Interplay of Environmental Forcing and Growth-Selective Mortality in the Poor Year-Class Success of Delta Smelt in 2005

Final Report

"Fish Otolith and Condition Study 2005"

Prepared for the
The Pelagic Organism Decline Management Team

by

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Background

Delta smelt, *Hypomesus transpacificus*, is one of the most imperiled and controversial fish species due to its impact on California water policy and world-class economy. Record-low abundance of this threatened species combined with their ongoing entrainment in state (SWP) and federal (CVP) water pumping plants, recently prompted a California Superior Court to order severe reductions in water exports to the state's multi-billion dollar agricultural industry and about 25 million residents. The current status of delta smelt has heightened tensions between water development and 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. Water export effects are embedded within a complex array of interacting factors, including reduced extent and quality of habitat, exotic species, multiple pollutants, numerous in-Delta water diversions, and climate variability (Bennett and Moyle 1996, Bennett 2005, Feyrer et al. 2006, Sommer et al. 2007, Nobriga et al. 2008). Entrainment in the export facilities, however, continues to reign as the most conspicuous and controversial issue, even as intensified research is accelerating our understanding of the magnitude of this problem (Kimmerer 2008, Grimaldo et al. *submitted*). Given the recent crisis, 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).

Here we report on our efforts to understand multiple, potentially interactive, factors that may be contributing to the extreme low abundance of delta smelt, focusing on the poor year-class of 2005. Our goal was to design and implement an effective program for integrating multiple sources of information from individual fish caught during routine monitoring by the Pelagic Organism Decline (PDO) initiative of 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 and micro-chemistry

respectively), as well as histopathology of key fish organs to assess factors influencing health (i.e. qualitative and quantitative evaluations of liver and gonads).
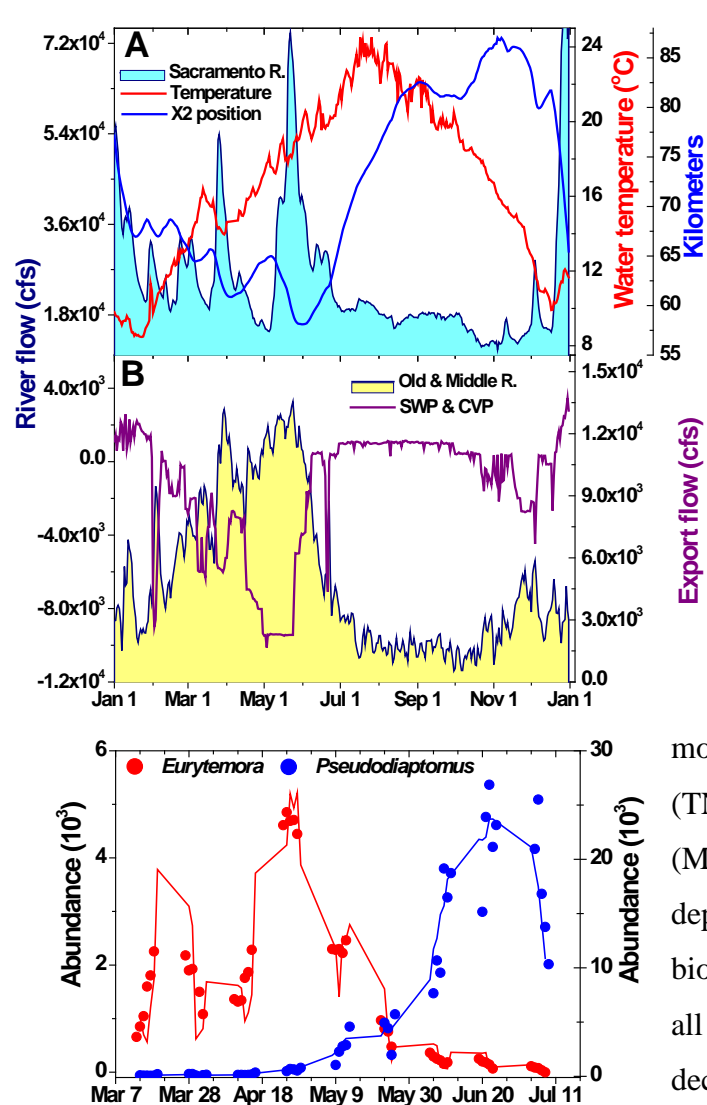
Extraction of growth information from otoliths and assessing tissue condition via histology are fairly routinely applied in fish resource assessments. Typically, however, they are implemented independently and somewhat haphazardly with respect to each other by researchers often from separate disciplines. Our approach differs considerably in that these tools are applied in a holistic fashion to each individual, where possible, in fish samples collected during monitoring. We envision that the 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* (low abundance index) observed at the population-level. Our previous work in this area has stressed the value of integrating similar complementary techniques (i.e. biomarkers) 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). We apply this interdisciplinary approach to delta smelt in an attempt to quantify the likely factors causing the 2005 year-class to be regarded as one of the most critically low recruitment years on record.

Methods

Environmental Data. A variety of data sources were used to complement our evaluations of fish condition and growth. Daily time trends of these variables over the period of study are shown in Figure 1. Data for Sacramento River flow, water export flows at the state and federal water projects, and position of the low salinity zone (i.e. X_2) were compiled from DAYFLOW (http://wwwiep.water.ca.gov/dayflow/index.html), and for flow in the Old and Middle rivers (that lead to the export facilities) from the USGS at:
(http://waterdata.usgs.gov/nwis/dv?cb_72137=on&format=html&begin_date=2007-01-01&end_date=2007-12-31&site_no=11313405&referred_module=sw
http://waterdata.usgs.gov/nwis/dv?cb_72137=on&format=html&begin_date=2007-01-01&end_date=2007-12-31&site_no=11312676&referred_module=sw) (Figure 1).

Data on water temperatures from four continuous monitoring stations located at Martinez, Pittsburg, Antioch, and Burns Cutoff were compiled 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 also used in estimating larval hatch-sizes and spawning-dates (as described below).

Finally, data on zooplankton abundance collected during delta smelt sampling was obtained from the IEP 20mm Survey (ftp://ftp.delta.dfg.ca.gov/Delta%20Smelt/). Abundances of *Eurytemora affinis* and *Pseudodiaptomus forbesii* were pooled for all stations recording salinity in the range 0-5psu and plotted in relation to sampling date in 2005 (Figure 1).

Figure 1. Environmental trends in 2005. (A) Sacramento river flow, daily mean water temperature in Suisun Bay and Delta, and daily mean position of the salt-field (2psu, or X2) are shown with (B) total water export flows in the state and federal pumping facilities, as well as water flows in Old and Middle rivers adjacent to the pumping facilities. Also plotted (C) are trends in traditional copepod prey showing the seasonal abundance minimum (or, notch) produced by the phenology of these species.

Field Sampling. All delta smelt were collected in the course of routine fish monitoring by the Summer Tow net Survey (TNS) and Fall Mid-water Trawl Survey (MWT) conducted by the IEP. 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 that pioneered the application of 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 Backcalculations. Otolith size was converted to fish body size using a modified version of 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 for fish sampled at a specific life stage, the relationship between otolith and fish body growth typically changes between early and later life. Previously, we modified Campana's method to handle such differences between delta smelt larvae and juveniles by fitting separate linear regression models to the otolith/fish size relationship (Hobbs et al. 2007). Determining daily growth from otoliths is rarely attempted with older (pre-adult) fish. Therefore, in the current study we further refined a novel method, modifying this general procedure by fitting a segmented, or broken-line, linear

regression to identify biological intercepts, or change-points, in the otolith/fish size relationship (Figure 2A). This involves using an optimization procedure (function "optimize" in S-Plus) to estimate the point at which the otolith / fish size relationship changes slope, along with the standard regression coefficients (Venables and Ripley 2002). Using all 2005 samples (N =167), the procedure estimated biological intercepts at x=5.19mm (y = -12.55 days) and at x= 45.5mm (or 98 days post-hatch) that correspond to biologically meaningful life history stages, fish hatch-size and size at the juvenile to pre-adult transition, respectively (Figure 2A). Size at age for larger fish was then back-calculated to 45.5mm, before using an individually-based weighting for the intercept at hatch-size based on estimated water temperature during the incubation period (see below).
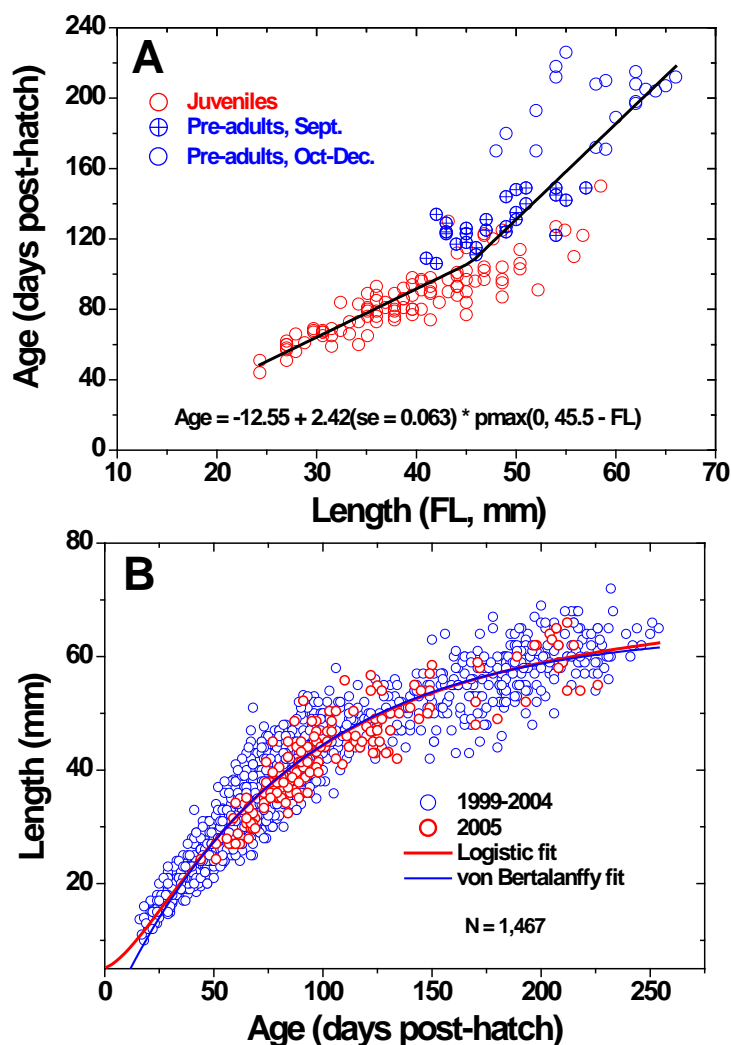

Figure 2. Delta smelt growth. (A) Otolith size to body length relationship used to back-calculate size at age. Data fitted with a broken-line regression (see Methods) to account for change in body growth from the juvenile to pre-adult life stage. (B) Body size at age relationship fitted using two standard growth functions.

