Additional Scientific Information, Recommended Changes to the Bay-Delta Water Quality Control Plan, and Recommendations to Address Scientific Uncertainty and Changing Circumstances

Workshop 1: Ecosystem Changes and the Low Salinity Zone

Prepared by:
Jonathan Rosenfield, Ph.D.
The Bay Institute

Submitted to the State Water Resources Control Board on behalf of:
The Bay Institute
Natural Resources Defense Council
American Rivers
Pacific Coast Federation of Fishermen's Associations

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Question 1. What additional scientific and technical information should the State Water Board consider to inform potential changes to the Bay-Delta Plan relating to ecosystem changes and the low salinity zone that was not addressed in the 2009 Staff Report and the 2010 Delta Flow Criteria Report? For large reports or documents, what pages or chapters should be considered? What is the level of scientific certainty or uncertainty regarding the foregoing information? What changes to the Bay-Delta Plan should the State Water Board consider based on the above information to address existing circumstances and changing circumstances such as climate change and BDCP?

Response to Question 1: Additional Scientific Information & Recommended Changes to the Bay Delta WQCP

In its 2010 final report on "Development of Flow Criteria for the Sacramento-San Joaquin Delta Ecosystem" (2010 Flow Criteria Report), the State Water Board found that "*the best available science suggests that current flows are insufficient to protect public trust resources.*" The scientific literature and other sources of data that have become available since the 2010 report continue to overwhelmingly and conclusively support this finding.

In addition, several recent studies reinforce the finding that large-scale changes in the Delta's low salinity zone (LSZ) and Delta outflows – changes that are known to be deleterious to numerous native aquatic species and other Public Trust values of the Delta ecosystem – are largely caused and controlled by human activities to divert, export, and store water that are subject to the Board's authority.

Other stressors, such as loss and degradation of physical habitats, water quality impairment, and the effects of introduced species, are a legitimate source of concern regarding their potential contribution to recent and long-term declines in public trust resources, However, the research published in the past few years has tended to either:

(1) reinforce the Board's caution that "… *flow and physical habitat interact in many ways, but they are not interchangeable*." [2010 Flow Criteria Report, p. 1],
(2) question the scientific basis for and/or importance of these other factors, and/or
(3) demonstrate the critical role of freshwater flows in meditating these other stressors.

In short, there is a high and increasing degree of certainty that increased Delta freshwater outflows (relative to available annual runoff) are absolutely necessary (even if not sufficient alone) to protect and restore estuarine habitat and fish and wildlife beneficial uses and public trust resources of the Bay-Delta estuary's low salinity zone. In this written submission, we review and summarize the findings of the new publications, studies, and data and conclude that these new studies and publications support the Board's findings in the 2010 Flow Criteria Report, including:

(1) Existing flows are inadequate to protect Public Trust resources;
(2) Winter/Spring outflows should be substantially increased and should be implemented as a percentage of unimpaired flows occurring in a narrow averaging period;
(3) Fall (and possibly summer) outflows should be increased to provide sufficient habitat following wetter year types; and,

(4) Non-flow measures (such as physical habitat) interact with flow, but are not interchangeable and cannot substitute for flow.

Based on this conclusion and the extensive scientific record on which it rests, and on the fact that the estuarine habitat and fish and wildlife beneficial uses are the most sensitive uses of the Delta's waters, and that these ecological resources are severely imperiled by the current highly degraded condition of the Low Salinity Zone, the Board should analyze the effects of implementing the 2010 Delta outflow criteria as new water quality objectives in the Bay-Delta Plan, make such modifications as may be necessary to avoid or minimize potential unintended consequences to Public Trust resources upstream, and should ultimately adopt outflow objectives that ensure restoration and maintenance of both upstream and downstream Public Trust resources.

The Board should also complement these changes to the Bay-Delta Plan objectives with the adoption and implementation of a clear, transparent, and fully-defined adaptive management strategy that establishes specific, measureable, achievable, relevant and time-bound targets for protection of estuarine habitat, fish and wildlife, and Public Trust values (including but not limited to biocriteria) that the Plan's objectives are intended to achieve; performance monitoring and evaluation protocols to measure whether the objectives are achieving the desired targets; adaptive management triggers for modification of the objectives; and decision pathways that describe how corrective actions will be implemented when necessary. (This recommendation is addressed in our response to the section responding to the Board's second question in the workshop notice).

I. WINTER-SPRING DELTA OUTFLOW AND LOW SALINITY ZONE HABITAT CONDITIONS

A. *New Information Regarding Changes in Delta Outflow Over Time and Causes*

SUMMARY: Central Valley water management activities have caused large-scale changes in the location and size of the LSZ during winter and spring and the magnitude and timing of Delta outflow during this period.

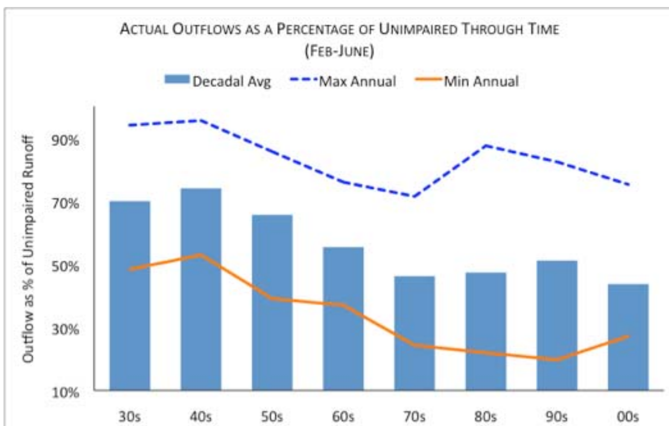

Figure 1: Trends in the mean, minimum, and maximum percentage of unimpaired flow that becomes actual delta outflow, across 8 decades.

The 2010 Flow Criteria report clearly recognized the effect of human water management activities on flows into, through, and out of the Delta as well as the deleterious effect on ecosystem processes related to these alterations in flow. Taken together with publications and testimony previously entered into the record, publications and data developed since 2010 demonstrate unequivocally that winter-spring freshwater flows into, through, and out of the Delta have deteriorated substantially over time (even following promulgation of previous water quality standards) as a result of water diversions throughout the Central Valley (Figure 1).

[San Francisco Estuary Partnership [SFEP]. 2011. The State of San Francisco Bay 2011. Available at: http://sfestuary.org/StateofSFBay2011/][1]

The State of San Francisco Bay Report (SFEP 2011) summarizes the effect of water management on freshwater flows in the upper estuary and the impact of these flow alterations on Public Trust resources in this area. This peer-reviewed report condenses multiple data streams into easy-to-understand metrics that track ecosystem health. The report also analyzes trends in the underlying data that contribute to the synthetic metrics so that readers can understand the root causes of trends in the Bay-Delta's ecological conditions.

SFEP's index of freshwater flows reveals a consistent decline in conditions over time such that a 10-year running average of this indicator has been "poor" since the late 1960's.

SFEP reports:

> *Since 1993, when the San Francisco Estuary Partnership's CCMP called for increasing freshwater availability to the Estuary and restoring healthy estuarine habitat, overall inflow conditions have … generally declined. Similarly, new water quality and flow standards established by the SWRCB in 1995 have not had a detectable effect on the Freshwater Inflow Index.* [p. 23].

and

> *Based on results of the Freshwater Inflow Index, the health of the San Francisco Estuary is critically impaired. Reductions and alterations in freshwater inflow have their greatest impacts in the upstream regions of the Estuary and Suisun and San Pablo Bays where the mix of fresh and salt water creates productive open water estuarine habitat.* [p. 23].

Not surprisingly, given the decline in freshwater flows through the Delta, the extent and quality of low salinity zone habitat declined significantly through time. The report explains:

> *Results of this analysis reveal a steady decline in springtime estuarine open water habitat, from consistently good or fair conditions prior to the 1960s to mostly poor conditions by the 1990s … Conditions improved during the late 1990s, during a sequence of unusually wet years but declined again in the 2000s* [p. 26]

[1] New scientific publications and studies are highlighted in gray for ease of reference. We have provided online

In appendices, SFEP disaggregates the findings presented in the summary indices. This exercise clearly demonstrates that water diversions from the Central Valley have increased relative to available runoff ("unimpaired flow") in the watershed over time, a pattern that was also revealed in our 2010 submission to the State Board (e.g. TBI et al. #1, Fig. 4). Figure 2 reveals that the percentage of available Central Valley runoff that makes it out of the Delta declines as conditions get drier (to the left of the graph) – diversions from the ecosystem remain relatively constant across hydrological conditions and dry years become disproportionately treacherous for native aquatic species.[2]

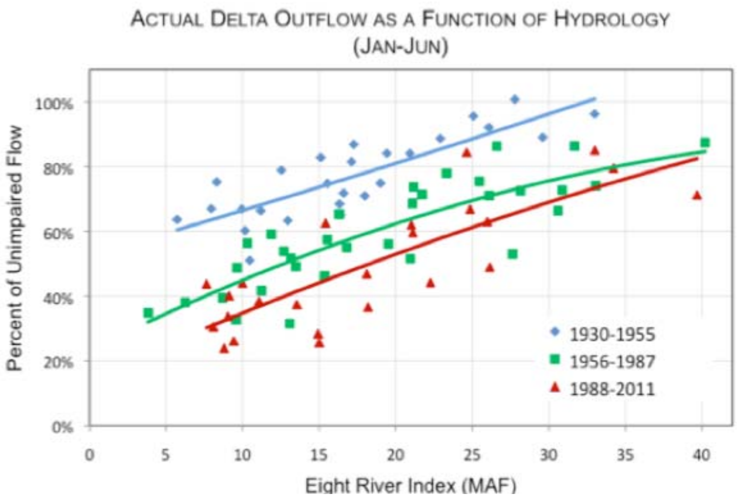

Figure 2: Percentage of available winter-spring runoff in the Central Valley (8-river index) that appears as actual Delta outflow across three time periods. Actual outflow has declined as a fraction of available runoff and has always been lower in drier years than in years with wetter conditions. Modified from SFEP 2011.

