%) in gut contents for 129 delta smelt from 2005 and 2006.
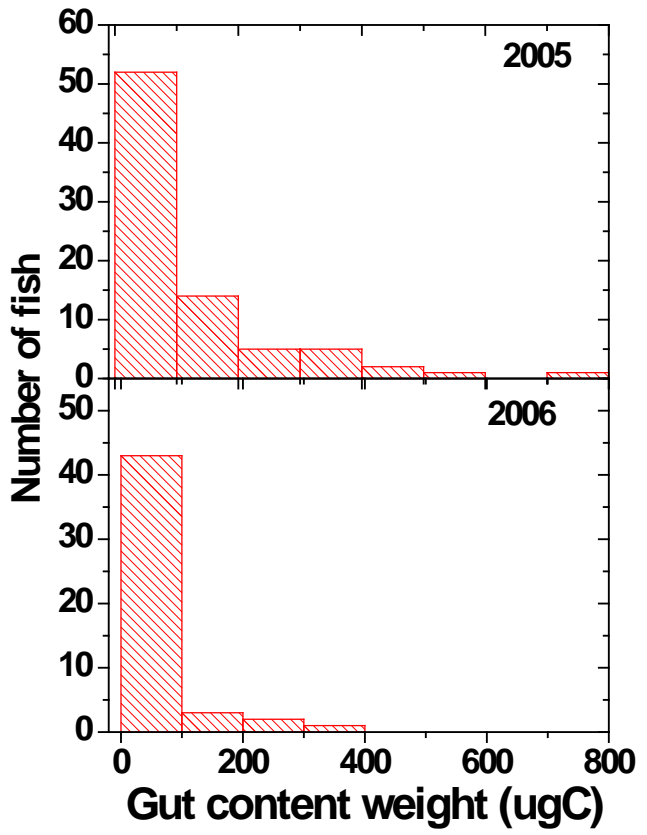


**Figure 7.** Distribution of gut content weights (*ug*C) for delta smelt examined in 2005 and 2006.

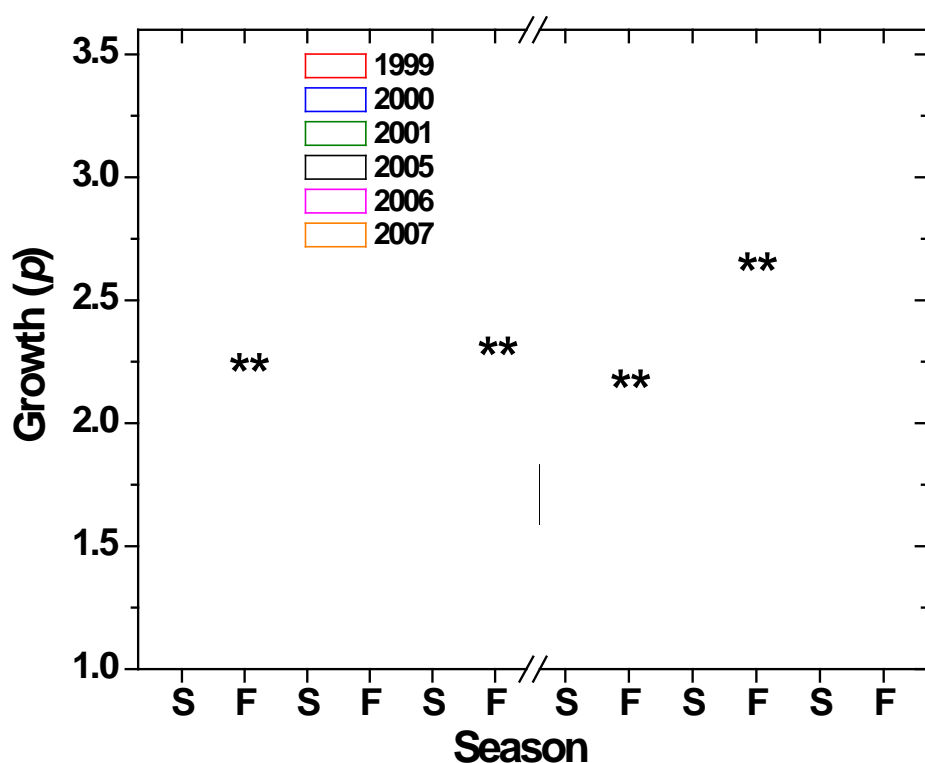*Growth Selection*. Comparisons between summer and fall growth indicated that *p* was significantly higher for fall fish in 1999, 2001, 2005, and 2006 (Welch's Modified Two-Sample *t*-Test, all p < 0.0001, Figure 8).



**Figure 8.** Box plots showing distributions of the growth parameter (*p*) for delta smelt caught in spring and summer versus fall. Asterisks indicate significant (p<0.0001) comparisons.

Following the initial PCA on the largest data set (N = 1322) containing all fish evaluated for growth, a scree plot of cumulative variance indicated that four principal components explained 89% of the variance in that data set (Figure 9). The loadings of the first component reflect the major trends in the data (Table 2), including long-term decline in both summer and fall habitat quality, and increases in the number of days with water temperature >22oC, glycogen depletion in fish livers, as well as in early growth rate (*p*).  The second component loads most heavily on growth (*p*), with the third component loading heavily on improved liver and glycogen condition, and the

**Table 2.** Factor loadings from principle components analyses.Data set 1 includes all specimens analyzed for growth; Data set 2 includes all fish analyzed for growth and histology; and, Data set 3 includes all fish analyzed for growth, histology, and weight of gut contents.

| Variable | Data set 1 (n=1322) | | | | Data set 2 (n=466) | | | | Date set 3 (n=127) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PC2 | PC1 | PC3 | PC4 | PC1 | PC2 | PC3 | PC4 | PC1 | PC2 | PC3 | PC4 |
| Temp. >22oC | 0.324 | -0.172 | 0.452 | 0.105 | 0.553 | -0.018 | -0.029 | -0.183 | 0.502 | 0.042 | 0.011 | 0.236 |
| Sum. habitat | -0.490 | 0.101 | 0.237 | 0.305 | -0.504 | -0.246 | 0.035 | 0.033 | 0.502 | 0.042 | 0.011 | 0.236 |
| Fall habitat | -0.501 | 0.090 | -0.128 | 0.106 | -0.543 | -0.175 | 0.185 | 0.229 | 0.502 | 0.042 | 0.011 | 0.236 |
| Lm | -0.243 | 0.216 | 0.278 | -0.742 | -0.266 | 0.202 | -0.650 | -0.385 | 0.230 | 0.225 | 0.584 | -0.499 |
| p | 0.332 | 0.524 | -0.044 | 0.443 | 0.218 | -0.698 | 0.158 | 0.068 | -0.327 | 0.442 | -0.116 | 0.486 |
| Liver | -0.282 | 0.238 | -0.562 | 0.054 | 0.057 | -0.049 | -0.508 | 0.516 | 0.170 | 0.406 | -0.439 | 0.017 |
| Glycogen | 0.364 | -0.051 | -0.544 | -0.328 | 0.153 | 0.035 | -0.265 | 0.643 | 0.062 | 0.215 | -0.534 | -0.530 |
| Gut wt. | na | na | na | na | na | na | na | na | 0.164 | 0.295 | -0.148 | -0.248 |
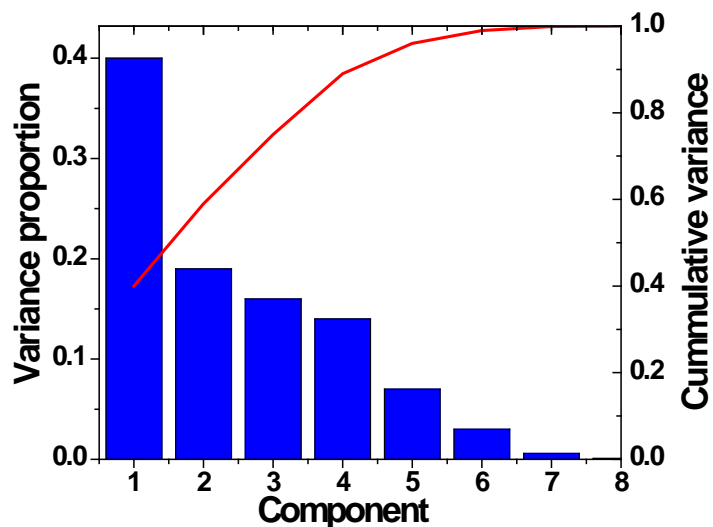
**Figure 9.** Results from principal components analysis of Data set 1, showing scree plot and factor scores for individual delta smelt along components 1 and 3.