Delta smelt growth was then modeled using the von Bertalanffy and Logistic growth equations (Figure 2B) using size at age estimates for fish collected from 1999-2005 (N = 1,467, Figure 2B). Akaike's Information Criterion (AIC) was then used to compare the fit of each model (Burnham and Anderson 2002). The logistic function (AIC = 8,147) provided a better description of delta smelt growth than von Bertalanffy (AIC = 8,173), with the lower AIC indicating it more accurately represented relatively slow

growth during the early larval stage (Figure 2B).
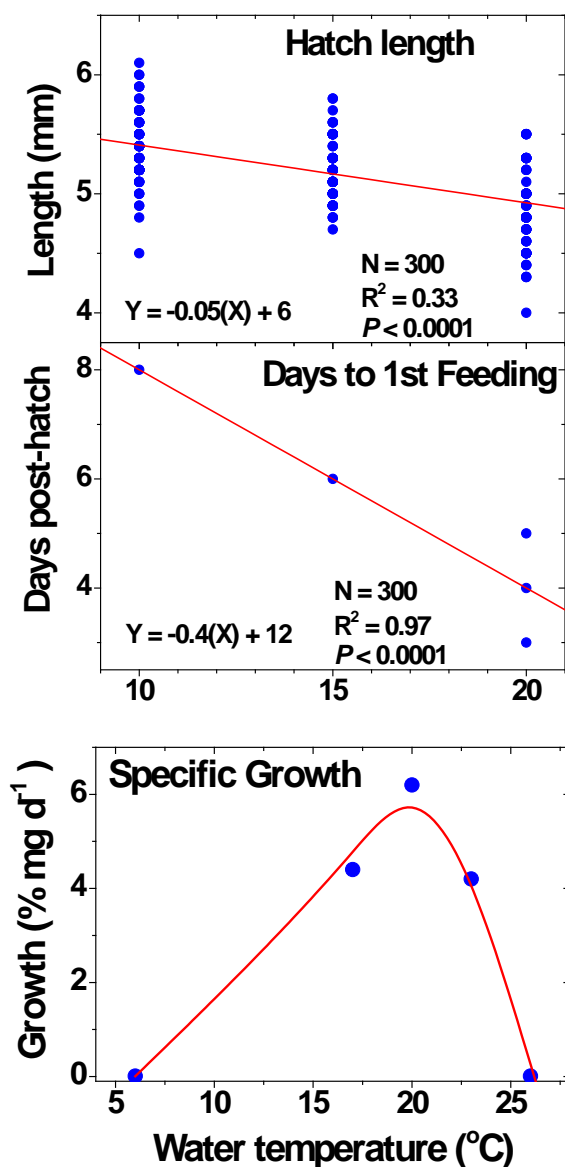
Laboratory studies have shown that egg incubation time, hatch-size, and initial feeding success are strongly influenced by water temperatures that typically range widely (about 10°C) over the delta smelt spawning season (Baskerville-Bridges et al. 2004).

Figure 3. Water temperature influences on hatch length, and development to first-feeding, as well as growth in the juvenile stage (i.e. specific growth) from studies by Baskerville et al. (2004) during aquaculture. Data on hatch-length and development fitted using linear regression. A cubic spline model was fitted to approximate the functional form of specific growth with respect to water temperature.

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 $\pm$ 0.24 SD, for fish incubated 15°C in that study, which corresponds well with our calculated biological intercept, 5.19 $\pm$ 1.8 SD (Figure 3). We then substituted water temperatures averaged over the week prior to hatching (see Figure 1) into the regression equation to estimate hatch size for each fish (Figure 3). 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, shown here as days to first-feeding, Figure 3).

Finally, we also developed a preliminary specific growth relationship with respect to water temperature (Figure 3). Specific growth relationships reflect the percent change in fish body weight per day given adequate food over a broad range of water temperatures (Brett 1979, Elliot

1994). Given the data available, we developed a prototype relationship by first plotting specific growth measures for juvenile delta smelt estimated at the aquaculture facilities at 4.4 % d^{-1} (17°C), 6.2% d^{-1} (20°C), and 4.2% d^{-1} (23°C) by Baskerville-Bridges et al. (2004) with the approximate thermal limits for delta smelt: about 26 ^{o}C on the high-end (mean = 25.5 ^{o}C, Swanson et al. 2002), and about 6 ^{o}C on the low-end, or an approximate minimum water temperature fish might experience in the Delta. The plotted values were then fit with a cubic-spline smoother to approximate the specific growth relationship (assuming a hyperbolic relationship, in the sense of Brett 1979) for juvenile delta smelt (Figure 3).

Otolith Strontium Isotope Analyses. Micro-chemical methods generally follow those outlined in our previous work (Hobbs et al. 2005). Following otolith increment analyses (or at least after photographing) they were washed with 1% nitric acid for 5 to 10 seconds, rinsed in an ultrasonic water-bath for 5 minutes, and then allowed to dry. Strontium isotopic compositions were then measured at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry (http://icpms.ucdavis.edu). At this facility, a multi-collector inductively coupled plasma mass spectrometer (Nu Plasma HR from Nu Instrument Inc.) is interfaced with a Nd:YAG 213 nm laser (New Wave Research UP213) for measuring strontium isotopes by laser ablation (i.e. LA-MC-ICP-MS). Single spots at the core and the mid-point of the otoliths were assayed using a laser beam (60 micron dia. Typical 88Sr signals of 2-6 Volts were obtained during the analyses. Helium gas was used as a carrier to maximize sensitivity and minimize sample deposition at the ablation site, and was mixed with additional argon after the laser sample cell. Gas blank and background signals were monitored and then subtracted from the measured signals automatically. The 86Sr/88Sr ratio, 0.1194, was used to correct for instrumental fractionation in accordance with the exponential law, and peak intensities for 88Sr, 87Sr, 86Sr, 85Rb, and 84Sr were measured simultaneously. The 85Rb peak was also monitored to correct for any 87Rb interference on 87Sr. We also measured strontium isotopes from water samples collected at 18 locations throughout the Delta and into Suisun Bay. These measurements were used with a simple water mixing model to help provide a baseline from which to assign rearing locations according to the strontium ratios measured in the otoliths of pre-adult delta smelt from the MWT.

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 (Figure 4), to reflect the increasing likelihood of mortality or reproductive failure.
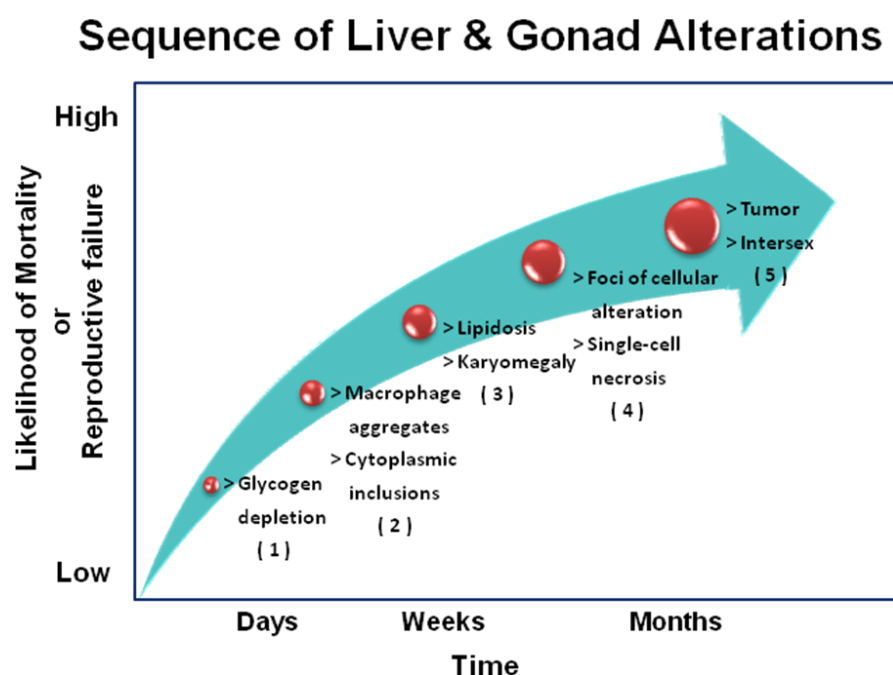

Figure 4. Conceptual diagram showing the temporal sequences among the typical cellular abnormalities scored during histological assessment of delta smelt tissues. Likelihood of mortality or reproductive failure increases as altered cells become more difficult to repair over time (i.e. alteration increases in severity). Numbers in parentheses are weightings.