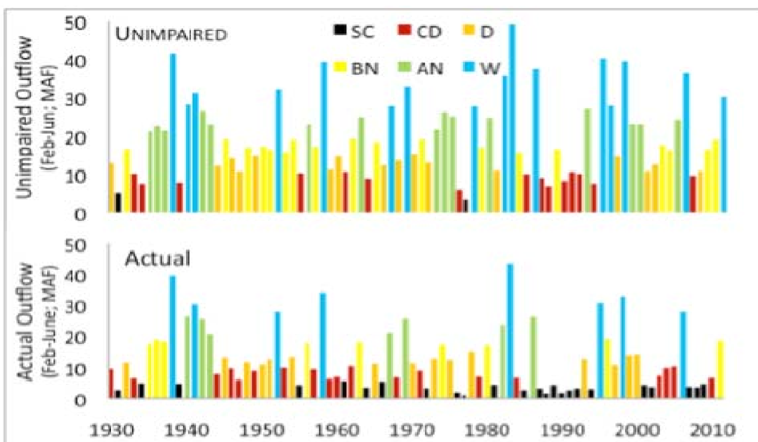

Figure 3: Unimpaired Delta outflow v. Actual Delta outflow through time. Colored bars indicate water year types from "super critical" (black) through "wet" (blue). Diversions result in a chronic drought for the ecosystem. Modified from SFEP 2011.

The result of the increasing water development in the watershed has been a prolonged, severe, and human induced "drought" for the Delta ecosystem (Figure 3). Although natural hydrology in the Central Valley (represented here by "unimpaired flows") is highly variable, the estuary has experienced drought conditions for most of the recent past as a result of water impoundment and diversions. Far from the claim that "we've tried improving flows and it hasn't worked," actual inflows to the Delta have been extremely (super-critically) dry in nearly half (12) of the last 25 years. For comparison, such extremely low Delta outflows occurred naturally only twice in the past 82 years. Wetter conditions ("Above Normal" (green bars) and "Wet" (blue bars) years) have occurred naturally in 10 of the last 25 years (40%) but such benign outflow conditions have materialized in the estuary far less frequently (4 years out of 25). It is no wonder that indicators of low salinity zone conditions, such as populations of pelagic species, have declined to levels associated with a long-term drought.

[2] The Board's 2010 formulation of flows necessary to protect the public trust as a consistent percentage of unimpaired would alleviate the disproportionate impact to the ecosystem of water diversions in drier years.

In addition to reducing the magnitude of ecologically essential freshwater flows through the ecosystem, water storage, diversion and export operations change the timing of those flows dramatically (Figure 4). Because fish and other native organisms have evolved to capitalize on seasonal pulses in flow, the loss of those pulses at the appropriate time, can be devastating to a species' population viability. In particular, by truncating the duration of pulse flows, water export operations cut short the window of time that native species have to complete flow-dependent transitions in their life cycle. Salmon juveniles that historically migrated to the Bay over a multi-month period of elevated flows (e.g. Williams 2006), are now constrained to migrating during regulated "pulse flow" periods that last just a few days or weeks. This has several effects: (1) fish that attempt to migrate outside the pulse-flow window (and the genetic and life history variation they represent) are selected against, which weakens the long-term resilience of the run (Miller et al. 2011) and (2) the entire year class is jeopardized if the fish that do migrate during the pulse window arrive in the bay or ocean at a time when conditions are sub-optimal. Simply put, the life history variation that occurs naturally among native fishes of the Central Valley would only exist if it had produced success (survival and reproduction) with some frequency in the past; eradicating that genetic and life history variation by limiting the duration of key flow conditions jeopardizes the continued existence of native fish populations in the future (Miller et al. 2011; Rosenfield 2010; NMFS 2009; Williams 2006).[3]

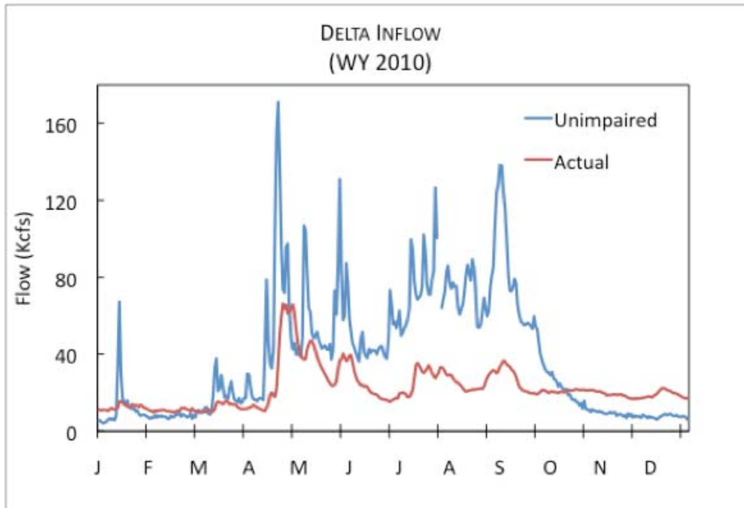

Figure 4: Unimpaired vs. actual inflow Delta hydrographs. Water management activities result in reduced average flows and reduction in flow variation that organisms use to trigger life history transitions. Modified from SFEP 2011.

[Enright, C. and S. Culberson. 2010. Salinity trends, variability, and control in the northern reach of the San Francisco Estuary. San Francisco Estuary and Watershed Science, 7(2). Available at: http://escholarship.org/uc/item/0d52737t].

Studying long-term data to determine which drivers influence Delta outflow and salinity at various locations in the Delta and Suisun Marsh, Enright and Culberson (2010) concluded that the State and federal water projects influence the trends in outflow and salinity across years and in specific months. This analysis reveals that, despite an almost 10% increase in rainfall measured in the Central Valley during the post-water project period, annual Delta outflow has decreased and salinity has increased at locations throughout the northern part of the San Francisco Estuary. These findings reinforce previous conclusions (referenced here and in previous testimony) that operations of the State and federal water projects exert a major

[3] The Board's 2010 recommendation that freshwater flows to protect the public trust ought to reflect percentage of unimpaired based on a narrow time window, such as a 14-day running average, would go a long way to restoring the natural hydrographic patterns in timing and pattern of freshwater flow.

influence on the size, position, and seasonal patterns of the estuary's low salinity zone, and that the operations of the State and federal water projects has made the upper reaches of the estuary more saline, not fresher (as is commonly asserted). Figure 5 (Enright and Culberson 2010's Figure 5) shows different resolutions of long-term trends in precipitation (hydrological conditions), actual Delta outflow, and salinity (environmental conditions), with the right panel revealing the long-term trend after accounting for seasonal (left column) and decadal (center column) patterns – the vertical dashed line divides the pre- and post-project periods. Enright and Culberson (2010) conclude, in part:

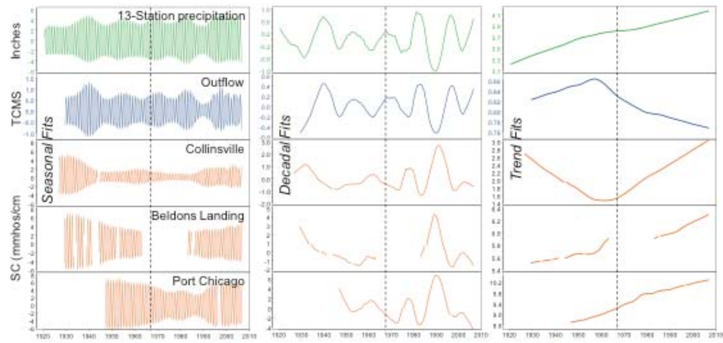

Figure 5: Variation in rainfall, Delta outflow, and salinity in the northern SF Estuary at three different temporal resolutions. At a decadal scale (right column) increasing precipitation, decreasing Delta outflow, and increasing salinity trends are apparent. [Enright and Culberson 2010, Figure 5].

> *The state and federal water projects decoupled long-term trends in annual mean outflow and salinity from long-term trends in precipitation.*

and

> *The water projects dampen seasonal and annual outflow and salinity variability.* [p. 10].

B. ***New Scientific Information Regarding Effects of Outflow on Fish Populations and Lower Trophic Levels***

SUMMARY: Changes in Delta outflow during the winter-spring are closely linked to population dynamics of numerous Public Trust resources -- less outflow corresponds to fewer aquatic organisms.

As the Board recognized in the 2010 Flow Criteria Report, freshwater flows out of the Delta in the winter and spring are strongly (orders of magnitude), significantly, and persistently (over many decades) correlated with populations of pelagic fish and other aquatic organisms in the Bay-Delta Estuary. The scientific evidence is simply overwhelming that freshwater flows control one or many physical, chemical, and biological processes that directly influence abundance and distribution patterns of many native species in the Bay-Delta. As a result, almost all scientists working on the Bay-Delta estuary subscribe to this view. As SFEP (2011; cited above) notes:

> *Scientists now consider poor freshwater inflow conditions to be one of the major causes for the ongoing declines of fish populations observed in the upper Estuary* [p.23].

[National Research Council. 2012. Sustainable water and environmental management in the California Bay-Delta. National Research Council. The National Academies Press, Washington, DC. Available at: https://download.nap.edu/catalog.php?record_id=13394]

The National Research Council's report on sustainable water and environmental management in the Bay-Delta found that evidence strongly supported a dominant role of winter-spring Delta outflows in driving population dynamics of native pelagic species. The NRC panel wrote:

> *Given that the position of X_2 for different periods of time appears to be important for different species, one can argue that water operations should be designed to preserve as much of both the volume of outflow and timing of that volume that would be observed in the absence of diversions (Moyle et al. 2010, SWRCB 2010). In light of the nature of the connection between flow and the position of X_2, this may necessitate limiting available water supply, especially in dry years.* [NRC 2012:63]

In addition, the panel concluded that:

> *… it appears that if the goal is to sustain an ecosystem that resembles the one that appeared to be functional up to the 1986-93 drought, exports of all types will necessarily need to be limited in dry years, to some fraction of unimpaired flows that remains to be determined. Setting this level, as well as flow constraints for wetter years, is well beyond the charge of this committee and accordingly we suggest that this is best done by the SWRCB, which is charged with protecting both water rights holders and the public trust.* [NRC 2012: 105]

1. New Scientific Information Regarding Effects of Outflow on Longfin Smelt

The Board's findings in the 2010 Flow Criteria Report were based in part on our work to identify the fresh water flow needs of individual species' life stages as they related to particular attributes of population viability – abundance, spatial distribution, life history diversity, and productivity (TBI et al. 2010 Exhibits 1-4; McElhany et al. 2000). With particular regard to the low salinity zone, we presented substantial new analyses of the population response of longfin smelt (*Spirhinchus thaleichthys*) to levels of Delta outflow during the winter and spring and demonstrated that flows that would be sufficient to restore longfin smelt populations in this estuary would also be protective of other aquatic organisms in the pelagic (open water) areas downstream of the Delta.