Legend:
- '99-Fall
- '99-Summer
- '00-Fall
- '00-Summer
- '01-Summer
- '01-Fall
- '05-Summer
- '05-Fall
- '06-Summer
- '06-Fall
- '07-Summer
- '07-Fall
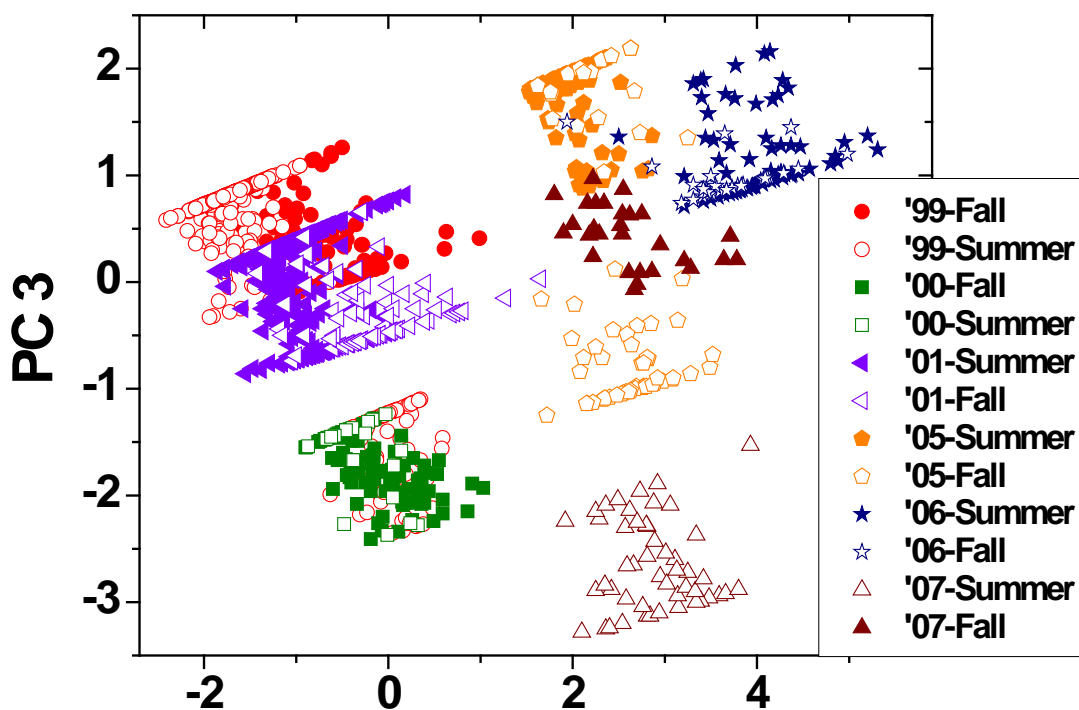
fourth on long term decline in size at maturity. Plotting the scores for individual fish for components one and three against one another indicates the individuals from each year tend to cluster tightly together with modest seasonal (summer vs. fall) separation along component three (Figure 9). Thus, differences in liver and glycogen depletion between summer and fall caught fish were most prominent in 1999, 2005, and 2006 (Figure 9).

The second PCA, using all individuals with growth and histology information (N=466), produced a similar scree plot indicating that four principal components explained 85% of the total variance (Figure 10). The loadings of the first component again reflect the major trends (Table 2),
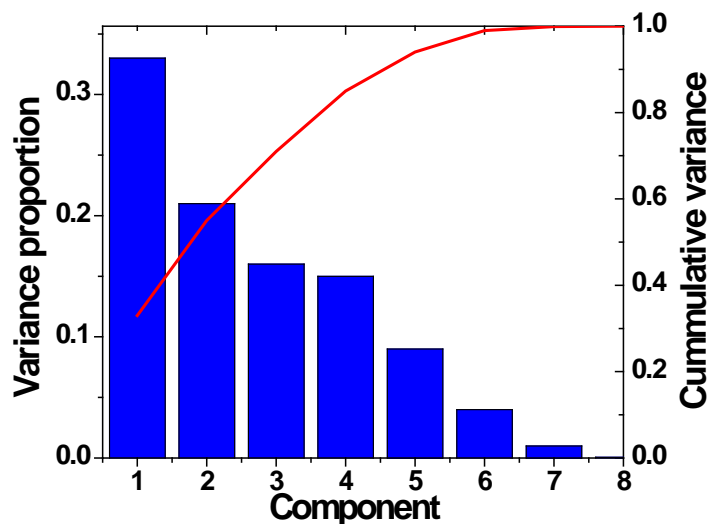
**Figure 10**. Results from principal components analysis of Data set 2, showing scree plot and factor scores for individual delta smelt along components 1 and 4. See Figure 9 for factor score legend.
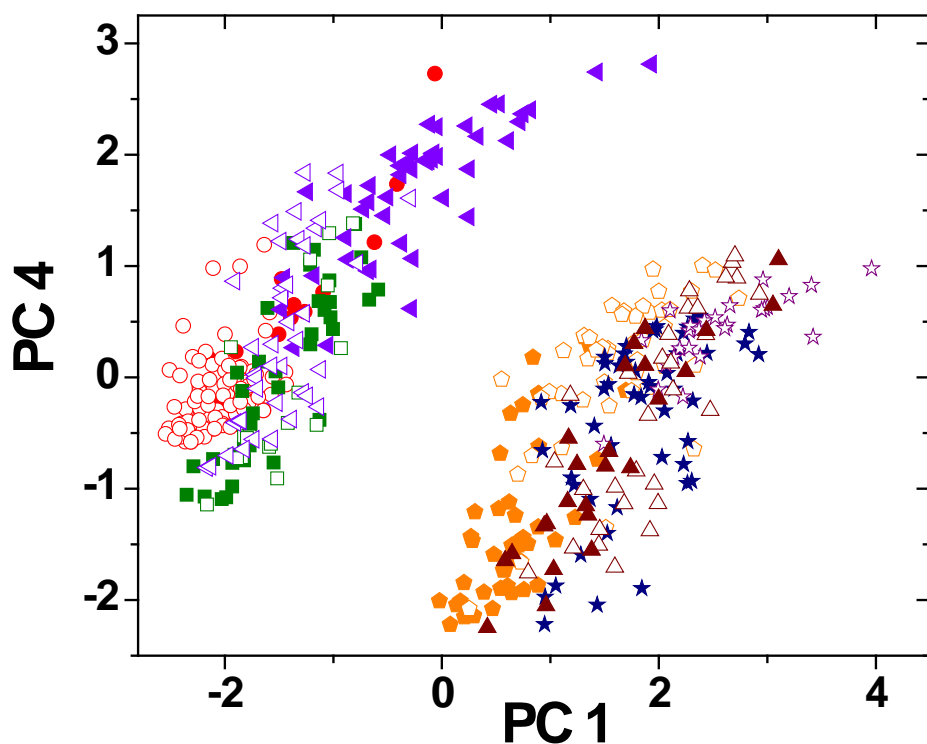
increasing summer water temperatures, and declining habitat quality. The second component loads heavily on deceasing growth rate ($p$), whereas the third reflects declining size at maturity (*Lm*). The fourth component loads heavily on poor liver and glycogen condition. A plot of the scores for individual fish for components one and four indicates that the three early years (1999-2001) and later years (2005-2007) cluster tightly together, with the early years overall scoring higher on component four (Figure 10). Within each year, there is modest separation between fish caught in summer versus fall primarily on the fourth component.