Statistical Analyses

Fish Size and Growth Selection. Potential size or growth selective mortality was first assessed by determining size-at-age, or weekly growth-at-age, for individuals during potentially critical life history stages among successive sampling periods for delta smelt in 2005 (Table 1). First, to account for ontogenetic influences on these distributions, size and growth at age estimates were standardized to zero mean and unit variance among all individuals (N = 167). Assessing relative survival requires comparisons of fish among successive sampling periods because each sampling effort can only collect those fish surviving to that point in time. For example, growth rate during

the first 2 weeks post-hatch was compared among sampling periods from June through December to test if initial growth may have influenced survival to the adult stage. A shift up in the distribution of the standardized values of this trait in successive samples would suggest that individuals with rapid growth during their first 2 weeks of life had higher survival relative to fish growing more slowly. Similarly, a shift down among successive samples would suggest fish initially growing slower had higher survival. Essentially, this approach estimates potential directional selection, or the nonlinear selection differential (in quantitative genetic terminology, Lande and Arnold 1983), for higher or slower initial growth rate. Potential selective mortality was also assessed in this manner for initial size (i.e., otolith core size), growth rate during the approximate week of swim-bladder inflation (age 30-43 days post-hatch), growth rate from May 21-28 (approximate week of seasonal low in copepod food abundance, Figure 1), as well as for growth rate in successive weeks of July: e.g. June 29-July 5; July 5-July12; July 13-20; and July 20-27. We envisioned that potential selective mortality is likely to have been more important during July, because environmental conditions appeared least favorable during this period of the summer (Figure 1), with suitable habitat likely limited (Bennett 2005, Nobriga et al. 2008). Potential shifts in size or growth were then assessed with respect to sampling period and hatch-date using ANOVA, or generalized linear modeling.

Estimating Natural Selection. We then estimated the intensity of natural selection between successive periods using the relationships developed by Arnold and Wade (1984a,b). Essentially, these equations were designed to estimate the amount of linear (S_i) and nonlinear (C_i) selection, or degree of phenotypic change in the mean and variance of specific traits, where

$$S_i = z_{after}\text{-}z_{before} / SD_{before} \qquad \text{and} \qquad C_i = Var_{after} - Var_{before} + S_i^2,$$

with z_{after}, z_{before}, and Var_{after}, Var_{before} as the mean and variance, respectfully, of standardized size or growth (z), with SD_{before} as the standard deviation of z before selection, during each sampling period.. Changes in the distribution of size or growth (z 's) between successive periods were then evaluated using Two-sample Kolmogorov-Smirnov tests.

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

$h(z) = e^u/1-e^u$,

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

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

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 varied widely during 2005, especially with respect to the timing and magnitude of freshwater inflows, water export flows, and water temperatures (Figure 1). Most striking was the unusual timing of annual precipitation which arrived in May through mid-June, rather than in winter. This highly unusual weather pattern resulted in extremely high Sacramento River flows from May-July (peak flows were 72,900 cfs on May 22nd) (Figure 1). Water export flows were also high during winter, maintaining high negative flows in Old and Middle rivers until the VAMP pumping restrictions engaged from mid-April to mid-May (Figure 1); export flows increased dramatically after this period and persisted through the fall. After the unusual storm period, freshwater inflows returned to normal in mid-June. However, water temperatures and salinity intrusion (indexed as X2 position, Figure 1) also increased dramatically, reaching 24°C and 80km, respectively by mid-July. Typical zooplankton prey for delta smelt also varied widely in abundance. *Eurytemora affinis* abundance was variable during spring, but then declined sharply through May, about one week before the seasonal rise in *Pseudodiaptomus forbesii* abundance, producing a seasonal low in the combined relative abundances of these species during the last week of May.

Field Sampling. Our analyses included the majority of delta smelt caught in the TNS and MWT in 2005. Juveniles caught in the TNS, from late-June through August, came primarily from stations located in the north channel of Suisun Bay from Grizzly Bay (station 602) through Suisun Cut (stations 501, 519), Chipps Island (station 508) and over to the lower Sacramento river (station 704); only 4 fish were caught in the southern ship channel (station 504). Pre-adults caught from September to December were similarly distributed, ranging a bit more widely from Benicia (station 411) to Prospect Island area of the Sacramento River (station 716). Age and growth were successfully analyzed from 104 of the 120 (87%) juveniles, and from 47 of the 55 (85%) pre-adults (Table 1). This included analysis of 27 fish caught during a special MWT in September to evaluate gear selectivity. These fish apparently passed through the mesh of the standard sampling net and were retained by an outer covering of smaller mesh size. Specimens not analyzed were either retained for other POD studies, were damaged, or otherwise culled during processing. Strontium isotopes so far have been analyzed in 18 otoliths (33%) from pre-adult fish in the MWT, with additional analyses ongoing to increase this sample size. Histopathology of liver and gonad tissues was performed on 42 juveniles (40%) from the TNS, and for 45 pre-adults (82%) from the MWT, including the special September survey (Table 1). The remaining fish are archived at UCD pending future analysis.

Table 1. Delta smelt collected and analyzed in the TNS and MWT in 2005. Entries are numbers of fish collected/otoliths analyzed (not analyzed or used in other POD studies) by station and sampling cycle for each survey. Also shown are percent of total samples analyzed for growth (% otolith), strontium isotope ratios (% micro), and histopathology (% histo) for each survey.

| Station | Summer Tow net Survey (TNS) | | | | | | Fall Mid-water Trawl Survey (MWT) | | | | | |
	1 06/13	2 06/27	3 07/11	4 07/25	5 08/08	6 08/22	3 Sept*	3 Sept	4 Oct	5 Nov	6 Dec	Total
411							1					1
414							1					1
418							2					2
501		2			7	12(4)						21(4)
504				1	1	2						4
507									2			2
508			1	5								6
509										1		1
510								0(1)				0(1)
512									1		1	2
513		0(1)		1	1		1				0(1)	3(2)
515							1					1
516							7					7
517							1				1	2
519		4	9	7(1)	18							38(1)
602	1(2)			16	5(1)	2(2)	2	0(1)				26(4)
603							6					6
604							2		1			3
606											2	2
609	0(1)											0(1)
701									0(1)			1
703											1	1
704		3	2			1(1)	1		1			8(1)
706		2				0(2)	1		3(1)	0(1)	1	7(4)
709										1		1
715									2			2
716							1	0(1)				1(1)
801	0(1)		1									1(1)
802								0(1)	1			1(1)
804										1		1
Total	1(4)	11(1)	13	30(1)	32(1)	17(9)	27	0(4)	8(2)	6(1)	6(1)	151(24)
% otolith						87					85	86
% micro						0					33	15
% histo						40					82	73

Otolith increment analyses. Estimated hatch-dates ranged from March 9 to June 15 for fish in the TNS and from March 10 to May 20 for MWT fish, with the majority of larvae hatching during the first week of May (Figure 5). Fish that hatched in March were caught only in the June TNS surveys (1 and 2), presumably because they had grown large enough (over 50mm) to avoid the TNS gear by July, and then reappeared in the catch of the October MWT. Fish caught in the September gear effectiveness study had hatched primarily during the first week of May, thus were generally too small (about 45-50mm FL) to be retained by the standard MWT gear. Estimated spawn-dates tend to overlap with those in which ripe females were sampled in the spring Kodiak Trawl survey (Figure 5).
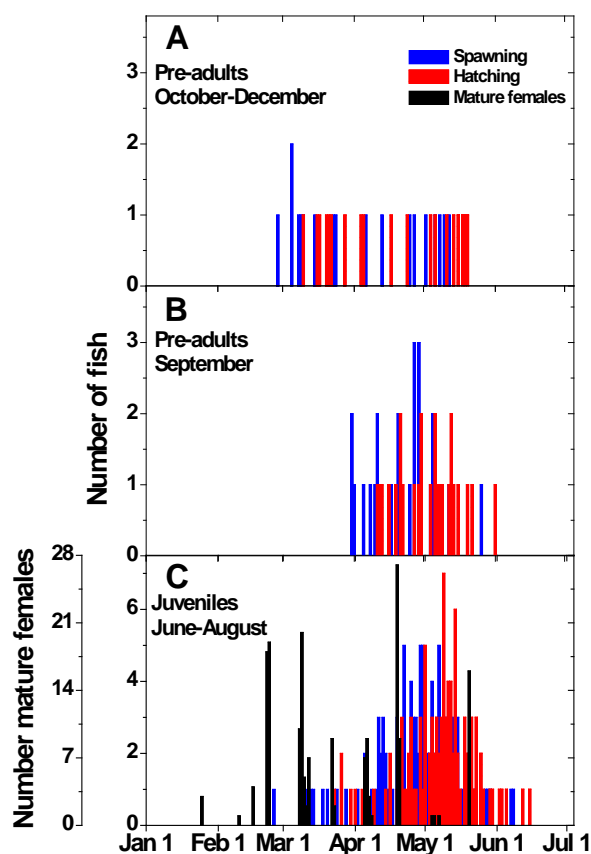

Figure 5. Distribution of estimated hatch-dates, and spawn-dates for (A) pre-adults from the MWT and (B) the special September MWT sampling, with (C) juveniles from the summer TNS. Also shown is the distribution of mature (ripe) females caught in the spring Kodiak trawl survey in 2005 (i.e. spawning females).