New publications, data, and analyses of the estuary's longfin smelt population strongly support the conclusions and recommendations of our 2010 analysis (TBI et al. 2010 exhibit #2). Indeed, the case for improving winter-spring Delta outflows (or, equivalently, locating X_2 further downstream) in order to protect and restore longfin smelt populations has been bolstered in the past two years.

[Thomson, J.R., W.J. Kimmerer, L.R. Brown, K.B. Newman, R. Mac Nally, W.A. Bennett, F. Feyrer, and E. Fleishman. Bayesian change point analysis of abundance trends for pelagic fishes in the Upper San Francisco Estuary. Ecological Applications 20:1431-1448. Available at: http://online.sfsu.edu/~modelds/Files/References/ThomsonEtal2010EcoApps.pdf]

This study used Bayesian and linear regression models to examine trends in abundance of four pelagic fish species (delta smelt, longfin smelt, striped bass, and threadfin shad) and identify the biotic or abiotic covariates most closely associated with the trends in abundance. The multispecies model identified step changes in abundance for 3 of the 4 species in the early 2000s and concluded that the decline of longfin smelt was a continuation of a longer-term decline. For longfin smelt, the individual species model identified the decline in abundance as responses to increases in spring X2 (reductions in spring outflow). The authors concluded, in part:

> *"… at the estuary scale, abiotic factors (water clarity, X2, exports) may have more influence on interannual variation in abundances of the four species than do biotic variables."* [p. 1445].

Acknowledging that their results reinforced previous evidence regarding the likely causes of pelagic species population declines in this ecosystem, Thomson et al wrote:

> *"The covariates we identified as strongly associated with pelagic fish abundance, namely X2, water clarity, and export flows, previously have been hypothesized to affect abundance."* [p. 1443].

[Mac Nally, R., J.R. Thomson, W.J. Kimmerer, F. Feyrer, K.B. Newman, A. SIH, W.A. Bennett, L. Brown, E. Fleishman, S.D. Culberson, and G. Castillo. 2010. Analysis of pelagic species decline in the upper San Francisco Estuary using multivariate autoregressive modeling (MAR). Ecological Applications 20:1417-1430. Available at: http://online.sfsu.edu/~modelds/Files/References/MacNallyetal2010EcoApps.pdf]

This study utilized multivariate autoregressive modeling to analyze what factors were contributing to the declines of four pelagic fish species (delta smelt, longfin smelt, striped bass, and threadfin shad). The authors studied 54 covariates, including water exports, spring and fall X_2, prey abundance, predator abundance, and water temperatures. They concluded:

> *The position of* [X_2] *(a measure of the physical response of the estuary to freshwater flow) and increased water clarity over the period of analyses were two factors affecting multiple declining taxa (including fishes and the fishes' main zooplankton prey).* [pp. 1417]

High summer water temperatures, spring water exports, abundance of largemouth bass, abundance of summer calanoid copepods, and winter water exports were negatively associated with delta smelt abundance to some degree. The modeled covariates explained 51% of the variability in abundance, and the authors concluded that water exports and X2 are associated with the declines and can be managed.

[Rosenfield, J.A. 2010. Conceptual life-history model for longfin smelt (*Spirinchus thaleichthys*) in the San Francisco Estuary. California Department of Fish and Game, Sacramento, CA. http://www.dfg.ca.gov/ERP/conceptual_models.asp].

The CALFED Ecosystem Restoration Program initiated an effort to screen and evaluate potential restoration actions in the Delta, known as the Delta Regional Ecosystem Restoration Implementation Program (DRERIP). One part of that evaluation program involved the creation of conceptual models that summarized the state of knowledge regarding species' life histories, ecosystem processes, habitats, and stressors. Final versions of these models are now published and available through CDFG and should be incorporated into the Board's record in these proceedings.

One of these DRERIP models focuses on what is known about the life history of longfin smelt and the magnitude and scientific certainty surrounding the impact of different stressors on the species' life history and ecology. This conceptual model clearly emphasizes the wealth of research demonstrating the high magnitude effect of freshwater flow on longfin smelt population abundance – the relationship between winter-spring Delta outflow and success of longfin smelt eggs, larvae, and juveniles is rated as "high" as is the scientific certainty of that impact. Fresh water outflow and salinity (which is directly modified by fresh water outflow) are the only two stressors identified as having both high magnitude impacts on longfin smelt survival and a high certainty of impact (Rosenfield 2010; Table 3 – "stressor matrix"). Of particular relevance are the figures that depict the likelihood and magnitude of impact of different stressors on the probability that longfin smelt will transition from one lifestage to the next (Rosenfield 2010; Figures 3-5, pp. 34-36). These schematic diagrams clearly identify Delta freshwater outflow as an ecosystem process with both high magnitude and high certainty effects on numerous stressors of longfin smelt populations – in every case, increased freshwater flow results in stressor reduction.

Furthermore, the model describes how Delta outflow affects the spatial distribution of spawning adult and larval longfin smelt. This mechanism largely determines longfin smelt entrainment rates at the South Delta pumps as lower winter-spring Delta outflows position longfin smelt nearer to the pumps and typically result in much higher entrainment rates than higher outflow conditions that help distribute longfin smelt further to the west.

US Fish and Wildlife Service. 2012. Endangered and Threatened Wildlife and Plants; 12-month Finding on a Petition to List the San Francisco Bay-Delta Population of the Longfin Smelt as Endangered or Threatened. 50 CFR Part 17. [Docket No. FWS-R8-ES-2008-0045]. Available at: http://www.fws.gov/cno/es/speciesinformation/Longfin%20Smelt%2012%20month%20finding.pdf

In 2012, The US Fish and Wildlife Service (FWS) issued a 12-month finding in response to a petition to list the San Francisco Bay-Delta population of longfin smelt under the federal Endangered Species Act (ESA; this population is already listed as a threatened species under the state ESA). The FWS determined that this population of longfin smelt warranted listing as a threatened species, though administrative priorities precluded formal listing at this time.

While describing the threats to this species in the Bay-Delta, FWS wrote:

> *In the Bay-Delta estuary, increased Delta outflow during the winter and spring is the largest factor positively affecting longfin smelt abundance … During high outflow periods, larvae presumably benefit from increased transport and dispersal downstream, increased food production, reduced predation through increased turbidity, and reduced loss to entrainment due to a westward shift in the boundary of spawning habitat and strong downstream transport of larvae (CFDG 1992; Hieb and Baxter 1993; CDFG 2009a). Conversely, during low outflow periods, negative effects of reduced transport and dispersal, reduced turbidity, and potentially increased loss of larvae to predation and increased loss at the export facilities result in lower young-of-the-year recruitment*. [p. 38].

The 12-month finding goes on to describe the effect of freshwater flow on longfin smelt populations and, in particular, the effect of water diversions on longfin smelt success. For example, the finding states:

> *Because longfin smelt spawn in freshwater, they must migrate farther upstream to spawn as flow reductions alter the position of X_2 and the low-salinity zone moves upstream. Longer migration distances into the Bay-Delta make longfin smelt more susceptible to entrainment in the State and Federal water pumps (see Factor E: Entrainment Losses). In periods with greater freshwater flow into the Delta, X_2 is pushed farther downstream (seaward); in periods with low flows, X_2 is positioned farther landward (upstream) in the estuary and into the Delta. Not only is longfin smelt abundance in the Bay-Delta strongly correlated with Delta inflow and X_2, but the spatial distribution of longfin smelt larvae is also strongly associated with X_2. As longfin hatch into larvae, they move from the areas where they are spawned and orient themselves just downstream of X_2. Larval (winter-spring) habitat varies with outflow and with the location of X_2, and has been reduced since the 1990s due to a general upstream shift in the location of X_2. The amount of rearing habitat (salinity between 0.1 and 18 ppt) is also presumed to vary with the location of X_2. However, as previously stated, the location of X_2 is of particular importance to the distribution of newly-hatched larvae and spawning adults. The influence of water project operations from November through April, when spawning adults and newly-hatched larvae are oriented to X_2, is greater in drier years than in wetter years.* [p. 39]

The FWS finding obviously indicates that the continued existence of this unique and ecologically important species in the Bay-Delta ecosystem is threatened. By not listing this longfin smelt population, FWS left it uncovered by the protections afforded threatened and endangered species under the federal ESA, even though those protections are warranted. What is more, the most recent reviews of the latest draft Bay Delta Conservation Plan (BDCP), which is supposed to contribute to the recovery of longfin and other native fish species, indicate that the BDCP as currently envisioned will have a substantial negative impact on this species (*see below*). Thus, actions to protect the Bay-Delta's longfin smelt population (and the estuarine habitat on which it and numerous other pelagic species rely) are all the more imperative – new outflow and other objectives promulgated by the Board may be the last best hope for conserving what was formerly among the most abundant fish in the Bay-Delta.