**Figure 11**. Results from principal components analysis of Data set 3, showing scree plot and factor scores for individual delta smelt along components 1 and 2. See Figure 9 for factor score legend.
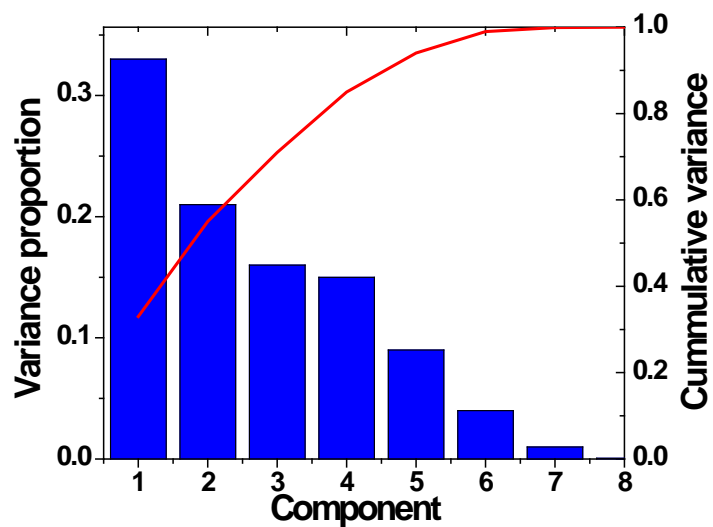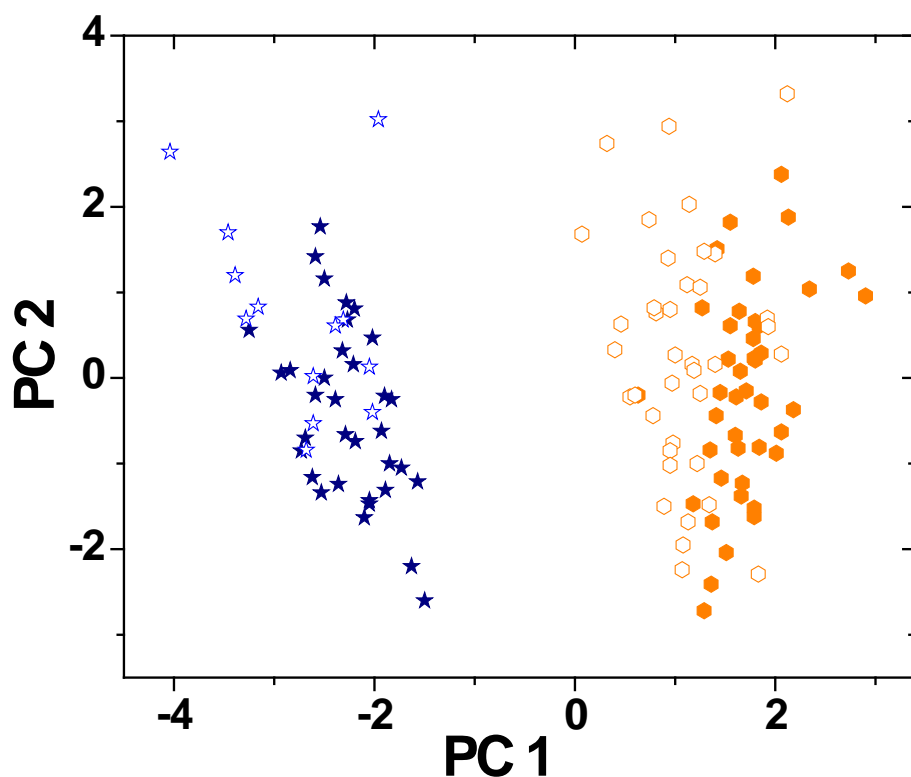


The third PCA, using the data set with growth histology and gut content information (N=129), again produced a scree plot indicating that four components explained 85% of the variance in the data set (Figure 11). The first component again loaded heavily on the major trends (Table 2), increasing water temperature and declining habitat, with the second loading on growth (p), poor

liver condition, and gut content weight. The third component loads heavily on increasing size at maturity (Lm), as well as improved liver and glycogen condition, and the fourth on improved glycogen condition, decline in gut content weight, and in size at maturity. The scores indicate that fish cluster together by year (2005, 2006), as well as exhibit modest separation by season along the first component. In each year, fish separate evenly with fall caught individuals loading slightly higher on component 2 (Figure 11).

Generalized linear modeling on each of these data sets consistently indicated that the growth, *p*, and size at maturity, *Lm*, parameters substantially reduced the residual deviance in each model (Table 3). Also important in Data sets 1 and 2 was the trend in declining suitable habitat, however, with only a sample size of two years it was not considered in the model for Data set 3 (Table 3). Both models for Data sets 2 and 3 indicate that liver condition and to a lesser degree glycogen depletion, and gut contents were important in reducing residual deviance (Table 3). Selection curves further demonstrate the intensity and non-linear form of natural selection during summer (Figure 12). Overall, selection favoring individuals with higher growth were more likely to survive through the summer in four of six years of study, whereas in 2000 and to a lesser extent 2007, natural selection appears to have been disruptive, favored both slower and faster growers (Figure 12).

## DISCUSSION

Our results clearly demonstrate that growth selective mortality during summer was a persistent and significant process causing mortality of delta smelt as the population declined over the last decade. Distributions of the growth parameter, *p*, shifted markedly higher for fish caught in fall versus spring and summer, and the steep slopes of the fitted spline curves for these same years suggest that growth selection was directional and intense. Difference in growth among individuals and years was apparently influenced by summer habitat conditions as defined by the probabilities of fish occurrence due to the predictors, water temperature, salinity, and turbidity in a generalized additive model. Also apparent is that the process of growth selection was reflected in the condition of fish livers and their glycogen content, as well as gut content weight. Although not an important predictor distinguishing summer and fall caught fish, hatching date and size would naturally bear strongly on the ability to survive through a juvenile bottleneck during summer. In addition to being older, larvae hatching in early spring also encounter much cooler water temperatures which slow incubation and early growth allowing for improved development (Bennett 2005). Thus, they are likely to be better at foraging and withstanding adverse summer conditions. Overall, these findings suggest growth selection was influenced more by physical habitat and food web processes rather than other factors, e.g., exposure to pollutants.
A key assumption underlying our results is the degree to which patterns in growth and condition from the IEP fish sampling reflect the entire population. Clearly, relatively fewer delta smelt were caught during regular monitoring in recent years. While this unfortunately reflects the poor status of the population, the catch of delta smelt is also known to be affected by gear selectivity

**Table 3**. Residual deviance, approximate coefficients of determination, and significance level resulting from generalized linear modeling (GLM) of factors potentially related to the summer bottleneck period for delta smelt. Data set 1 includes all specimens analyzed for growth; Data set 2 includes all fish analyzed for growth and histology; and, Data set 3 includes all fish analyzed for growth, histology, and weight of gut contents. All GLMs used a binomial error distribution to fish condition and factors occurring in summer versus fall.