Otolith Strontium Isotope Analyses. Strontium isotope ratios (87Sr:86Sr) exhibit an exponential relationship with salinity in water samples taken at various locations in the Delta and Suisun Bay (Figure 6A). This suggests a conservative mixing of fresh and marine water with respect to strontium ratio (Figure 6A). We then used these measures to predict strontium ratios likely occurring in Delta water which typically reflects a mixture of the Sacramento River (Sr ratio at 3-mile slough = 0.7063) and San Joaquin River water (Sr ratio at Mossdale = 0.7072) (Figure 6B). Measured strontium ratios in the north Delta more often resemble Sacramento River water, whereas those from the south Delta are closer to San Joaquin River values. However, seasonal hydrology and water export operations can substantially alter this pattern. To determine approximate natal history using

strontium ratios measured from delta smelt otoliths, we judged values < 0.7065 to reflect the north Delta, 0.7066-0.7070 as the central Delta, and 0.7071- 0.7072 as a south Delta signature. Strontium ratios >0.7073 and of concentration >3ppm presumably reflect low-salinity water, and thus derived from Suisun Bay.
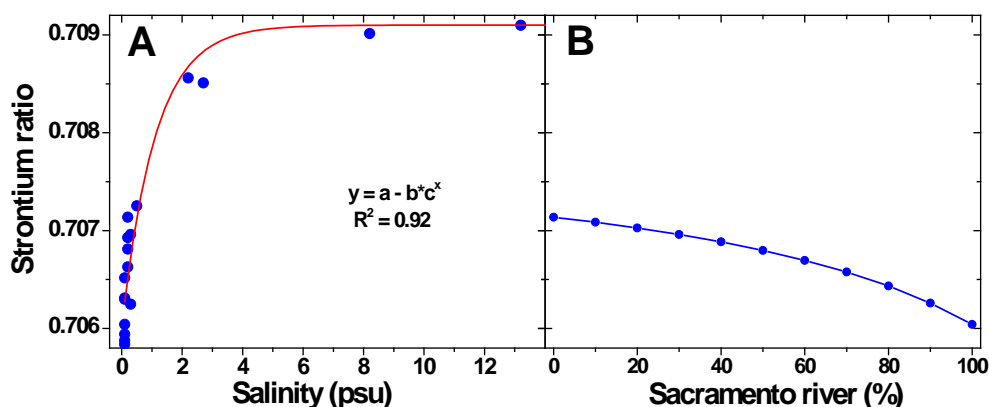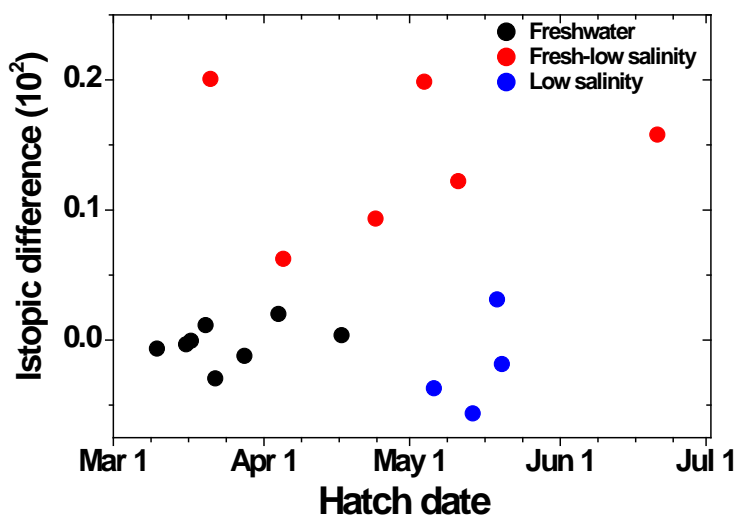

Figure 6. (A) Strontium Isotope ratios and salinities measured in water samples taken from various locations in Suisun Bay and the Delta, with (B) ratios predicted for the Delta depending on the proportion of Sacramento river water occurring at a particular location or time.

Strontium isotope ratios measured from otoliths suggest three distinct patterns of natal origin and rearing history for delta smelt in 2005 (Figure 7). Overall, strontium ratios measured at the core and midpoint of the otoliths varied from 0.7061-0.7084. After assigning each fish to natal location based on its respective core ratio (Figure 6), as well as the difference between the core and mid-point ratios, delta smelt appear to have hatched and reared

Figure 7. Difference in strontium isotope ratio measurements taken at the core and mid-point of adult delta smelt otoliths in relation to estimated hatch-date.

either (1) exclusively in the Delta, or (2) at higher salinity (i.e., Suisun Bay), or (3) had hatched in the Delta and then reared at higher salinity (i.e. had large differences between their

mid-point and core ratios) (Figure 7). Fish rearing in the Delta hatched earlier in the season than those that hatched and reared at higher salinity (i.e., Suisun Bay), whereas some fish consistently hatched in the delta but then reared at higher salinity throughout the spawning season (Figure 7).

Histopathology Analyses. Histologic scoring indicated a substantial proportion of the delta smelt caught in 2005 had depleted glycogen or otherwise poor liver condition. Out of the 42 juveniles from the TNS examined, almost three-quarters (74%) showed some signs of glycogen depletion (index > 0, Figure 8D), with over one-half (55%) of the fish exhibiting severe shortages (index >7). Almost one-half (48%) of the fish also exhibited other liver alterations, primarily single-cell necrosis, found in 28% of these individuals (Figure 8C). The majority of pre-adult fish (n = 47) from the MWT survey exhibited similar, or worse, condition. Over 80% showed some indication of glycogen loss (Figure 8B), and over one-half (60%) were severely depleted at the time of capture (Figure 9). Similarly, 85% of the fish had other liver abnormalities, with 48% exhibiting signs of single-cell necrosis, and 3 individuals were diagnosed with ovatestis, or intersex, including 1 out of only 6 fish caught in the December survey (Figure 9).
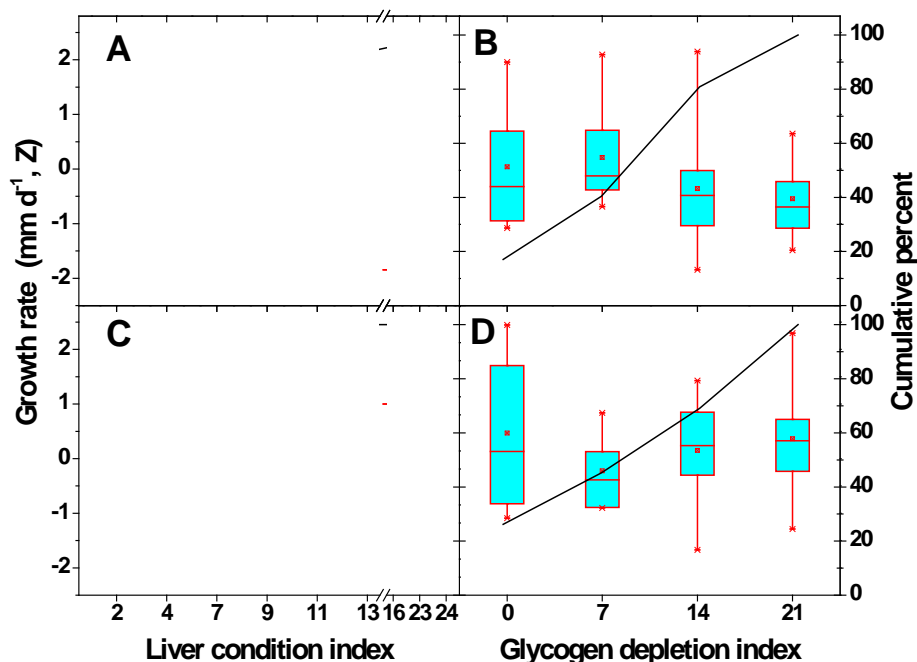

Figure 8. Relationships between growth rate (one week prior to capture) and histological diagnosis of delta smelt condition. Recent growth with overall liver condition for (A) MWT and (C) TNS fish; and with energy reserves (glycogen in hepatocytes) for (B) MWT and (D) TNS samples. Also shown are cumulative percent of condition index scores for individuals from each survey (black line).

Poor liver condition was generally associated with reduced growth rate during the week prior to capture. Growth rates in juvenile fish were more sensitive to glycogen depletion, with lower than average growth occurring in individuals with only moderate loss (index = 7), whereas pre-adult fish appear more

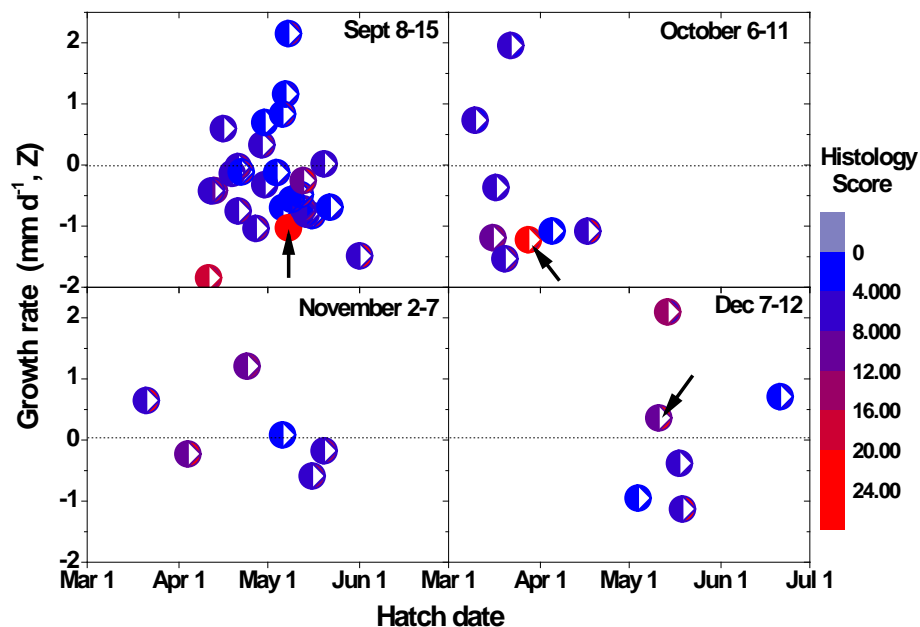tolerant showing reduced growth only after severe depletion (index = 14, Figure 8B, 9).