[BDCP "Red Flag" Documents [California Department of Fish and Game; US Fish and Wildlife Service; and National Marine Fisheries Service. April 2012 BDCP EA (Ch. 5) Staff "Red Flag" Review Comprehensive List. Available at: http://baydeltaconservationplan.com/Libraries/Dynamic_Document_Library/Effects_Analysis_-_Fish_Agency_Red_Flag_Comments_and_Responses_4-25-12.sflb.ashx]

In February 2012, BDCP released a draft Effects Analysis of a project proposal that was designed to minimize the direct impacts of water export operations (e.g. entrainment, stranding), but was projected to increase water diversions over recent norms. The fish and wildlife trustee agencies were asked to comment on the proposed project and Effects Analysis. These preliminary reviews, known collectively as the "Red Flag Staff Reviews," were highly critical of the draft BDCP (both the Plan itself and its Effects Analysis – see below for complete discussion). In particular, the agencies expressed grave concerns about the effect of anticipated reductions in Delta outflow arising from State and federal water project operations described in the Draft plan. The FWS wrote:

> *The Low Salinity Zone (LSZ) is the primary habitat for delta smelt and the primary rearing habitat for larval longfin smelt and juvenile to adult splittail. The Preliminary Proposal modeling indicates that Delta outflows during February-June will more frequently be near the minima required by the SWRCB under D-1641. This will represent a substantial negative project effect on longfin smelt. The effects analysis and Net Effects only partly address this issue, reporting that Preliminary Project is expected to provide a large, positive impact to food resources that will offset the negative impact to "transport flows". But there are multiple mechanisms by which Delta outflow can affect longfin smelt recruitment; transport flow is only one of them. Transport flows might be managed via gates or other engineering solutions. The other mechanisms for which there is stronger scientific support are kinetic energy mechanisms (low-salinity zone habitat area and retention from gravitational circulation in the estuary). The problems that reduced outflow creates by changing these processes do not have reasonable engineering solutions, and at present appear to be manageable only via outflow* [pp. 12-13].

2. New Scientific Information Regarding the Effects of Outflow on Other Fish Species

The longfin smelt is not the only native fish species in the Bay-Delta Estuary whose population responds strongly to Delta outflows in the winter-spring period. The populations of several other species also display long-term, statistically significant, and high-order relationships with winter-spring Delta outflows (or X_2; e.g., Jassby et al. 1995; Kimmerer 2002; Kimmerer et al 2009; TBI et al. 2010, Exhibit #2). In 2010 and 2011, these species responded to hydrological conditions exactly as one would expect given their life histories and their well-established historical response to freshwater flow variations.

[Population Response in Water Years 2010 & 2011 – data from CDFG. Available at: http://www.dfg.ca.gov/delta/data/].

Water years 2010 and 2011 were wetter than the three years leading up to publication of the 2010 Flow Criteria Report (Figure 6). In combination with high flows, operational constraints required under biological opinions for Delta smelt and anadromous species maintained more favorable in-Delta hydrodynamic conditions (e.g. less net negative flow from Old and Middle River) for a longer period than has occurred recently. These protective regulations notwithstanding and despite the fact that WY 2011 was only in the top ~80 percent of years in terms of available runoff (i.e., WY 2011 was wet, but not extremely wet), water exports from the South Delta reached an all-time high.

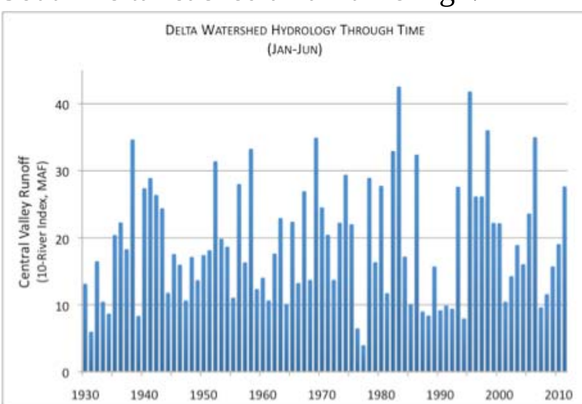

Figure 6: Central Valley Hydrology through Time

Native fish populations responded positively to improved Delta outflows and Delta hydrodynamic conditions (Figure 7) – not surprisingly given the long-term, significant, high magnitude, and widespread response of the Delta ecosystem to increased Delta inflow, through-flow, and outflow (e.g. Stevens and Miller 1983; Jassby et al. 1995; Kimmerer 2002; Kimmerer et al. 2009). Water years 2010 and 2011 illustrate the point made by almost 5 decades of fish and flow data from this estuary: increased freshwater flow into, through, and

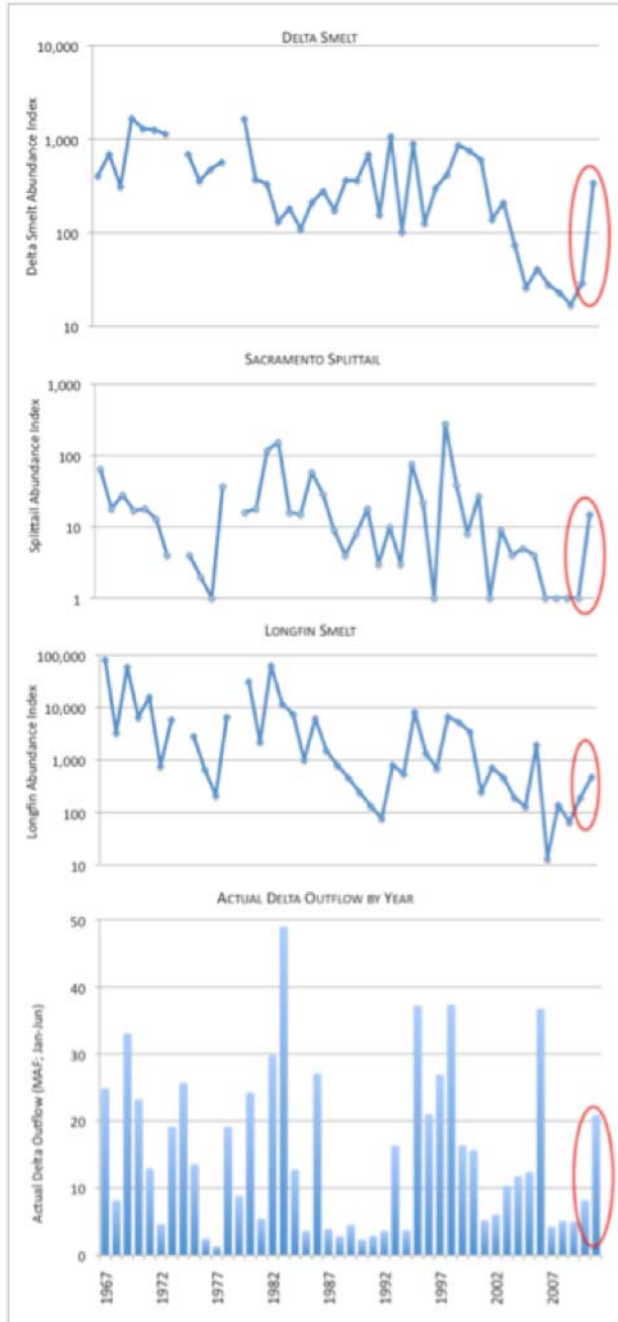

Figure 7: Response of three pelagic fish species to variation in winter-spring Delta outflow. Ovals highlight population and outflow increases in the 2 years since the State Board issued its 2010 Flow Criteria Report.

out of the Delta generally leads to increased abundance of native fishes. Although 2011 is only one year (albeit, one with a pattern very similar to that seen in "wet" years throughout the ~45 year fish population sampling record), the population responses of organisms as ecologically diverse as Delta smelt, longfin smelt, striped bass, Sacramento splittail should be somewhat independent of one another, unless one assumes that each of these populations is affected by the same drivers – drivers like freshwater flow. It is worth noting that the moderate population increases seen in 2010 and the more dramatic response in 2011 could not have been a response to restoration of shallow water habitat; reduction in ammonium discharge from the Sacramento Municipal Wastewater Treatment facility: or reduction in the abundance or extent of fish predators – none of those other stressors changed substantially in 2010 and 2011. Similarly, mortality due to entrainment at the South Delta water export facilities was relatively high in 2011 for some species; nearly 9,000,000 Sacramento splittail and 27,000 Sacramento sucker were "salvaged" at the south Delta export pumps (a record for each of these endemic fish species) and over 200 white sturgeon where captured at the pumps, their worst year since 1998 (TBI 2012). The only change in major ecosystem drivers that improved in 2011 compared to the previous 4-years was the volume of water that flowed into, through, and out of the San Joaquin Delta during the winter and spring and the improvement in fall habitat conditions.

3. New Scientific Information Regarding the Effects of Outflow on Lower Trophic Levels and Food Webs

In addition to fish species, the 2010 Flow Criteria Report recognized that Delta freshwater outflow significantly affects populations of numerous invertebrate species that form the base of the food web for fish and other species (*see also* Kimmerer 2002; Kimmerer et al. 2009). Freshwater flow, through its myriad effects on the Low Salinity Zone, structures and controls the distribution of aquatic species assemblages in the upper reaches of the estuary and beyond. Recent scientific studies and publications confirm the validity of this finding (e.g. Mac Nally et al. 2010; NRC 2012; Peterson and Vayssieres 2010; Winder and Jassby 2010)

For instance, the analysis by Mac Nally et al (2010) found a strong relationship between X_2 and the abundance of both calanoid copepods and mysids (both of which are part of the food web for delta smelt, longfin smelt, and other native fish species). When X_2 is more seaward in the spring (lower X_2 values), the spring abundance of copepods and mysids is greater, "*which also would propagate back through those food pathways*" to delta smelt and longfin smelt (Mac Nally 2010:1426). Because "*Longfin smelt abundances had strong negative correlations with calanoids in spring and summer and mysids in spring*," and Delta smelt abundance had a weaker relationship with calanoid abundance in summer, the authors concluded that X_2 "*seems to have a profound effect on the declining fish and on their prey*," (Mac Nally 2010:1428).