| Term | Data set 1 (n=1322) | | | Data set 2 (n=466) | | | Date set 3 (n=127) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Residual deviance | Coefficient | Pr(Chi) | Residual deviance | Coefficient | Pr(Chi) | Residual deviance | Coefficient | Pr(Chi) |
| Null | 1615 | | | 633 | | | 172 | | |
| $p$ | 1001 | 38 | 0.00 | 457 | 28 | 0.00 | 87 | 49 | 0 |
| $L_m$ | 1195 | 26 | 0.00 | 512 | 19 | 0.00 | 118 | 31 | 0 |
| Habitat | 1492 | 8 | 0.00 | 597 | 6 | 0.00 | na | | |
| Glycogen | 1594 | 1 | 0.64 | 610 | 4 | 0.00 | 77 | 55 | 0.49 |
| Liver | 1595 | 1 | 0.43 | 452 | 28 | 0.03 | 78 | 55 | 0.00 |
| Days $>22^{o}$C | 1595 | 1 | 0.02 | 629 | 0.5 | 0.06 | na | | |
| Hatch size | 1601 | 1 | 0.00 | 609 | 4 | 0.42 | 166 | 3 | 0.18 |
| Year | 1611 | 0.2 | 0.06 | 452 | 29 | 0.49 | 168 | 2 | 0.05 |
| Gut wt. | na | | | na | | | 87 | 49 | 0.82 |
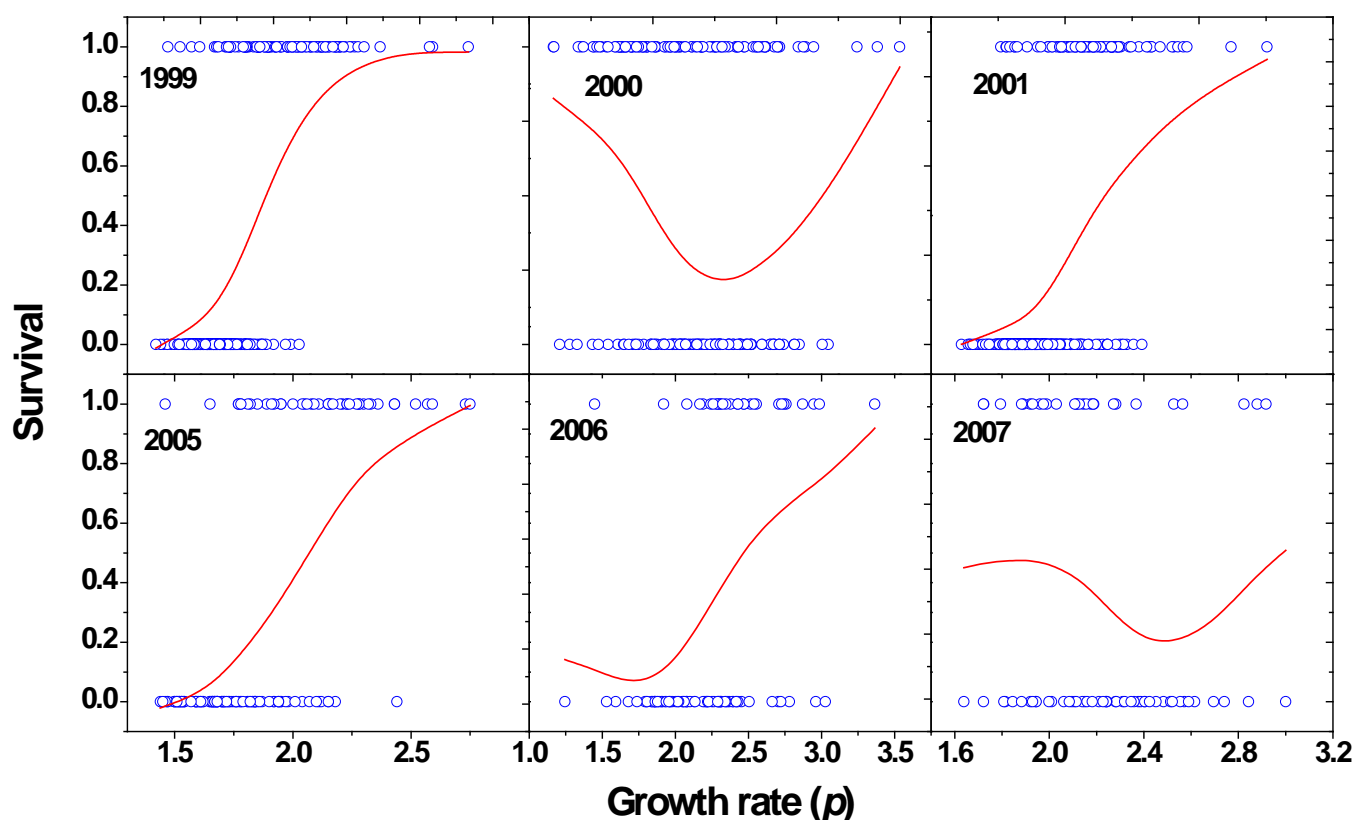
**Figure 12.** Changing intensity and form of natural selection for the growth parameter (*p*) from Logistic growth models fitted to individual delta smelt. Fitted lines are cubic-splines following Schluter (1988) as modified by Anderson (1995) (see methods).

of the sampling nets. Moreover, our analyses were limited primarily to only 2 of the IEP surveys; regular analysis of younger fish (e.g., 20mm survey, salvage) would allow estimation of food-limitation and intensity of selective mortality, implied by patterns of slower growth. Subtle shifts in habitat use (i.e. to areas off the sampling grid) may be another source of bias, especially if it influenced growth or condition. Nevertheless, even though relatively few fish were sampled at any given period, the observed patterns in selective mortality were quite robust among years increasing the likelihood that they reflect important processes affecting recruitment.

*Management Implications*. To our knowledge this approach and findings are unique. Rarely is growth from otoliths and histological condition of organs integrated within individuals. One similar study (Catalan et al. 2006) employed these techniques to assess growth and condition of larval pilchard (a sardine), but applied them separately. This study highlights the added value of integrating techniques within individuals to provide a more holistic view of the interactions between environment and organism that contribute to patterns in year-class success. The findings

of this study highly recommend that our approach become an integral part of the long-term fish monitoring programs. The information provided by our study approach is critical for understanding fish population dynamics, and especially the status of an endangered species. Our experience clearly indicates that the effectiveness of this type of investigation is marginalized by intermittent funding opportunities, as well as by short-sighted management focus. This is especially compelling given that by most accounts the years for which we have little or no data (2002-2004) were the critical years in the recent decline of delta smelt.

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# Unnatural Selection by Massive Water Extraction Enhances Decline of an Endangered Estuarine Smelt.

## INTRODUCTION

Human exploitation of natural resources can impose unintended and unnatural selection pressure, altering life history traits and causing evolutionary change in populations of a various organisms (Allendorf and Hard 2009, Darimont et al. 2009, Hendry et al. 2011). Activities such as fishing or trophy hunting, routinely harvest larger, older, fast growing or bold individuals, removing desirable traits associated with higher survival and fitness in a given set of environmental conditions, often with deleterious consequences (Coltman et al. 2003, Swain et al. 2007, Edeline et al. 2007, Allendorf and Hard 2009). Such unnatural selection pressure often opposes and overrides natural processes by removing otherwise successful individuals (phenotypes) which eventually erodes the genetic integrity of populations (Edeline et al. 2007). In aquatic systems, body size typically scales positively with fecundity, and inversely with mortality, such that growing-fast and maturing at a larger size in a given environment is often advantageous (Roff 1992). However, predictions from life history theory and various empirical and experimental studies indicate that selective harvest leads to reductions in size, slower growth, maturation at younger ages, causing poorer survival that impacts population viability (Roff 1992, Conover and Munch 2002, Hutchings and Reynolds 2004, Jorgensen et al. 2007).