Figure 9. Growth rates (one week prior to capture) in relation to hatch-date for individuals collected in the MWT. Plotted points are color-shaded to represent histological scoring of liver condition (left-half), and glycogen depletion (right-half). Arrows designate 3 fish diagnosed with ovatestis (intersex).

Natural Selection for Fish Size and Growth. Our assessment of size and growth distributions among the different sampling periods suggests selective mortality was important during the summer of 2005. Clear shifts in the distributions of several size and growth characteristics emerge during late-August to September and maintain until December (compare Figures 10-17). Fish surviving to the pre-adult stage had significantly smaller otoliths at hatching (Figure 10) and slower growth rates during the first 2 weeks post-hatch (Figure 11), as well as during the period of low food abundance in late May (Figure 13, Table 2). Growth also tended to be slower during the time of swim-bladder inflation (Figure 12) although the trend is not significant at the 0.05 level (Table 2). As juveniles, however, the survivors were those also able to maintain higher than average growth rates during the first 2 weeks of July (Figures 13-17). This seemingly counterintuitive set of characteristics appears to have been a prerequisite for fish survival to the pre-adult stage.

Adding fish hatch-dates as a factor with sampling period in the ANOVA modeling further indicated that selection tended to also favor individuals hatching earlier in the spawning season (Table 2). Overall, individuals hatching before May tended to grow more slowly as larvae, but as juveniles were then apparently better able to maintain faster growth during July, which

disproportionately enhanced their survival relative to fish spawned later during May-June (Figures 11-17, Table 2). Thus, selection for size and growth appears to have reversed direction during ontogeny; larval and juvenile characteristics influencing survival are negatively correlated between these life history stages (Figure 18).
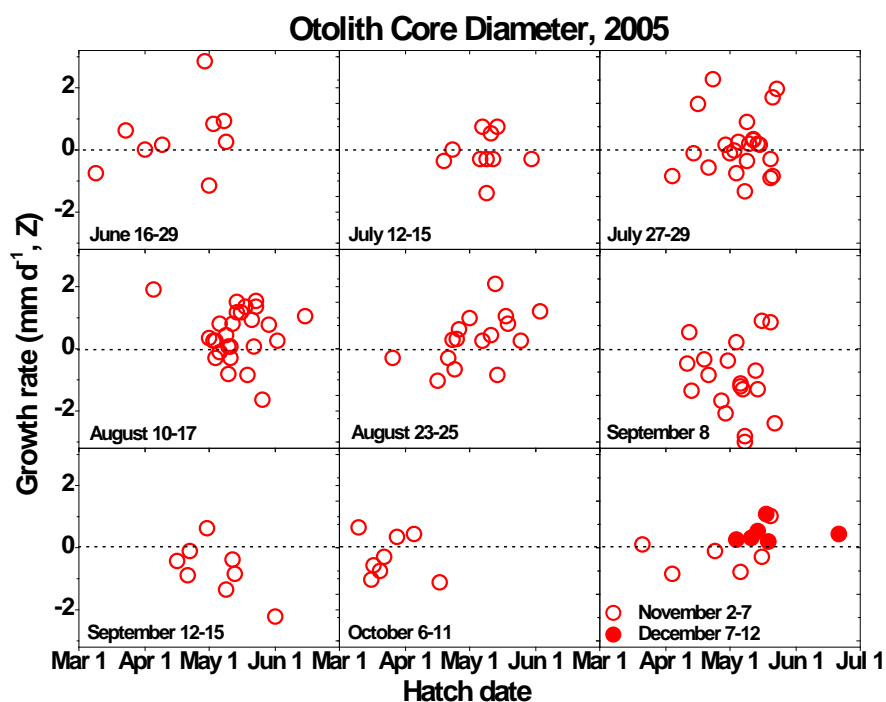

Figure 10. Otolith core sizes at hatching in relation to hatch-date for fish caught during successive sampling periods.
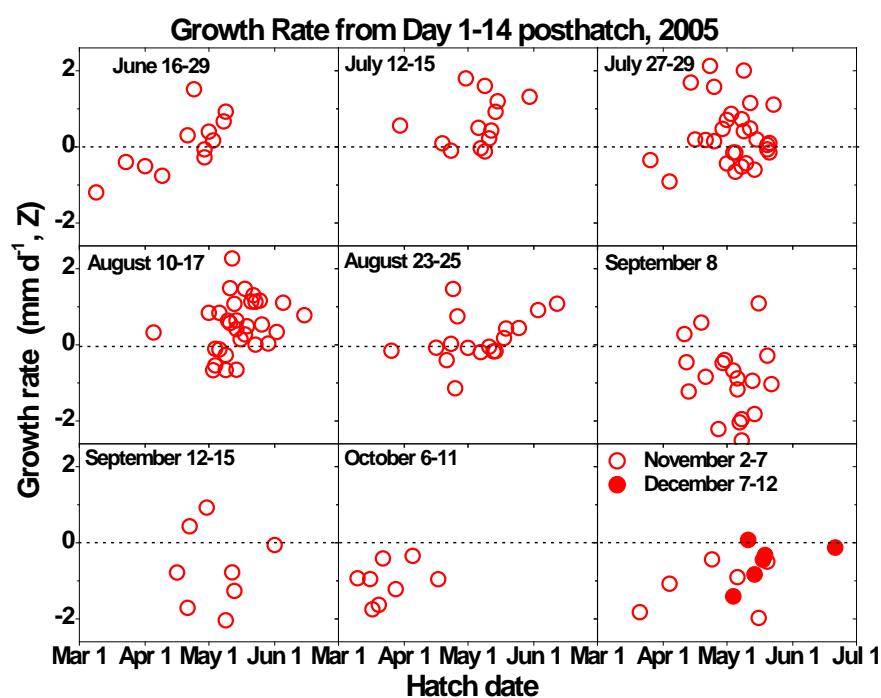

Figure 11. Growth rates during the first 2 weeks post-hatching in relation to hatch-date for fish caught during successive sampling periods.
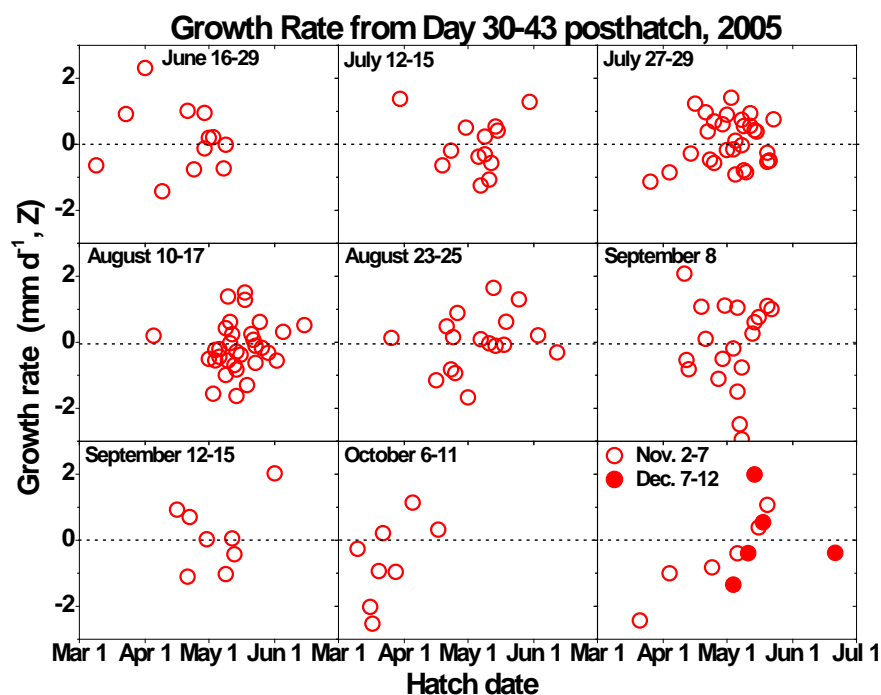
Growth Rate from Day 30-43 posthatch, 2005

Figure 12. Growth rates during the period of swim-bladder inflation in relation to hatch-date for fish caught during successive sampling periods.