The National Research Council (NRC 2012) reviewed the effects of X_2 on aquatic resources, including food webs. They reviewed work by Jassby that found that X_2 affected "*the abundance/biomass of a number of organisms, including the total production of particulate organic carbon by phytoplankton in Suisun Bay, the shrimps Neomysis mercedis and Crangon franciscorum, and several fishes*," (NRC 2012:58, citing Jassby 1995). They reviewed the Mac Nally et al. 2010 study, stating that it found that, "*The position of X_2 in the spring ("spring X_2")*

[s]trongly influences the abundance of mysids, longfin smelt, and calanoid copepods." (NRC 2012:60). After reviewing work by Jassby, Kimmerer, and Mac Nally, the NRC report concluded that:

> *Thus, while the mechanisms behind the influence the of position of X_2 on the abundance of a variety of biota remain hypothetical, the statistical relations reported in several papers show that abundance of a number of species at different trophic levels found in the Delta and San Francisco Bay is higher when X_2 is farther downstream. This implies that sufficient reductions in outflow due to diversions would tend to reduce the abundance of these organisms.* [p. 60].

The National Research Council's report also included an appendix prepared by Dr. Wim Kimmerer, which discussed changes in zooplankton composition and abundance over time. The appendix notes that:

> *Opportunities to reverse the declines in zooplankton are severely limited, at least with our current knowledge of their ecology. Producing more food for them is impracticable because adding more phytoplankton to the system would probably just produce more clams. There may be opportunities to enhance populations of some zooplankton through manipulations of freshwater flow, and control of nutrient inputs to the Delta may improve growth conditions for phytoplankton and reduce the frequency of harmful algal blooms. These are active areas of research which will help to clarify the potential responses to these changes.* [NRC 2012:201].

Several other studies provide additional information on the relationship between outflow and lower trophic levels.

[Winder, M. & A.D. Jassby. 2010. Shifts in zooplankton community structure: implications for food-web processes in the upper San Francisco Estuary. Estuar. Coasts. Available at: http://www.springerlink.com/content/b30544u2xx0l235u/fulltext.pdf]

This paper reported analysis of long-term patterns in zooplankton abundance, distribution, and species composition in the Bay-Delta estuary. They note the correspondence between major changes in the estuary's zooplankton assemblage and native fish populations that occurred during the 1987-1993 drought, but found no evidence that more recent declines in fish populations (i.e. the POD) were caused by changes in zooplankton abundance. They wrote:

> *While the long-term decline of diverse fish populations in the upper SF Estuary coincided with reduced primary and secondary production (Cloern 2007), our analysis showed that the sudden drop of many pelagic fishes in 2002 (Sommer et al. 2007; Thomson et al. 2010) was not accompanied by an equivalent decrease in the quantity of zooplankton carbon. Substantial zooplankton and mysid declines occurred in the mid- to late 1980s, and biomass of both groups remained at low levels thereafter, without significant changes in the early 2000s when pelagic fish densities dropped substantially (Table 2). This suggests that changing prey*

> *quantity was not a dominant factor contributing to the recent fish declines.* [Winder & Jassby 2010:686-87]

[Peterson, H. and Vayssieres, M. 2010. Benthic Assemblage Variability in the Upper San Francisco Estuary: A 27-Year Retrospective. San Francisco Estuary and Watershed Science, 8(1). Available at: http://escholarship.org/uc/item/4d0616c6]

Peterson and Vayssieres (2010) studied 27 years of data on benthic assemblages along the major axis of the northern estuary and concluded:

> *Hydrologic variability was associated with significant changes in benthic assemblage composition at all locations. Benthic assemblage composition was more sensitive to mean annual salinity than other local physical conditions. That is, benthic assemblages were not geographically static, but shifted with salinity, moving down-estuary in years with high delta outflow, and up-estuary during years with low delta outflow, without strong fidelity to physical habitat attributes such as substrate composition or location in embayment vs. channel habitat.* [p. 1].

As a result, the authors found that species assemblages at specific geographic locations such as Grizzly Bay varied dramatically between high outflow and low outflow years. (Peterson & Vayssieres 2010:19-20).

4. New Scientific Information Regarding Relationships Between Outflow and "Other Stressors"

SUMMARY: New scientific information casts further doubt upon the hypothesized connection between certain "other [non-flow] stressors" and decline of native fish species asserted in 2010.

During the Board's Delta Flow Criteria proceedings in 2010, several parties promoted the hypothesis that factors other than fresh water flows were driving declines in populations of public trust species. Many of these putative stressors involved different mechanisms for suppressing the pelagic food web in the Bay-Delta. However, none of the arguments presented in support of this hypothesis provided any basis for making a direct connection between the hypothesized cause of a decline in primary productivity and the fish populations that were alleged to be affected by this decline. Most of these species are secondary consumers, that is, they feed two levels away from phytoplankton in the food web. Although it is intuitively attractive to argue that a decline in production at the base of the food web would lead to a decline in production of secondary consumers (or, in some cases, predatory fish), this kind of linkage has not been demonstrated in this system (e.g. Kimmerer 2002). Indeed, with regard to trophic interactions, Thomson et al. (2010; cited below) found:

> *"…the strongest effects generally were ''top-down,'' with fish apparently having more influence on prey biomass than vice versa"*. [p. 1445].

i. New Scientific Information Regarding the Effects of Ammonium Loadings

[Cloern, J.E, A.D. Jassby, J. Carstensen, W.A. Bennett, W. Kimmerer,, R. Mac Nally, D.H. Schoellhamer, M. Winder. 2012. Perils of correlating CUSUM-transformed variables to infer ecological relationships (Breton et al. 2006; Glibert 2010). Limnol. Oceanogr., 57:665–668. Available at: http://kjiwuqx.aslo.org/lo/toc/vol_57/issue_2/0665.pdf]

and by reference

[Glibert, P. 2010. Long-term changes in nutrient loading and stoichiometry and their relationships with changes in the food web and dominant pelagic fish species in the San Francisco Estuary, California. Rev. Fish. Sci. 18: 211–232. Available at: http://sustainabledelta.com/pdf/GlibertReviewsFisheriesScience.pdf]

During the State Water Board's 2010 Delta Flow proceedings, a manuscript by Glibert (2010) was offered as evidence to suggest that ammonium loadings, through their putative effect on the pelagic food web, were driving declines in the fish fauna of the Sacramento Estuary and that freshwater flows were relatively unimportant. Although we do not dispute the potential value of addressing ammonium inputs (or other potential toxins) to the estuary, the analysis by Cloern et al. (2012) demonstrates that the correlations between ammonium and fish populations reported by Glibert (2010) were artifacts of an unwarranted and invalid statistical technique.

Cloern et al. 2012 explored and invalidated the statistical approach employed by Glibert (2010) and the conclusions Glibert posited regarding ecosystem processes in this San Francisco Estuary. Specifically, Glibert (2010) presented an unorthodox statistical analysis that led her to conclude that recent zooplankton and fish species declines were driven by a single factor, increased ammonium inputs from the effluent of municipal wastewater treatment plants. Cloern et al (including eight of the most respected and experienced ecological researchers who have studied the San Francisco Estuary) found:

> "*…no history for regression (or correlation) analyses on CUSUM-transformed variables prior to its use by Breton et al. (2006), and we have found <u>no theoretical development or justification for the approach</u>. We prove here that the CUSUM transformation, as used by … Glibert (2010), violates the assumptions underlying regression techniques. As a result, high correlations may appear where none are present in the untransformed data… Regression analysis on CUSUM-transformed variables [the method used by Glibert 2010] is, therefore, <u>not a sound basis for making inferences about the drivers of ecological variability</u> measured in monitoring programs.* [Emphasis added] [p. 665]

Cloern et al (2012) conclude:

> *"… Glibert (2010) inferred a strong negative association between delta smelt abundance and wastewater ammonium from regression of CUSUM transformed time series. However, the Pearson correlation (r = -0.096) between the time*

> *series … is not significant, even under the naive … assumptions (p = 0.68). In short, correlations between CUSUM-transformed variables should not be used as a substitute for analysis of the original untransformed variables."* [Emphasis added] [p. 668]

ii. New Scientific Information Regarding the Effects of Invasive Benthic Grazers

Peterson and Vayssieres (2010; cited above) discounted the potential linkage between putative stressors such as benthic grazing (e.g. clams), pesticides, or microcystines from toxic algal blooms and the recent pelagic organism decline (POD). For example, they found:

- "[Benthic] *assemblage structure during the POD years was not significantly different from other post-invasion years at any of the stations,"*
- *"…no evidence from the benthic abundance data that the influence of benthic grazing underwent a significant change coincident with the POD,"*
- *"…no decline in amphipod species (… important prey for pelagic fish) was evident during the POD …"* [and thus that] *"…the role of pesticides in the POD may be limited,"* and
- *"… that microcystines probably did not have a broad effect in the upper estuary"*. [p: 22]

Winder and Jassby (2010; cited above) reported analysis of long-term patterns in zooplankton abundance, distribution, and species composition in the Bay-Delta estuary. They note the correspondence between major changes in the estuary's zooplankton assemblage and native fish populations that occurred during the 1987-1993 drought, but found no evidence that more recent declines in fish populations (i.e. the POD) were caused by changes in zooplankton abundance. They wrote:

> *While the long-term decline of diverse fish populations in the upper SF Estuary coincided with reduced primary and secondary production (Cloern 2007), our analysis showed that the sudden drop of many pelagic fishes in 2002 (Sommer et al. 2007; Thomson et al. 2010) was not accompanied by an equivalent decrease in the quantity of zooplankton carbon. Substantial zooplankton and mysid declines occurred in the mid- to late 1980s, and biomass of both groups remained at low levels thereafter, without significant changes in the early 2000s when pelagic fish densities dropped substantially (Table 2). This suggests that changing prey quantity was not a dominant factor contributing to the recent fish declines.* [pp. 686-687]

In addition, new scientific information regarding effects of fall outflow on *Corbula amurensis* and other invasive benthic species is discussed *infra*.

iii. New Scientific Information on the Relationship Between Physical Habitat and Flow

In the 2010 Flow Criteria Report, the Board correctly noted that "…*flow and physical habitat interact in many ways, but they are not interchangeable*." Put another way, increased flows of fresh water into, through, and out of the Delta, at appropriate times of year (particularly, the winter and spring) are absolutely necessary, if not sufficient on their own, to restore the public trust values and protect beneficial uses of the Bay-Delta ecosystem.