Phenotypic changes also tend to occur rapidly in harvested populations, in contrast to the traditional notion that evolutionary change occurs very slowly (Reznick and Ghalambor 2005). The likelihood for rapid change is in part due to the realization that the levels of unnatural selection pressure typically exerted by human activities are much stronger than those typically imposed by natural processes on populations (Darimont et al. 2009, Hendry et al. 2011). For example, in a series of simulated harvest experiments using Atlantic silversides, weight at age dropped by about 40% over only four generations (Conover and Munch 2002). In several time series studies of exploited fish populations, trait changes predicted by life history theory occurred in only a few generations of exploitation by humans (Edeline et al. 2007, Swain et al. 2007, Jorgensen et al. 2007). Although changes in various life history traits have been reported, shifts to an earlier age and/or smaller size at maturity appear to occur faster than others in experimental and empirical studies (Conover and Munch 2002, Edeline et al 2007, Hendry et al. 2011).

In this study, the likelihood for a change in size at maturity due to human exploitation were investigated for the imperiled delta smelt, *Hypomesus transpacificus* , in the northern San Francisco Estuary. This endemic species is currently protected under the California and U.S. Endangered Species Acts following serious declines in abundance in the 1980s and then after 2001. Delta smelt have also become a major focus of contentious debates between conservation efforts and operations of the State Water Project and Central Valley Project.  These massive

facilities export from 30-60% of the freshwater flowing into the delta smelt habitat to fuel a $25 x $10^9$ agricultural economy and drinking-water for over 23 million people (Grimaldo et al. 2009). In the process, however, water export pumping alters estuarine flow patterns and directly kills large numbers of delta smelt (Kimmerer 2008) such that conservation efforts currently involve controversial reductions in operations, interfering with freshwater allocations throughout California (Service 2007, Sommer et al. 2007).

Here I examine whether mortality of delta smelt in water export operations has acted as an agent of unnatural selection, producing a reduction in size at maturity that also may have contributed to the decline and poor resilience of the population. This so-called, "Big Mama Hypothesis"[1], and the potential for selection in the population builds from several premises. First, delta smelt are an endemic, closed population, and primarily live one year, with a small percentage of individuals living two years. They exhibit substantial variability among individuals in several key life history traits that scale with maternal body size (i.e. maternal effects) including, fecundity, egg volumes and yolk quality, viability and ability of larvae to withstand stress (Bennett 2005, Bennett et al. Chap#1, this Report). Maternal effects such as these are widely considered to be associated with a higher probability of survival and eventual reproductive success of larvae (Green 2008). Second, larger females (~ 70-85mm FL) also appear to spawn earlier in spring as water temperatures rise above 12$^o$C, relative to smaller females that do not mature until they reach 60mm FL (Bennett 2005). Spawning early increases the chances for larger females to produce a second clutch before water temperatures exceed 20$^o$C, which are poor for larval survival (Bennett 2005, Bennett et al. Chap#1, this Report).

Spawning early, however, also coincides with peak water- export operations. Upwards of 20-30% of larval production for delta smelt was estimated to have been lost annually before mid-April from 1995-2005 (Figure 1A, from Kimmerer 2008). Thus, water export operations are more likely to have been impacting the progeny of larger, early-spawning females, removing a larger proportion of individuals each year that were presumably better provisioned to survive the annual recruitment process. Over the past two decades, export operations typically peak from late-winter until mid-April, then they are sharply ramped-down to protect salmon out-migrating in the San Joaquin River: a conservation action known as the Vernalis Adaptive Management Program (VAMP, Figure 1B, Grimaldo et al. 2010). Operations typically resume by late-May, near the end of the delta smelt spawning season as water temperatures rise above 20$^o$C in the Delta (Figure 1B, Bennett 2005). Given that this seasonal pattern of water diversion and larval mortality has occurred since at least the 1990s (Figure 1B), it may constitute a unique an unintended source of unnatural selection lasting for at least ten generations and disproportionately impacting the progeny of larger delta smelt (Allendorf and Hard 2009). This source of unnatural selection pressure has likely varied in strength relative to the amount of freshwater outflow, position of the salt-field, and strength of water export pumping (Grimaldo et al. 2010).
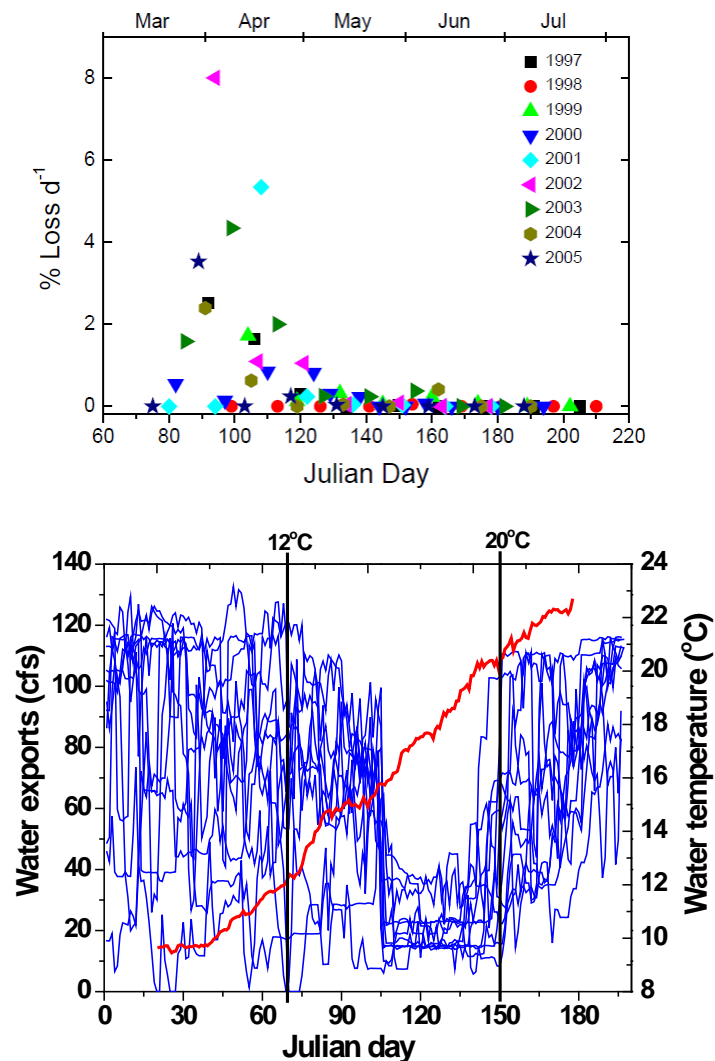
**Figure 1**. Estimated percent loss per day of larval delta smelt (A, from Kimmerer 2008) with annual patterns of water export pumping (B). Also shown are mean water temperatures in Suisun Bay, bracketing the delta smelt spawning season, 12-20°C.

## METHODS

*Field Samples and Otolith Analysis -* Delta smelt were evaluated for age and growth using otoliths from specimens collected in the course of routine fish monitoring primarily from the Summer Tow net Survey (TNS) and Fall Mid-water Trawl Survey (MWT) conducted from 1999-2007 by the Interagency Ecological Program (IEP). In the field, after deploying and retrieving the sampling nets, biologists onboard identified and measured all delta smelt. Then each fish was decapitated, the head was individually labeled and preserved in 75-100% ETOH and then transported to UCD. Otolith analysis generally followed the procedures outlined in our previous work (Hobbs et al. 2007, Bennett et al. 2008). Sagittal otoliths were dissected, cleaned and then mounted on glass slides. After being sanded and polished, they were photographed at

200-400x magnification under a light microscope. Each otolith was then aged by enumerating and measuring daily growth rings from the photographs using imaging software (Image Pro 6.0 ®). Readings typically started with measuring the first increment from the core, or nucleus, and continued out to the dorsal edge of the otolith (as this was often the clearest trajectory). All otoliths were similarly measured by at least two, and typically three independent readers. If the age estimates differed by more than 10%, the otolith was re-read, and if subsequent readings did not resolve the difference, the otolith was culled from the study. Overall, age estimates varied by 3-5% and reflect the mean of 2-4 otolith readings. Age is interpreted as the number of days from hatching (post-hatch). Otolith increment measurements at age were translated (back-calculated) into fish length (size) at age first using the methods developed by Vigliola and Mechum (1999) to verify a linear relationship between otolith size and fish length, which then justified applying the Biological Intercept Method developed by Camapana (1990).