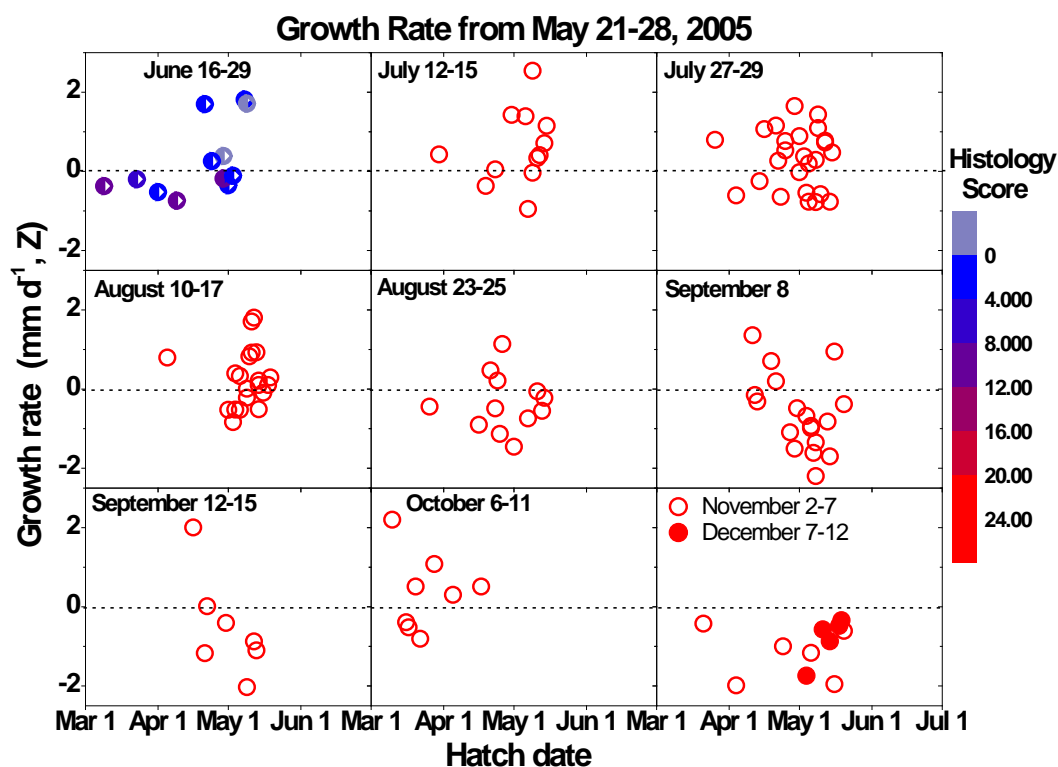## Growth Rate from May 21-28, 2005

Figure 13. Growth rates during the seasonal low in collective abundances of the zooplankton prey species, *Eurytemora affinis* and *Pseudodiaptomus forbesi*, in relation to hatch-date for fish caught during successive sampling periods. Plotted points are color-shaded for the June sampling period to show histological scoring of liver condition (left-half), and glycogen depletion (right-half) in relation to growth (about one month earlier).
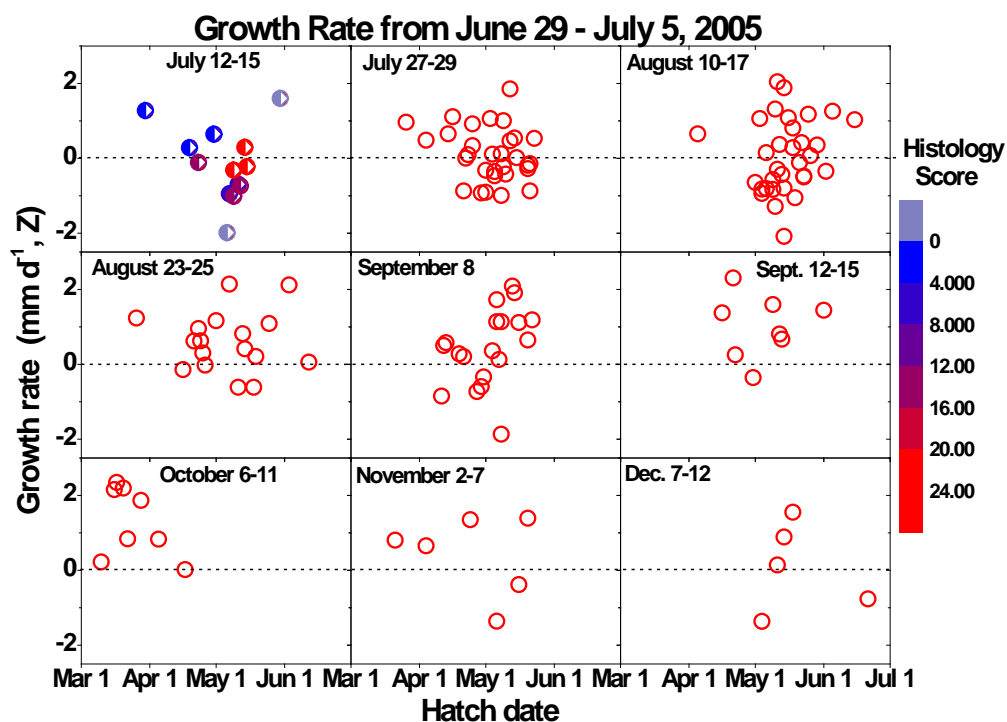
Growth Rate from June 29 - July 5, 2005

Figure 14. Growth rates during late-June and first week of July in relation to hatch-date for fish caught during successive sampling periods. Plotted points are color-shaded for the July 12-15 sampling period to show histological scoring of liver condition (left-half), and glycogen depletion (right-half) in relation to growth (about two weeks earlier).

Growth Rate from July 5-12, 2005

Figure 15. Growth rates during early July in relation to hatch-date for fish caught during successive sampling periods. Plotted points are color-shaded for the July 12-15 sampling period to show histological scoring of liver condition (left-half), and glycogen depletion (right-half) in relation to growth (over the week prior to capture).

Figure 16. Growth rates during mid-July in relation to hatch-date for fish caught during successive sampling periods. Plotted points are color-shaded for the late-July sampling period to show histological scoring of liver condition (left-half), and glycogen depletion (right-half) in relation to growth (about 2 weeks prior to capture).
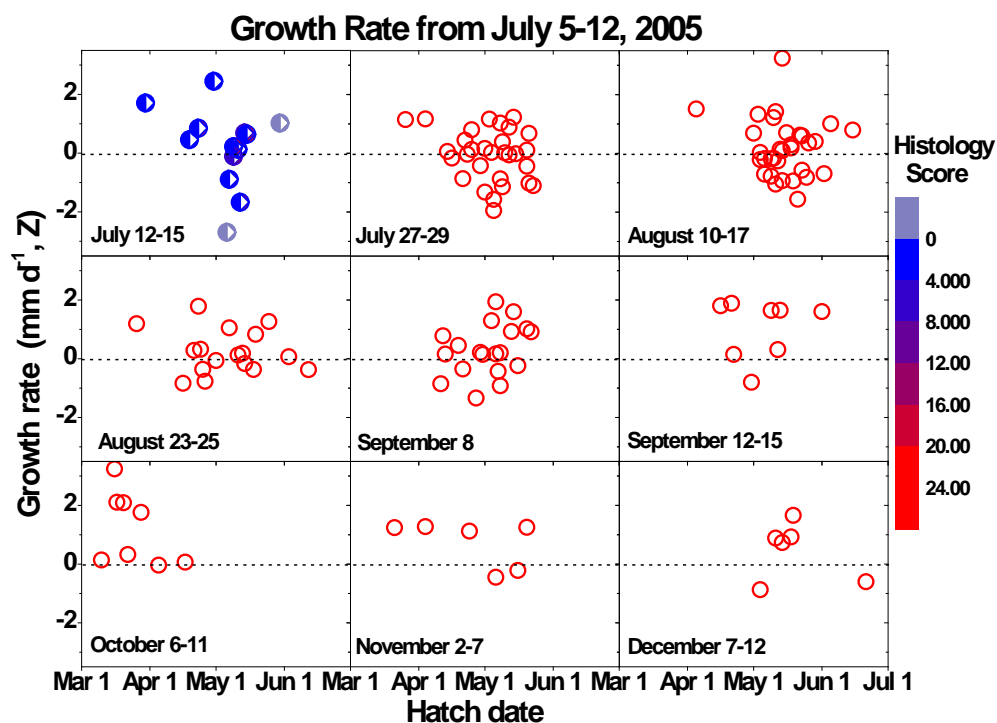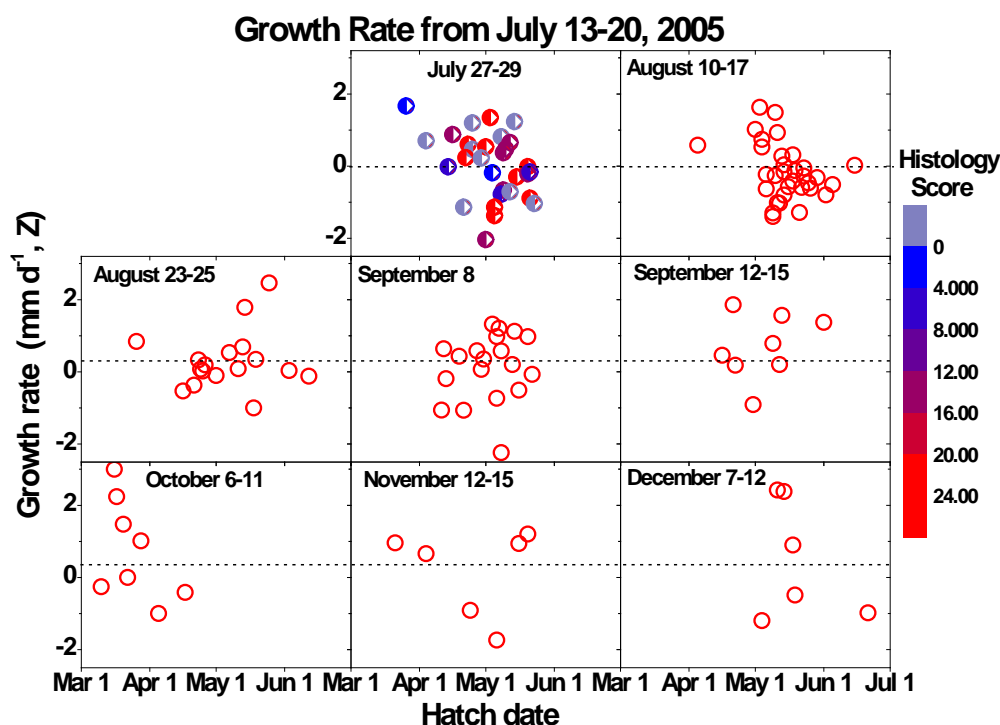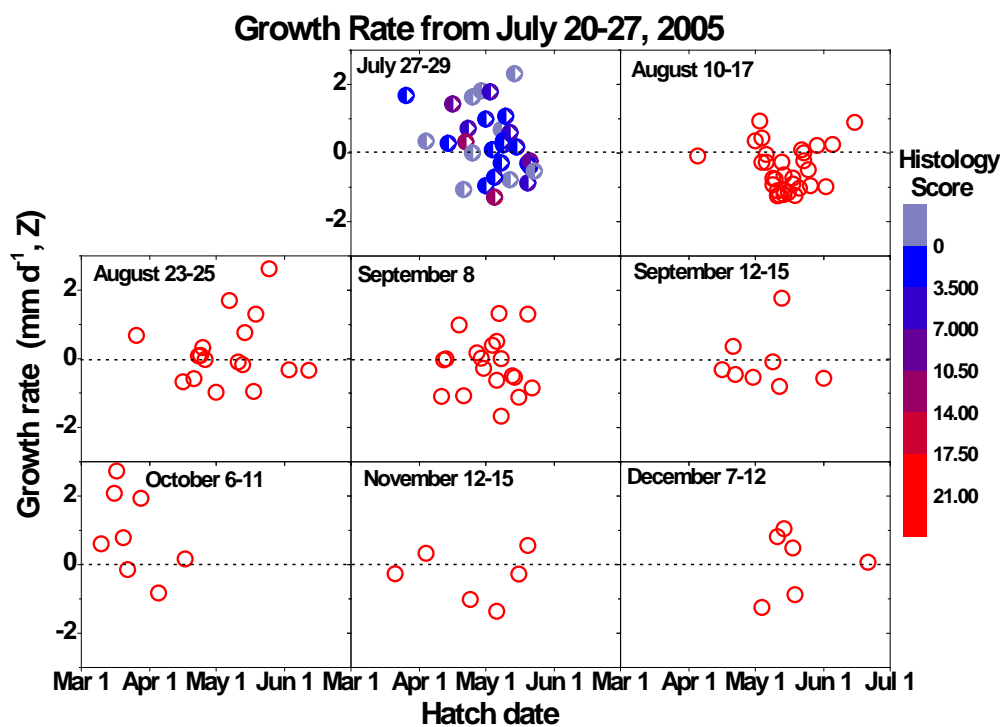