In its review and revision of the Bay-Delta Plan, the Board has indicated that it anticipates using relevant information from the environmental documentation being prepared on the effects of the proposed Bay Delta Conservation Plan (BDCP). A central assumption of the BDCP to date has been that negative impacts of increased Delta exports by the state and federal water projects can be offset by restoring tens of thousands of acres of shallow tidal and floodplain habitats in the Delta. This assumption, and the failure to adequately analyze it in the environmental documents thus far, has been widely criticized by the scientific community. All of the federal and state fish and wildlife trustee agencies as well as various independent scientific review panels have commented repeatedly that the BDCP's projected impact on freshwater flow conditions as a result of increasing exports will be deleterious to native fish species (regardless of the fact that the new diversion would be equipped with improved fish screening technology that would presumably reduce the direct impact of entrainment at the new diversion) and that its plan to mitigate these impacts by improving habitat conditions are speculative at best. Below, we describe the feedback on recent versions of the BDCP as it is relevant to current arguments about the magnitude and likelihood of potential non-flow related solutions to the decline in the Bay-Delta's public trust resources.

[Parker, A., Simenstad, S., George, T., Monsen, N., Parker, T., Ruggerone, G., and Skalski, J. 2012. Bay Delta Conservation Plan (BDCP) Effects Analysis Phase 2 Partial Review, Review Panel Summary Report. Delta Science Program. Available at: http://deltacouncil.ca.gov/sites/default/files/documents/files/BDCP_Effects_Analysis_Review_Panel_Final_Report_061112.pdf]

This independent scientific peer review of the BDCP Effects Analysis was highly critical of the Plan's analysis of food webs, habitat restoration, and effects on phytoplankton production, concluding that, "*the BDCP treats restoration as a 'given' positive, without considering to much extent that the same actions will also 'create' habitat for trophic consumers (e.g., Egeria invading and providing habitat for predators of threatened fish), or trophic competitors (e.g., filter-feeling clams creating permanent phytoplankton sinks)*" and stating further that, "*the treatment of food resource availability is grossly incomplete and overly simplistic*" (Parker 2012: 22, 27).

The panel found that restored tidal marsh habitat could lead to increased Submerged Aquatic Vegetation and introduced species, and that the BDCP's Effects Analysis failed to consider the potential that:

> *…these shallow open water habitats will be new habitat for expansion of invasive clams such as Corbicula. Under this scenario, the new shallow water habitats would likely act as net sinks for pelagic phytoplankton, and not sources of phytoplankton, thus acting as a net negative effect for food resource availability.* [p. 28]

In addition, the authors found that restored tidal marsh habitat could lead to eutrophication, harmful algal blooms, or low dissolved oxygen, and that "[*t]his would most likely occur in shallow open water habitats with poor flushing / long residence times, i.e., the type of habitats being proposed under the BDCP*," (Parker et al. 2012:28). The peer review also noted that currently:

> *…some of the shallow open water habitats (i.e., Mildred Island) with long residence time are habitat for cyanobacteria, including Microcystis spp. While specific drivers of Microcystis blooms are still not resolved (but see Lehman 2005; Lehman et al. 2008), water temperature and residence time have been implicated. Under future climate scenarios, shallow open water habitats may promote Microcystis and other harmful cyanobacteria species (e.g. Anabaena, Aphanizomenon).* [pp. 28-29]

Finally, the authors specifically looked at the potential impacts to longfin smelt from BDCP proposals that would include habitat restoration but reduce winter/spring outflow. The peer review strongly recommended that BDCP must avoid further declines in the longfin smelt population "*while waiting for possible beneficial effects of habitat restoration*," noting that habitat restoration would take years to be accomplished and that "*the benefits of habitat restoration for longfin smelt are not highly certain*." (Parker 2012:39-40)

The BDCP Red Flag Staff Reviews (2012; cited above) were extremely critical of the assumption that habitat restoration elements of the Plan would more than mitigate for the project's increased water exports. For example, regarding the impacts of reduced flows resulting from BDCP on sturgeon, CDFG found:

> *The collective predicted negative river flow effects of the [Project]create the risk of a depressive effect on sturgeon production that may not be overcome by more favorable… aspects (e.g. reduced entrainment, increased food production supply). This suggests the need to modify the [Project] to reduce the magnitude and frequency of river flow reduction occurrences, in both upstream and downstream areas.* [p. 2]

Regarding the impacts of reduced flows resulting from BDCP on ecosystem processes in the low salinity zone, FWS found:

> *Reduction of flows (in full consideration of timing, magnitude, variability) is the most fundamental cause of stress and driver of change to the fishes and food web that have adapted to the tidal and freshwater mixing environment that is the Bay-Delta ecosystem. In addition, some of the other stressors listed and assumed to be*

> *addressed through the* [BDCP] *conservation measures are either directly or indirectly influenced by Delta inflows, exports, and outflows.* [p. 11].

and

> *Increased residence times and reduced flushing of the Delta by Sacramento River water appear likely to result in interior-Delta channels that are further dominated by agricultural runoff, invasive aquatic vegetation, warmer temperatures, and increased algal productivity with its associated dissolved oxygen swings.*[p. 14]

and

> *Both projected sea level rise and [BDCP] are also anticipated to cause the average location of X_2 to move upstream during the summer and fall. The effects analysis acknowledges this result, but … concludes that habitat restoration and food web enhancement will greatly offset this loss of habitat value. The conclusion is in part speculation and in part does not reflect current scientific understanding.* [p. 13].

In response to these critiques, the consultant preparing the BDCP Effects Analysis admitted that, "*The larger question regarding how flow and habitat restoration interact in terms of effects on covered fish, the information and tools we would need to address this issue in the EA do not exist.*" [p. 10]

[Grimaldo, L., Miller, R., Peregrin, C., Hymanson, Z. 2012. Fish Assemblages in Reference and Restored Tidal Freshwater Marshes of the San Francisco Estuary. San Francisco Estuary and Watershed Science, 10(1). Available at: http://escholarship.org/uc/item/52t3x0hq.pdf]

This paper documents the results of fish sampling in 1998 and 1999 at a reference marsh and at several tidal marshes that were unintentionally restored as a result of levee breaches. Fish were sampled in both shallow and deeper water. The surveys found that the flooded islands were dominated by introduced fish species (native species were only 2% of the total catch), particularly where submerged aquatic vegetation (SAV) was found. The authors found that, "*Flooded islands dominated by SAV will likely support an abundance of introduced fishes, especially centrarchids. Thus, lower priority should be given to potential restoration sites that are at elevations likely to favor SAV colonization,*"[Grimaldo et al. 2012:17]. Because islands in the central and south delta are substantially below sea level, the authors suggested that habitat restoration efforts may need to focus on the North Delta, where SAV concentrations are lower and potential restoration sites are near sea level. The authors cautioned that, "*Our study findings indicate that newly restored habitats in the Sacramento–San Joaquin Delta will be invaded by introduced fishes,*" [Grimaldo et al. 2012:1].

The National Research Council's 2012 report also raised concerns that habitat restoration may not yield substantial benefits for listed species, and could cause harmful impacts, particularly if newly restored habitats are colonized by *Corbula amurensis* and other invasive clams, stating that:

> *A more subtle effect of transport on primary production is that transport can couple regions of high productivity with regions that are strong sinks for primary production due to benthic grazing (Lucas et al. 2002), such that increasing*

> *residence time can reduce the accumulation of phytoplankton biomass. As an aside, this points to a possible problem with proposals (e.g., in the BDCP) to increase primary production in the system by increasing shallow water habitat: if that shallow water habitat includes a significant biomass of benthic grazers, it may become a net sink for primary production and so will decrease the total phytoplankton biomass available for pelagic grazers like zooplankton.* [NRC 2012:58].

The NRC panel also cautioned that restored habitats are likely to be dominated by nonnative species.

iv. New Scientific Information Demonstrates that Freshwater Flow Mediates "Other Stressors"

In the 2010 Delta Flow Criteria proceedings, much was made of the opportunities to better understand the mechanisms by which freshwater flow benefits native species. In some cases, these mechanisms were well understood at the time. For example, the Board heard evidence that Sacramento splittail spawning and growth of Chinook salmon juveniles is supported by floodplain inundation, presumably because of the creation of suitable incubation habitat for splittail and generation of food items for the salmon. In other cases, the mechanism driving the well established, durable, and high magnitude correlations between flow and abundance are less certain – probably because there is not a single mechanism but a complex, interconnected, and perhaps context-dependent set of factors driving the relationships.

The Board does not need a complete technical understanding of the mechanistic processes by which increased freshwater flow leads to positive population responses in order to establish protective objectives in the Bay-Delta Plan. The highly significant correspondence between flow and abundance (for instance) provides a more than adequate basis for such objectives. Furthermore, it is important not to regard freshwater flow and other stressors as unrelated, independent factors. Freshwater flow is integral to numerous ecosystem processes (e.g. related to temperature, transport, turbidity, particle retention, water quality, etc.); as a result, reductions in freshwater flows tend to exacerbate other stressors to fish populations and vice-versa. For example, Dugdale et al. (2007) recognized that the impacts of their hypothesized ammonium loading mechanism would be strongly influenced by Delta flow rates and Jassby and Van Nieuwenhuyse (2005) revealed the impact of reduced Delta inflow on the dissolved oxygen barrier to fish migration that prevails in the Stockton Deepwater Ship Channel.