*Age-length analysis-* Age-length keys were used to increase the likelihood that observed patterns in size and age reflect those in the overall delta smelt population. Sample sizes and size ranges of fish analyzed for age and growth using otoliths vary markedly among years due to declining catch and availability afforded by the IEP. Numbers of aged fish range from only 74 fish in 2007 to 398 fish in 2001, and are limited in size range (fork length, FL) from 14-50mm FL in 2004 to 12-70mm FL in 2001 (Table 2). No fish samples were available for aging analysis in 2003. Thus, age-length keys were calculated to estimate age for the large numbers of samples for which only length information is available in the majority of fish surveys implemented by IEP (Table 1).While this analysis substantially increases sample size and provides more representative size distributions among years, this approach is limited to estimating the ages of fish that occur within the size range of the otolith -aged fish in each year (Table 2).

**Table 1.** Data sources for delta smelt used in age-length keys from 1999-2007.

| Fish Survey (institution*) | Years | Months (frequency) | Locations (stations) | Life stages |
|---|---|---|---|---|
| Mid-water trawl (MWT, CDFG) | 1967-present | September-March (monthly) | San Pablo Bay-Delta (53-113) | Juvenile-adult |
| Tow-net (TNS, CDFG) | 1959-present | June-August (bi-weekly) | Suisun Bay-Delta (~30) | Juvenile-adult |
| Plankton nets (20mm, CDFG) | 1995-present | April-June (bi-weekly) | Suisun Bay-Delta (~30) | 20mm post-larvae-juvenile |

| | | | | |
|---|---|---|---|---|
| Bay Study mid-water trawl (CDFG) | 1980-present | January-December (monthly) | So. San Francisco Bay-Suisun Bay (42) | Juvenile-adult |
| SWP & CVP Water projects (CDWR, USBR) | 1979-present | January-December (daily) | South Delta near Tracy, CA (2) | 20mm post larvae-adult |
| Mid-water trawl (USFWS) | 1976-present | April-June (~weekly) | Chipps Island (1) | Juvenile-adult |
| Beach seine (USFWS) | 1977-present | ~January-June (~bi-weekly) | Delta-Sacramento river (23) | Juvenile-adult |
| Otter trawl (UCD) | 1979-present | January-December (monthly) | Suisun Marsh (~20) | Juvenile-adult |

*California Department of Fish and Game (CDFG); University of California, Davis (UCD); California Department of Water Resources (CDWR); U.S. Bureau of Reclamation (USBR); U.S. Fish and Wildlife Service (USFWS).

**Table 2.** Sample sizes and ranges used to estimate age from lengths of delta smelt collected by IEP monitoring surveys (see Table 1).

| Year | N(aged) | N(length) | Size range(FL, mm) |
|---|---|---|---|
| 1999 | 378 | 9,434 | 14-70 |
| 2000 | 226 | 5,742 | 20-69 |
| 2001 | 398 | 5,500 | 12-70 |
| 2002 | 199 | 2,274 | 22-55 |
| 2003 | 287* | 2,894 | 14-55 |
| 2004 | 89 | 1,516 | 14-50 |
| 2005 | 150 | 801 | 26-67 |
| 2006 | 93 | 926 | 20-57 |
| 2007 | 74 | 662 | 22-69 |

* Pooled age samples from 2002 and 2004.

Age-length keys were calculated using the procedure developed by Isermann and Knight (2005). Fish in the otolith-aged samples were assigned into 2mm FL categories. Contingency tables were then developed using the range of ages within each length category to estimate the probability that a fish of a given length (i.e. the non- aged fish) belongs to each age within each length

category. This procedure expanded estimates of fish age at length for the large numbers of fish samples taken each year by IEP, ranging from 662 fish in 2007 to 9,434 fish in 1999 (Table 2). For fish in 2003, ages were estimated by combining the otolith-age estimates for samples from 2002 and 2004 to provide a complete time series from 1999-2007.
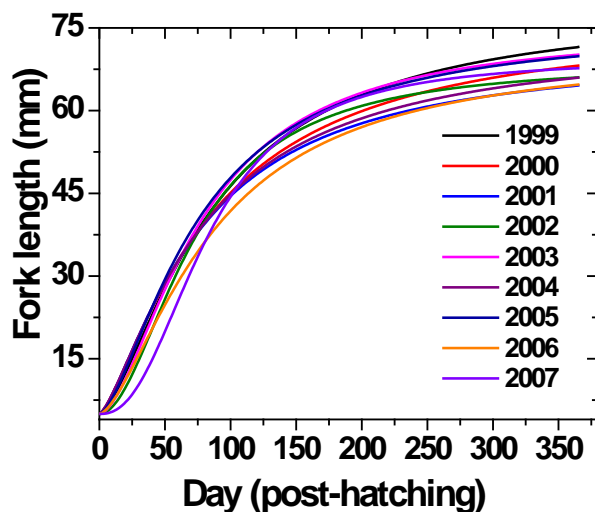


**Figure 2**. Logistic growth model fits to size at age data for delta smelt cohorts from 1999-2007.

*Fish Growth and Selection Analysis* - Delta smelt growth was then modeled by fitting the logistic growth equation to the size at age data from the age-length keys for each year from 1999-2007. The logistic equation was used because it provided a better description of delta smelt growth than other growth models, e.g. von Bertalanffy, by more accurately describing the relatively slow growth of fish during the early larval stage (Figure 2, Bennett et al. 2008). A four-parameter formulation of logistic model was used,

$$L_i = ( L_0 - L_m / 1 + (A_i /A_{mat})^p) + L_m$$

where $L_i$, is fish length at $age_i$ ($A_i$), $L_0$ is initial size, fixed at 5mm, the mean hatch size for delta smelt (Bennett 2005), $L_m$ is maximum length, and $A_{mat}$ is the age at the inflexion point, typically interpreted as the time when an individual begins to devote energy to reproduction and maturation. $L_m$ was modeled by constraining fitted values to occur within the 1% and 99% percentiles of the size distributions of delta smelt caught during February each year. In most years, such constraints had little influence on the fitted values for $L_m$, however, it was important in 2002-2004 when the available size range of otolith-aged fish was relatively narrow (Table 2).

Maximum length, $L_m$, is a key parameter of interest because it is an index of lifetime somatic growth among years, and used here to reflect size at maturity. Thus, $L_m$ constitutes a mean synthesis of the decisions among individuals regarding the tradeoffs between energy allocated for growth versus reproduction at the population-level among years. Potential changes in $L_m$ over time were then evaluated to assess whether they were consistent with those predicted from life history theory and unnatural selection. In a variety of commercial fisheries the effects of

harvesting larger individuals over many successive generations typically manifest in a decline in size at maturity (Allendorf and Hard 2009).