Figure 17. Growth rates during early July in relation to hatch-date for fish caught during successive sampling periods. Plotted points are color-shaded for the July 12-15 sampling period to show histological scoring of liver condition (left-half), and glycogen depletion (right-half) in relation to growth (over the week prior to capture).

Table 2. Summary of results from two-way ANOVA models evaluating the degree of change in fish size or growth characteristics among sampling periods and with respect to hatch-date. Values are F-statistics (mean square error), and shadings indicate significance level, $p < 0.05$.

| Size or Growth Character	Sampling Period	Hatch Date	Interaction
Core size	5.12 (4.98)	1.10 (1.07)	0.446 (0.505)
Growth day 1-14	28.2 (22.7)	10.0 (8.08)	0.784 (0.377)
Growth day 30-43	0.666 (0.649)	0.172 (1.83)	4.46 (4.34)
Growth 5/21-28	8.81 (7.61)	1.31 (1.13)	2.13 (0.147)
Growth 6/29-7/05	12.7 (11.8)	2.61 (2.44)	0.269 (0.251)
Growth 7/05-7/12	8.41 (7.74)	4.92 (4.53)	2.23 (2.06)
Growth 7/12-20	2.53 (2.48)	5.73 (5.63)	0.546 (0.537)
Growth 7/20-27	0.491 (0.448)	11.9 (10.8)	0.014 (0.013)
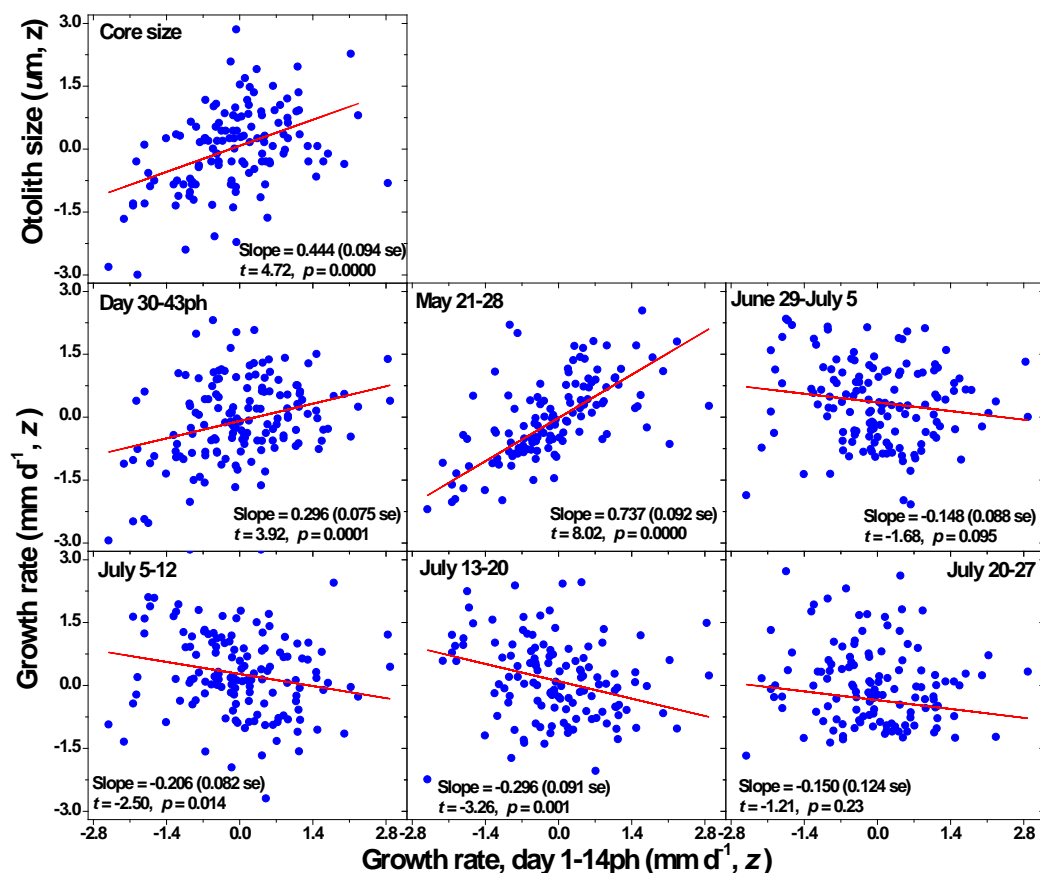
Figure 18. Relationships among life history characteristics evaluated in this study. Size and growth characteristics are positively correlated during the larval stage, but then switch and are negatively correlated with those influencing juvenile survival.

Similarly, formal measures of linear and nonlinear natural selection also suggest that selective mortality was intense during late summer relative to other time periods (Table 3). Overall, most values increase in absolute magnitude during August-September. Significant negative shifts between late-August and September indicated higher survival of individuals with smaller otoliths at hatch, and slower larval growth rates (Table 3). As juveniles, however, these fish were those that also grew faster than average during July, with significant shifts occurring from August to October (Table 3). Two other significant shifts were detected; fish surviving into late-fall were those that grew more slowly as larvae during the springtime low in food abundance, whereas fish surviving into August were growing more slowly in late-July: a shift that appears intermittent relative to subsequent periods (Figure 17, Table 3). Selection curves further demonstrate the intensity and non-linear form of natural selection during late summer (Figure 19), as well as the inverse relationship between larval and juvenile characteristics (Figure 18). Natural selection appears to have favored individuals with an opposing set of larval versus juvenile characteristics (Figure 19).

Table 3. Estimates of selective mortality between sampling dates from June to December (columns) based on size or growth periods from hatching to late July (rows). Values are the intensities of linear (change in mean) and nonlinear (change in variance) (in parentheses) selection between sampling dates, with shaded boxes reflecting significant changes in size or growth characteristics of the survivors (Kolmogorov-Smirnov test, $p < 0.05$).