Since 2010, new publications have highlighted the central role played by flow in the operation of numerous potential "other stressors" in the San Francisco Estuary. For example:

[Winder, M., A.D. Jassby, and R. Mac Nally. 2011. Synergies between climate anomalies and hydrological modifications facilitate estuarine biotic invasions. Ecology Letters 14: 749–757. Available at: http://online.sfsu.edu/~modelds/Files/References/Winder2011EcolLetters.pdf]

Winder et al. (2011) conducted retrospective analyses of the correspondence between species invasions and fresh water flow rates in this ecosystem and concluded:

> *Hydrological management exacerbated the effects of post-1960 droughts and reduced freshwater inflow even further, increasing drought severity and allowing unusually extreme salinity intrusions. Native zooplankton experienced unprecedented conditions of high salinity and intensified benthic grazing, and life history attributes of invasive zooplankton were advantageous enough during droughts to outcompete native species and colonise the system.* [p. 794]

Similarly, the life history conceptual model for longfin smelt (Rosenfield 2010; cited above) clearly show that Delta freshwater outflow is the dominant driver of survival ("transition probability") for early life stages of this native fish and that it drives many other potential stressors on longfin smelt abundance, productivity and distribution, including:

- quality and availability of incubation habitat,
- direct entrainment at water diversions,
- concentration and diversity of toxins,
- transport and spatial distribution of larval longfin,
- marine migrations, and
- availability of prey.

Also, in their "Red Flag Reviews" of 2011's draft Bay Delta Conservation Plan (cited above) the fish and wildlife trustee agencies, wrote:

> *Increased residence times and reduced flushing of the Delta by Sacramento River water appear likely to result in interior-Delta channels that are further dominated by agricultural runoff, invasive aquatic vegetation, warmer temperatures, and increased algal productivity with its associated dissolved oxygen swings. These environmental conditions favor nonnative/invasive species (e.g. Egeria densa, largemouth bass, water hyacinth, Microcystis) and disfavor native fishes. The Delta is already more biologically similar to a lake than it once was, due to the historical accumulation of human modifications. We expect that by reducing Delta flows, the Preliminary Project would likely facilitate the spread of habitat conditions that are unfavorable to delta smelt, and less favorable to other target fish species survival and recovery.* [pp. 14-15].

II. FALL DELTA OUTFLOW AND LOW SALINITY ZONE HABITAT CONDITIONS

The Flow Criteria Report concluded that increased fall Delta outflow was necessary to improve habitat conditions for Delta smelt, and it recommended that X_2 be located west of 74 km in Wet years and west of 81 km in Above Normal years ("Fall X_2 Action"),[4] with delta outflow for other water year types consistent with the 2006 Bay-Delta Water Quality Control Plan (SWRCB 2010: 98-99, 108-112). The report classified this as a Category B recommendation, calling for implementation in an adaptive management framework. Scientific studies and publications since

[4] This is consistent with the requirements of the U.S. Fish and Wildlife Service's 2008 biological opinion.

2010, particularly the preliminary monitoring and study results from implementation of the adaptive management plan for the Fall X_2 Action in 2011, provide additional support the Board's conclusions in 2010.

A. *New Information Regarding Changes in Fall Outflow Over Time and Causes*

[Enright, C. and S.D. Culberson. 2010. Salinity trends, variability, and control in the northern reach of the San Francisco Estuary. San Francisco Estuary and Watershed Science, 7(2).[5] Available at: http://escholarship.org/uc/item/0d52737t.pdf]

This paper documents a substantial reduction in delta outflow during fall months in the 1968 to 2006 period, concluding that the reductions in outflow during the fall "likely reflect increased fall pumping after the 1994 Delta Accord." This paper also documents how delta outflow has become decoupled from precipitation, with the reduction in outflow due primarily to water export operations, as discussed in detail above.

[Feyrer, F. 2012. Declaration of Frederick V. Feyrer In Support of Defendants' Opposition to Plaintiffs' Motion for Injunctive Relief, July 1, 2011. (Doc. 944)]

In this declaration, Feyrer compared average September to December X_2 locations following wet and above normal year types for the periods 1930-1967 (pre-project), 1968-1999 (post-project), and 2000-2009 (post-project) (Figure 8). He also examined changes in the CVP/SWP export: inflow ratio during these same periods (Figure 9). The analysis in this declaration shows that increased water exports and other CVP/SWP operations have reduced Fall X_2 in recent years.

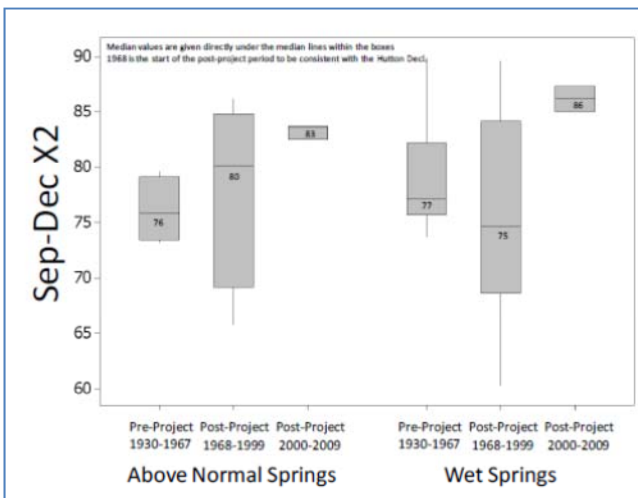

Figure 11. Box plots of X2 for three time periods following above normal and wet springs.

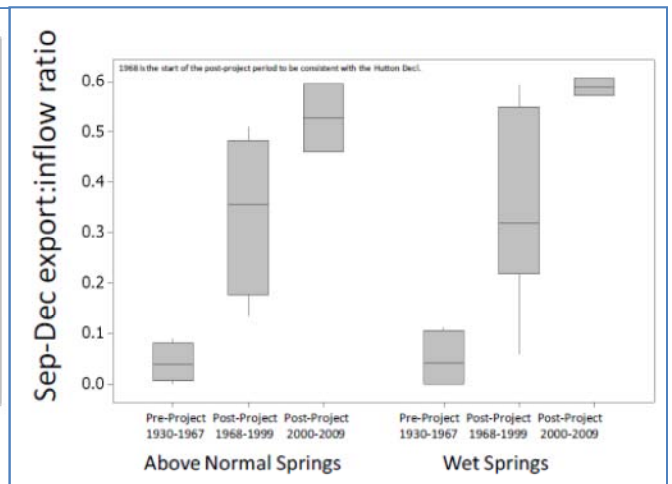

Figure 10. Box plots of export:inflow ratio for three time periods following above normal and wet springs.

Fig. 8 (Reprinted from Feyrer 2012)

Fig. 9 (Reprinted from Feyrer 2012)

[5] This paper was published during the 2010 proceeding, but it was not cited in the 2010 Flow Criteria Report.

B. *New Scientific Publications Regarding the Effects of Fall outflow on fish populations and lower trophic levels*

[Feyrer, F., K. Newman, M. Nobriga, and T. Sommer. 2010. Modeling the Effects of Future Outflow on the Abiotic Habitat of an Imperiled Estuarine Fish. Estuaries and Coasts. DOI 10.1007/s12237-010-9343-9. Available at: http://www.dwr.water.ca.gov/aes/docs/FeyrerNewmanNobrigaSommer2010.pdf]

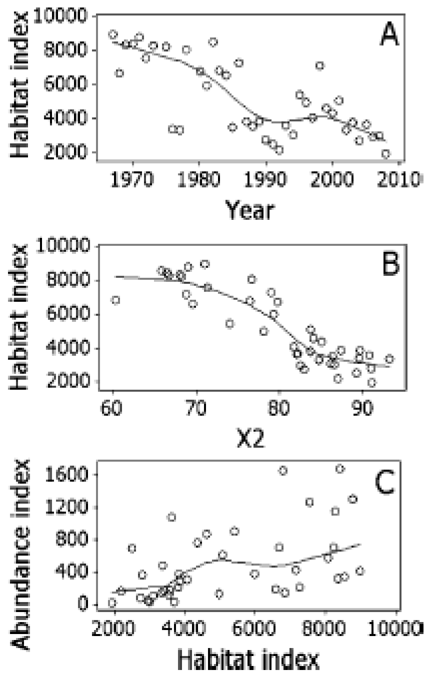

Fig. 2 Plots of the habitat index time series (**a**), relationship between *X2* (km) and the habitat index (**b**), and relationship between the habitat index and delta smelt abundance measured as the fall midwater trawl index. Curves are LOESS smooths

Fig. 10 (Reprinted from Feyrer et al 2010)

This paper documents a substantial decline in the abiotic habitat quality for delta smelt from 1967 to 2008, using a generalized additive model to relate habitat quality (temperature, salinity, and turbidity) with the probability of occurrence of delta smelt. The model predicts continued decline in fall habitat quality as a result of climate change. In addition, this habitat index was positively correlated with the Delta smelt abundance index, but there was more variability in abundance at higher habitat values; in other words, low habitat values are correlated with low abundance, but high habitat values are correlated with both high and low abundance (*See* Figure 10). The authors hypothesized that increased habitat area lessens the likelihood of density-dependent effects on the Delta smelt population and lessens the probability of stochastic events, such as a major pollution event that causes substantial mortality.

[T. Sommer, F. Mejia, M. Nobriga, F. Feyrer, L. Grimaldo. 2011. The Spawning Migration of Delta Smelt in the Upper San Francisco Estuary. San Francisco Estuary and Watershed Science, 9(2). Available at: http://escholarship.org/uc/item/86m0g5sz.pdf]

The paper concludes that the over the past 20 years, the distribution of delta smelt during the pre-migration period (fall) is in the low salinity zone, and that fish distributions in the fall are highly significantly related to the location of X2. The paper acknowledges that an unknown portion of the population occurs in the Cache Slough region, an area that is not consistently sampled in the FMWT, but concludes that the FMWT provides the best available information to analyze long term trends in Delta smelt abundance and distribution.

[Baxter, R. R. Breuer, L. Brown, L. Conrad, F. Feyrer, S. Fong, K. Gehrts, L. Grimaldo, B. Herbold, P. Hrodey, A. Mueller-Solger,T. Sommer, K. Souza. 2010. Pelagic Organism Decline

Work Plan and Synthesis of Results. Interagency Ecological Program, Sacramento, CA. Available at: http://www.water.ca.gov/iep/docs/FinalPOD2010Workplan12610.pdf]

This report summarizes the synthesis of research on the pelagic organism decline. It finds that fall habitat quality for delta smelt has significant declined, and that there is evidence that this habitat decline has had and continues to have population level consequences for delta smelt. It states that "there is good evidence for reduction in habitat availability and suitability during the fall and a linkage of these reductions with abundance." The report also proposes that changes in salinity and outflow are the most important environmental drivers of the pelagic organism decline, including the problem of recent fall outflows being low regardless of water year type.