The intensity of unnatural selection between successive years was also estimated using procedures developed for quantitative genetics by Arnold and Wade (1984a, b)., Estimates of linear, or directional ($S_i$), selection, reflecting the degree of phenotypic change in the mean of $L_m$ were estimated using,

$$S_i = L_{m\ after} - L_{m\ before} / SD_{before} ,$$

with $L_{m\ after}$, $L_{m\ before}$, as the mean, and $SD_{before}$ as the standard deviation before selection in $L_m$, the lifetime maximum growth of individuals in the delta smelt population from 1999-2007. Estimates of the amount of linear $S_i$ are also commonly referred to as selection differentials (Arnold and Wade 1984a, b). All statistical analysis was conducted using the S-Plus and R programming languages (Venables and Ripley 2002, R Development Core Team 2008).

Finally, selection differentials were associated with estimates of several environmental variables typically associated with water export pumping and delta smelt. Physical environmental variables were obtained from web-based databases maintained by the California Department of Water Resources (DWR) and the U.S. Geological Survey (USGS), and included water temperatures in Suisun Bay from C-Dec (DWR), position of the salt-field, or X2, from Dayflow (DWR), as well as water flows in Old River and Middle River (USGS). Habitat quality for delta smelt was quantified as the probability of fish occurrence from generalized additive modeling of survey data in summer (Nobriga et al. 2008) and fall (Feyrer et al. 2007). Food abundance was estimated by estimating the abundance of calanoid copepods in the spring 20mm survey (see Bennett 2005). Adult and larval entrainment estimates for delta smelt were obtained from Kimmerer (2008).

**RESULTS**

Coefficients of determination ($r^2$) for the fitted logistic growth models were high overall, ranging from 0.75-0.96, and generally reflected the large sample sizes provided by the age-length keys (Table 3). Parameter estimates for size at maturity, $L_m$ , reflecting the mean length attained for individuals in the delta smelt population, were variable but clearly declined over time from 1999-2007 (Table 3, Figure 2). Time trends in the other fitted parameters were also variable but declined and remained low for age at maturing, $A_{mat}$ until 2006 (Figure 3B), and 2007 when the growth exponent, $p$, rebounded (Figure 3C).

**Table 3**. Summary statistics for logistic growth models fitted to size at age data for delta smelt from 1999-2007.

| Year | Size at Maturity ($L_m$, mm) | Age at Maturing (inflexion, $A_{mat}$, days) | Growth exponent ($p$) | $n$ | $r^2$ |
|---|---|---|---|---|---|
| 1999 | 79.0 ± 0.6 | 85.5 ± 1.0 | 1.50 ± 0.02 | 9,812 | 0.96 |
| 2000 | 76.6 ± 0.6 | 83.8 ± 1.2 | 1.37 ± 0.01 | 5,968 | 0.95 |
| 2001 | 80.0 ± 0.6 | 93.0 ± 1.3 | 1.29 ± 0.01 | 5,898 | 0.97 |
| 2002 | 68.7 ± 3.4 | 72.6 ± 4.3 | 1.94 ± 0.01 | 2,400 | 0.75 |
| 2003 | 74.8 ± 2.4 | 77.0 ± 3.0 | 1.70 ± 0.04 | 3,179 | 0.87 |
| 2004 | 73.1 ± 4.7 | 78.4 ± 7.6 | 1.39 ± 0.06 | 1,612 | 0.85 |
| 2005 | 75.5 ± 1.8 | 75.4 ± 2.7 | 1.55 ± 0.07 | 959 | 0.85 |
| 2006 | 71.0 ± 1.7 | 86.0 ± 3.2 | 1.56 ± 0.04 | 1,018 | 0.94 |
| 2007 | 69.5 ± 0.8 | 83.0 ± 1.0 | 2.38 ± 0.07 | 729 | 0.92 |

Selection differentials measuring the linear (directional) component of selection in size at maturity, *Lm*, were also highly variable, declining sharply from 2001-2002, and then over time after rebounding in 2003 (Figure 4A). A similar temporal pattern was exhibited by the selection differentials for age at maturing (Figure 4B), whereas the growth exponent differentials exhibited an inverse trend, reflecting the inherent tradeoffs in these model parameters.
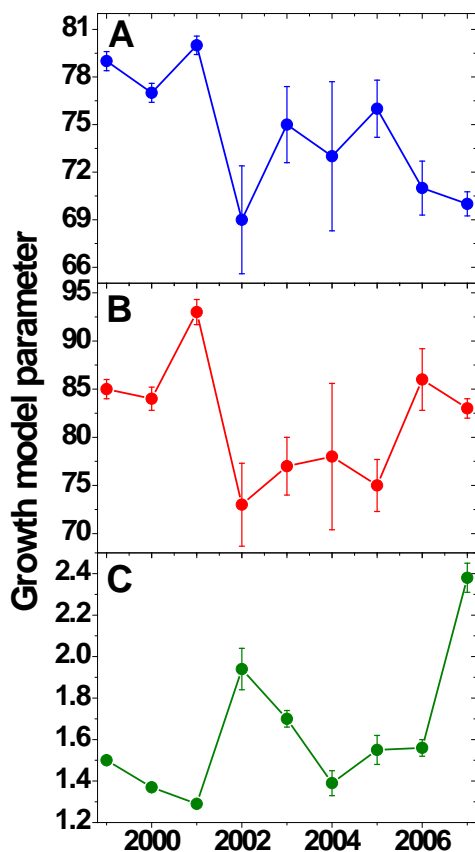


**Figure 3**. Trends in parameter estimates from Logistic growth modeling of size at age for delta smelt, including size at maturity (A), age at maturing (model inflexion point, B), and growth exponent (C).

Variables associated with entrainment effects largely exhibited negative trends with the selection differentials for size at maturity and age at maturing, including both adult and larval entrainment estimates and the position of X2 in March and April (Table 4). In contrast, variables reflecting spring food abundance and summer habitat quality exhibited positive trends, as did extreme water temperatures (number of days over 22°C) during summer (Table 4). Trends with all environmental variables and early growth, as reflected by Ω (Omega), the product of age at inflexion and the growth exponent (*p*), were negative, except for X2 (Table 4).
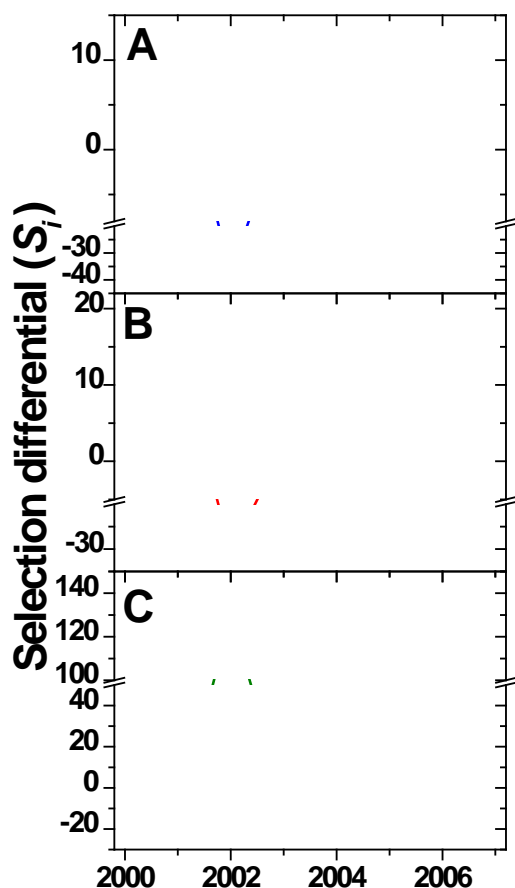
**Figure 4.** Trends in selection differentials ,estimating direction and strength of phenotypic change between parental and offspring cohorts in fitted parameters from growth modeling, including size at maturity (A), age at maturing (B), and growth exponent (C).