| Size/Growth Character	06/28-7/15 to 07/27-29	07/27-29 to 08/10-11	08/10-11 to 08/23-25	08/23-25 to 09/08	09/08 to 09/12-10/11	09/12-10/11 to 11/02-12/12
Core size	0.015(0.043)	0.318(0.093)	-0.170(0.002)	-1.581(3.12)	0.421(0.509)	0.842(0.469)
Gr. Day 1-14	0.074(0.276)	0.181(0.151)	-0.519(0.003)	-1.698(3.40)	0.054(0.255)	0.034(0.224)
Gr. Day 30-43	0.017(0.313)	-0.324(0.175)	0.218(0.212)	-0.133(1.01)	-0.114 (0.326)	0.297(1.63)
Gr. 5/20-28	-0.146(0.275)	-0.054(0.024)	-0.835(0.682)	-0.365(0.537)	0.590 (0.808)	-0.824(0.316)
Gr. 6/29-7/05	0.265(0.380)	-0.069(0.447)	0.561(0.004)	-0.195(0.439)	0.692 (0.201)	-0.673(1.88)
Gr. 7/05-12	-0.199(1.03)	0.253(0.230)	0.077(0.369)	0.072(0.209)	0.942(1.33)	-0.494(0.246)
Gr. 7/12-20		-0.210(0.173)	0.619(0.466)	-0.212(0.234)	0.726(1.51)	-0.340(0.181)
Gr. 7/20-27		-0.657(0.519)	0.526(0.193)	-0.380(0.087)	0.694(1.07)	-0.497(0.353)
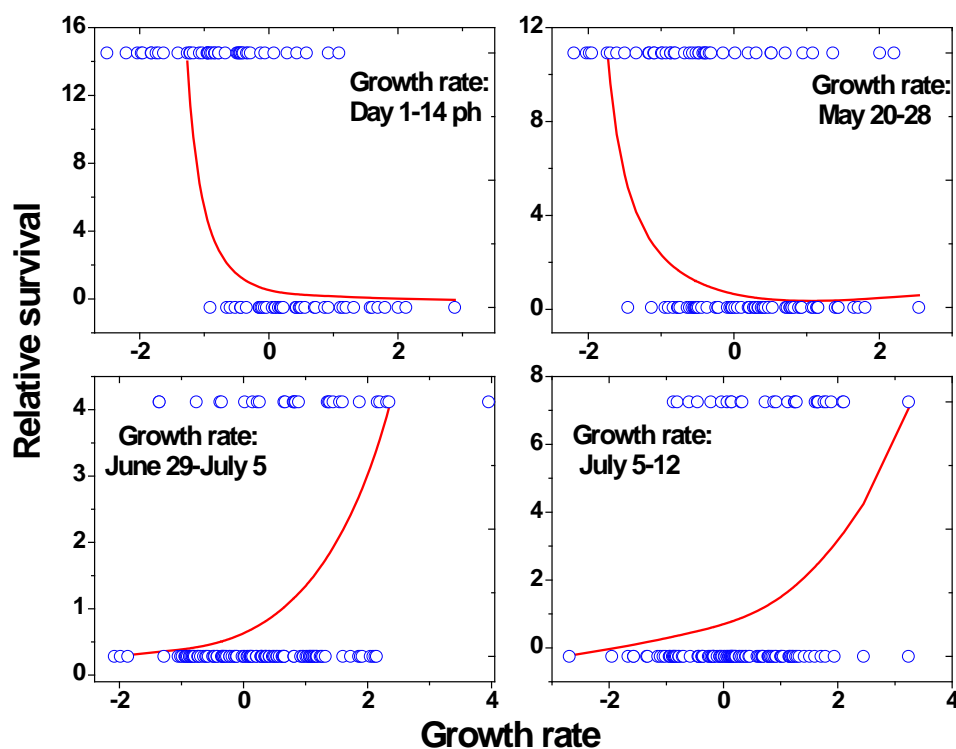
Figure 19. Changing intensity and form of natural selection for growth characteristics at the larval and juvenile stages. Fitted lines are cubic-spline models following Schluter (1988) as modified by Anderson (1995) (see methods).

Discussion

Our analyses of fish growth and condition clearly demonstrate the interacting role of selective mortality and environmental conditions in the poor survival of delta smelt during the summer of 2005. Dramatic shifts in the distributions of size or growth rate characteristics emerge during August and September and maintain into late-fall. These distributional shifts among sampling periods suggest that natural selection favored individuals with a specific set of characters, including relatively slow larval development, but then faster than average juvenile growth in July: a period with extremely high water temperatures and salinity encroachment (Figure 1). Many of these fish surviving into the pre-adult stage had also hatched earlier in the spawning season (i.e. before May). Overall, most individuals we examined in this study showed signs of reduced glycogen content (energy reserves) in their liver, and nearly one-half exhibited signs of single-cell necrosis (cell death). Taken together, these results suggest that selective mortality and poor body condition were intensified by extreme environmental conditions, elevating mortality during late-summer and contributing to poor year-class success. This provides a comprehensive explanation for patterns implying elevated summer mortality (or, a juvenile bottleneck) for delta smelt (Bennett 2005), as well as recent evidence for a long-term decline in suitable summertime

habitat (Nobriga et al. 2008).

Patterns of regional climate and local weather were highly unusual in 2005 (Schwing et al. 2006). A rare southward shift in the Jet Stream combined with periodically strong winds produced winter-time conditions during May; a storm-track brought multiple periods of rain, oceanic upwelling (the spring transition) was delayed about a month, and southern ocean water was transported northward by strong winds, producing anomalously high ocean water temperatures off central California and Oregon (Schwing et al 2006, Pierce et al. 2006). As a result, a variety of deleterious effects on marine populations occurred (Schwing et al. 2006, Barth et al. 2007). These unusually warm conditions region-wide likely contributed to the rapid rise in water temperatures in the delta smelt habitat following the unusual storm-events (Figure 1). Mean water temperatures in Suisun Bay and Delta rose from 20°C in late-June to over 24°C by mid-July, hovering close to the critical thermal maximum for delta smelt (25-26°C, Swanson et al. 2000). The majority of delta smelt caught during this period were in warm water (22-23°C) located along the northern channel of Suisun Bay and Suisun Marsh. Although this area is adjacent to Suisun Marsh which is known to harbor relatively high densities of copepod prey items (R. Schroeter and P. Moyle, UC Davis, *unpublished data*), water temperatures were well over the thermal optimum suggested by our specific growth relationship (Figure 3).

Above optimum water temperatures likely contributed to our histological results indicating poor fish condition during late-summer. Warm conditions are well known to elevate fish metabolic rates (Roessig et al. 2004). However, recent work has shown that many fishes cannot increase cardiac output in proportion, and quickly fail to meet the higher demands for oxygen imposed by a ramping metabolism (Portner and Knust 2007). This physiological limitation, combined with a declining concentration of dissolved oxygen in warming water, can markedly interfere with fish foraging and predator avoidance at water temperatures much lower than critical thermal maxima (Portner and Knust 2007). Our results showing widespread glycogen depletion and frequent instances of single-cell necrosis in delta smelt livers may indicate oxygen limitation. Fish become stressed in elevated water temperatures causing a rise in plasma glucose levels, thus depleting glycogen from liver cells (Van Dijk et al. 1999). Accordingly, frequent occurrence of single-cell necrosis may indicate increased oxidative damage to liver cells stemming from the

impairment of antioxidant enzyme activity at higher temperatures (Heise et al. 2006). Although single-cell necrosis may also reflect exposure to chemical pollutants, overall (1999-2006) we observe decreasing trend in such abnormalities, mirroring the shift in pesticide use to pyrethroids, which disrupt the endochrine system resulting in abnormalities, such as ovatestes (or intersex) observed here in 3 fish (Figure 9). The likelihood that extreme water temperatures contributed to these cellular abnormalities is further reinforced by the prolonged period of exposure (about a month); such chronic exposure has been shown to elevate mortality rates and lead to declining abundance and distributional changes in fishes (Portner and Knust 2007).

The inverse growth strategy between larval and juvenile stages exhibited by fish surviving through summer to the pre-adult stage appears counterintuitive for an annual species. Optimal life history strategies tend to maximize lifetime reproductive success, and thus age-specific survival and fecundity (Roff 1992). With a primarily annual life cycle, it would seem advantageous for delta smelt to grow as fast as possible given that the probabilities of reducing mortality, maturing and maximizing fecundity, tend to scale with body size (Bennett 2005). In contrast, natural selection appeared to favor individuals hatching early in the spawning season and developing slowly as larvae, but then growing faster than average as juveniles. This suggests strong compensatory growth (Atchley 1984), which is contrary to the typical expectations of the growth-mortality, or bigger-is-better, hypotheses (Anderson 1998). Typically, selection for growing slowly is associated with food-limited or otherwise stressful environmental conditions, such that development is provisioned to facilitate endurance (i.e. food shortages, or other stress). For example, at the inter-specific level, the "equilibrium" life history strategy of Winemiller and Rose (1992) predicts slower developmental rates in stressful environments (i.e. high competition for resources). In later life, growth must then be compensated so that juveniles catch-up, insuring some target size is reached for survival or maturity (Atchley 1984, Arendt 1997). Adoption of such a strategy by larval delta smelt is consistent with observations of widespread decline in calanoid copepod food resources in the estuary (Sommer et al. 2007); slower larval development may have also better prepared these individuals to endure higher temperature stress and maintain rapid growth in July.

A key assumption underlying our results is the degree to which patterns in growth and condition

from the IEP fish sampling reflects the population as a whole. Clearly, relatively few delta smelt were caught during regular monitoring in 2005. While this unfortunately reflects the poor status of the population, the catch of delta smelt is also known to be affected by gear selectivity of the sampling nets, as indicated by the special September survey samples we included in our analyses. Moreover, our analyses were limited to only 2 of the POD surveys; analysis of younger fish (the 20mm survey) would allow estimation of food-limitation and intensity of selective mortality, implied by patterns of slower larval growth. Subtle shifts in habitat use (i.e. to areas off the sampling grid) may be a 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 successive periods increasing the likelihood that they reflect important processes affecting recruitment.

Finally, our results have important implications for assessing the relative importance of entrainment losses to the major water export facilities. Overall, fish exhibiting strong growth compensation and higher survival to the pre-adult stage were also those spawned early in spring. High negative flow velocities in Old and Middle rivers with high export flows during this period suggests that many early-spawned fish were entrained (Grimaldo et al., *submitted*). In a recent analysis of export effects, Kimmerer (2008) estimated entrainment losses of delta smelt during this period at less than 5%, considerably lower than in the previous four years. However, these early-spawned fish were also those with a higher probability of surviving to the pre-adult stage. Thus, while the percentage of fish lost was relatively low, such early-season mortality would likely have had a disproportionate and additive effect on year-class success in 2005. To our knowledge this is the first study to assess growth and condition by integrating otolith and histological techniques 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.

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