**Table 4**. Spearman rank correlation coefficients derived from relationships between trends in selection differentials among growth model parameters and environmental variables from 1999-2007. Coefficients were not significant at the 0.05 level.

| Environmental variable | Selection differential | | Early Growth |
|---|---|---|---|
| | Size at Maturity | Age at Inflexion | $\Omega$ (Omega) |
| Water temperature >22oC | 0.54 | 0.56 | -0.10 |
| Summer habitat quality | 0.11 | 0.13 | -0.48 |
| Fall habitat quality | -0.07 | -0.09 | -0.40 |
| Spring calanoid copepods | 0.10 | 0.38 | -0.53 |
| Adult entrainment % | -0.21 | -0.34 | -0.19 |
| Larval entrainment % | -0.38 | -0.41 | -0.30 |
| X2 March-April | -0.11 | -0.24 | 0.61 |
| Old-Middle R. flow Feb.-April | 0.09 | 0.31 | -0.05 |

The declining trend in size at maturity also mirrors that observed in the pre-adult abundance index (mid-water trawl) since 1999 (Figure 5). Moreover, a similar trend in effective population size from 2003-2009 (Fisch et al. 2011) employed the linkage disequilibrium method, which

estimates inbreeding based on levels of random allelic correlations at unlinked loci from a single sample (Palstra and Ruzzante 2008).
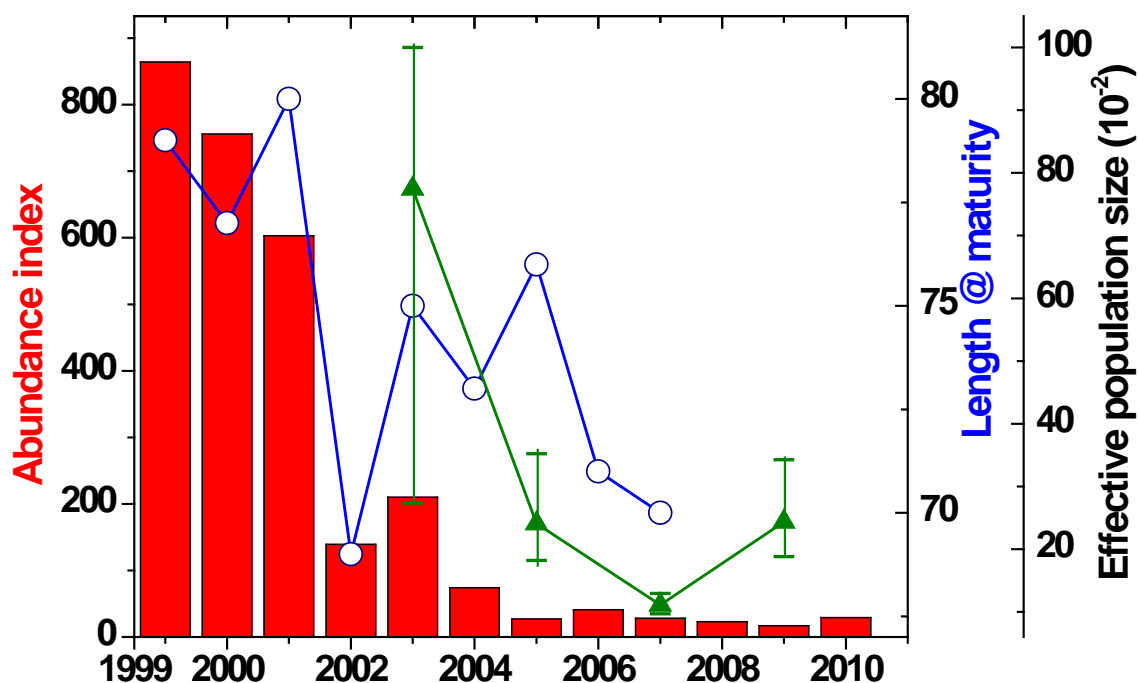


**Figure 5**. Trend in abundance indices for delta smelt (red columns), with that for estimated size at maturity (blue circles) and effective population size, $N_e$ (green triangles, from Fisch et al. 2011) . Error bars are 95% confidence limits.

## DISCUSSION

Declining size or age at maturity are diagnostic trends exhibited by a population that has sustained routine harvest of larger individuals (Conover and Munch 2002, Allendorf and Hard 2009, Hendry et al. 2011). During peak water-export operations in early spring, a high percentage of larvae were entrained and lost from the delta smelt population from about 2000-2004 (Kimmerer 2008); a trend that may have occurred regularly since the early 1990s considering the typical pattern in seasonal export operations (Figure 1).  Given that there is an apparent size threshold for maturity of >60mm, it is likely that progeny from larger females were disproportionately impacted on the fairly regular basis during the first portion of the delta smelt spawning season. After mid-April, when export pumping is ramped down considerably, entrainment mortality on larvae is substantially reduced until water temperatures exceed 20oC (Bennett 2005). Although the severity of entrainment mortality has varied annually, largely due to the amount of freshwater outflow in late winter and spring (Kimmerer 2008), it has occurred over a sufficient number of generations to qualify as an agent of selection.

A key concern with understanding the mechanisms underlying declines in size or age at maturity is in distinguishing phenotypic plasticity from genetic responses to environmental changes. Although variables reflecting change in the physical and biological environment, or those related to entrainment impacts were not significantly associated with the selection differentials, this result was likely due in part to the extremely small sample sizes (i.e., df = 6-7). Nevertheless, the directional signs of the trends are illustrative of potential underlying relationships. Variables representing spring food abundance and summer habitat quality, as well as extreme water temperatures exhibited positive trends, suggesting that adequate food supplies and habitat quality promote larger size at maturity. While extreme water temperatures are indicative of stressful conditions, it is generally accepted that warmer temperatures have a positive influence on growth. Overall, variables associated with entrainment effects exhibited negative trends with selection differentials, which suggest that both adult and larval entrainment estimates, as well as the position of X2 in March and April, promote a decline in size and age at maturity. Thus, even though this study represents changes occurring over 8-9 generations, at the annual time-step it is difficult to distinguish the relative importance of nature from nurture.

Additional information on population genetics may help understand the degree to which declines in size and age at maturity reflect ephemeral environmental responses or evidence of evolutionary change. Estimates of effective population size ($N_e$) and recent genetic bottlenecks using many of the same specimens in our growth analyses, indicated a sharp decline in $N_e$ as well as evidence for an ongoing bottleneck, indicating the population was losing genetic diversity from 2003-2009. Although their analyses did not detect a concurrent decrease in the number of rare alleles per locus, which typically occurs rather quickly, it is quite reasonable that such a loss occurred before 2003(Fisch et al. (2011). Our analyses support that idea, given the apparent dramatic decline in selection differentials estimated for size at maturity between 2001-2002.

Our results provide evidence for unintentional and unnatural selection due to a fixed seasonal schedule of water-export pumping against the progeny of delta smelt spawning in early spring. Both life history theory and our analyses (see Chap #1 this report) suggests that females spawning early are larger and exhibit significant maternal effects intended to increase the likelihood of success for their progeny, of which a substantial proportion are lost each year. We detected that a significant decline in size and age at maturity occurred as delta smelt population crashed to record-low levels after 2001. However our study was limited in the ability to distinguish whether unnatural selection was severe enough to produce evolutionary change or reflect phenotypic plasticity to other environmental factors. Although evidence for a sharply declining effective population size and an ongoing genetic bottleneck in the population provide further support for evolutionary change, further analyses of this data will be needed to definitively conclude that the observed changes were caused by unnatural selection by water-export operations. Nevertheless, the evidence presented here is sufficient to conclude that water export operations can cause significant harm to the population. Overall, as in many commercial

and recreational fisheries (Conover and Munch 2002, Allendorf and Hard 2009, Hendry et al. 2011) our results warrant higher consideration should be given to understanding the processes underlying life history variation in delta smelt: i.e., "which" individuals, rather than "how many" are entrained.

## LITERATURE CITED

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