[BDCP "Red Flag" Documents [California Department of Fish and Game; US Fish and Wildlife Service; and National Marine Fisheries Service. April 2012 BDCP EA (Ch. 5) Staff "Red Flag" Review Comprehensive List. Available at: http://baydeltaconservationplan.com/Libraries/Dynamic_Document_Library/Effects_Analysis_-_Fish_Agency_Red_Flag_Comments_and_Responses_4-25-12.sflb.ashx]

The BDCP "Red Flag Staff Reviews" (2012; cited above) comments by the regulatory agencies on the February 2012 draft BDCP identify the agencies' concerns regarding the exclusion of the Fall X_2 Action from that BDCP proposal. In particular, DFG concluded that there is reasonable evidence that recent changes in water management in the Delta have substantially degraded fall habitat for delta smelt, that this has contributed to the pelagic organism decline, and there is great uncertainty the potential benefits of tidal habitat restoration and food production will offset the negative effects of fall habitat degradation. FWS similarly stated that reduction in flows (in terms of magnitude, timing, and variability) is the most fundamental cause of stress and driver of change in the Delta ecosystem. FWS also stated that:

> *Both projected sea level rise and the Preliminary Proposal are also anticipated to cause the average location of X_2 to move upstream during the summer and fall. The modeling indicates that intra-annual variability would be lost for several months in the late summer and fall in all water year types; even wet years would functionally become dry years for a third of delta smelt's life cycle. The effects analysis acknowledges this result, but the Net Effects concludes that habitat restoration and food web enhancement will greatly offset this loss of habitat value. The conclusion is in part speculation and in part does not reflect current scientific understanding.*
>
> *This has several implications for delta smelt. First, under the preliminary project delta smelt habitat would less frequently lie in Suisun Bay and Marsh during summer and fall. The habitat suitability modeling shows that this would limit the capacity of tidal marsh restoration in the Suisun region to contribute to delta smelt production. Second, lower summer outflows would increase the length of time that seasonal delta smelt habitat constriction occurs and overlaps with physiologically stressful water temperatures. This means that more food production would be required to maintain current delta smelt growth and survival rates, even in areas where temperatures remain suitable. In areas where*

> *temperatures exceed physiologically suitable levels during the summer (~ 24°C), no amount of food production will increase growth or survival rates. Third, the restricted distribution of delta smelt during most summers and essentially all falls would increase the chance that a localized catastrophic event could pose a serious threat to the survival of the delta smelt population.* [p. 13].

[U.S. Fish and Wildlife Service 2012. First Draft 2011 Formal Endangered Species Act Consultation on the Proposed Coordinated Operations of the Central Valley Project and State Water Project. Available online at: http://www.usbr.gov/mp/BayDeltaOffice/docs/Signed_FINAL%202011-F-0043_%20SWP-CVP%20BO%20Dec%2014%20FIRST%20DRAFT.pdf]

The draft biological opinion released by the U.S. Fish and Wildlife Service in December 2011 acknowledged that several recent life cycle models (Mac Nally 2010; Thomson 2010) did not find correlations between Fall X_2 and Delta smelt abundance.[6] The draft biological opinion states there are reasonable explanations why the partial life cycle models (Feyrer 2007, Feyrer 2010) found such a relationship whereas the other models have not: whereas the life cycle models examined changes in the FMWT index from year to year, the Feyrer analyses examined changes from the FMWT index to the STNS index (from adult to juvenile abundance). As a result, "*This time step may therefore just be too long to track the population-level effects of fall habitat conditions – especially since the concurrent habitat influence on each year's FMWT index is already encompassed in the indices themselves*." (FWS 2012: 269). In the draft BiOp, the Service repeated the analysis in Feyrer 2007, using updated FMWT and TNS data and using average X_2 values instead of specific conductance; the draft BiOp finds that:

> *The linear regression showed that fall relative abundance is a highly significant predictor of the next generation's relative abundance (logTNS = 0.742*logFMWT – 1.34; r2 = 0.65; P < 0.000001; AICc = 16.21). Then, we re-ran the linear regression including fall X_2 as a covariate. Consistent with Feyrer et al. (2007), the analysis indicated that both fall relative abundance and fall X2 were significant predictors of the relative abundance of juveniles the next summer (logTNS = 0.703*logFMWT – 0.0252*X2 + 0.872; P < 0.000001; AICc = 14.20). Note that the AICc for the stock-recruit model including fall X2 is two units lower than the model without it, suggesting the regression model that includes X2 provides a better fit to the data (Burnham and Anderson 1998 as cited by Maunder and Deriso 2011).* [p. 270]

The draft biological opinion concludes that these analyses support a population-level effect of fall outflow conditions, but acknowledge that the full life cycle models do not show such an effect. (FWS 2012:271).

[6] The life cycle model prepared by Maunder & Deriso (2011) is also discussed in the draft biological opinion. The published life cycle model did not include Fall X2 as a covariate to analyze.

C. New Scientific Information From the Fall X2 Adaptive Management Plan Monitoring and Studies on the Effects of Fall Outflow on Delta Smelt and Lower Trophic Levels

SUMMARY: Preliminary results from 2011 Fall X2 Action Suggest the Action Contributed to Reduced *Corbula* Grazing Pressure, Increased Phytoplankton and Zooplankton Abundance, and Increased Delta Smelt Growth and Abundance

[Brown, L., R. Baxter, G. Castillo, L. Conrad, S. Culberson, G. Erickson, F. Feyrer, S. Fong, K. Gehrts, L. Grimaldo, B. Herbold, J. Kirsch, A. Mueller-Solger, S. Slater, T. Sommer, K. Souza, and E. Van Nieuwenhuyse. 2012. Synthesis of Studies in the Fall Low Salinity Zone of the San Francisco Estuary, September – December 2011. Available at: http://deltacouncil.ca.gov/sites/default/files/documents/files/FLaSH_combined_7_0_12.pdf][7]

[U.S. Bureau of Reclamation, 2011. Draft Plan: Adaptive Management of Fall Outflow for Delta Smelt Protection and Water Supply Reliability. Available at: http://deltacouncil.ca.gov/sites/default/files/documents/files/Materials_Reviewed_Fall_Outflow_Mgmt_Plan_2011_06_06_review_draft_b.pdf]

[Thompson, J., K. Gehrts, F. Parchaso, and H. Fuller. 2012. Going with the flow: the distribution, biomass and grazing rate of *Potamocorbula* and *Corbicula* with varying freshwater flow (May and October 2009-2011). Progress Report to U.S. Bureau of Reclamation, Sacramento, CA.]

[Teh, S. 2012. Fall X2 fish health study: contrasts in health indices, growth and reproductive fitness of delta smelt and other pelagic fishes rearing in the low salinity zone and Cache Slough regions. Progress Report to Environmental Restoration Program, California Department of Fish and Game, Sacramento, CA.]

[Baxter, R. and S. Slater. 2012. Delta Smelt Distribution & Diet Fall 2011. Presentation to the Delta Science Program independent scientific peer review of the Fall Low Salinity Zone (FLASH) Studies and Adaptive Management Plan Review. Available at: http://deltacouncil.ca.gov/sites/default/files/documents/files/Baxter_Slater_Flash_DeltaScienceJuly2012_v2.pdf]

Calendar year 2011 was classified as a wet water year type, triggering the Fall X_2 action and the associated scientific monitoring and adaptive management program (Brown 2012). The adaptive management plan for the Fall X_2 Action in 2011 included specific monitoring programs and scientific studies, and included testable hypothesis and predictions. Although the Fall X_2 Action was not fully implemented due to court injunctions, on average in 2011 X_2 was located at 75 km for the months of September and October (Brown et al 2012). This resulted in X_2 locations that are substantially different from the most recent previous wet year of 2006 (82 km) or 2010 (85 km) (Brown 2012).

[7] This draft report was publicly released as part of the Delta Science Program's independent scientific peer review of the Fall Low Salinity Zone (FLASH) Studies and Adaptive Management Plan Review. The document has not been finalized, and all conclusions therein are preliminary and subject to revision.

The analysis of monitoring and studies associated with the Fall X_2 Action in 2011 continues, and similar scientific studies and monitoring programs will be implemented in 2012, when no Fall X_2 Action will occur. The preliminary results from monitoring and scientific studies of the 2011 Fall X_2 Action required under the adaptive management plan are discussed below. In addition, Figure 11 on page 32 (reprinted from Brown 2012) provides a preliminary assessment of the Fall X2 Action measured against the testable hypotheses identified in the adaptive management plan.

1. **<u>Effects of Fall Outflow on Delta Smelt Abundance</u> –**

The 2011 adaptive management plan warned that, "*Delta smelt are rare, and a simple calculation reveals that we cannot expect to detect an abundance difference in the FMT after a single year of flow augmentation unless the abundance difference is very large*." (USBR 2011:18). The adaptive management plan predicted that implementation of the Fall X_2 Action at 74 km in 2011 would result in the fall abundance of Delta smelt reversing its declining trend. (USBR 2011). This prediction was more than met (Brown 2012; *see* Figure 7). According to the California Department of Fish and Game, the Fall Midwater Trawl Index of Delta smelt, a measure of abundance of delta smelt, was at its highest levels since 2001 and this "*improvement is likely due in large part to higher than usual Delta outflow which resulted in more and better habitat.*" (CDFG, 2011).

In addition, the adaptive management plan examined the relationship between the FMWT and the TNS survey, as an indicator of delta smelt present in the summer surviving into the fall. The plan hypothesized that the ratio of FMWT population index to TNS population index would be higher in years when X_2 is at 74 km, like 2011 (Brown 2012:36-37, 60-61). The report concludes that:

> *This ratio was well above the median in 2011; however, this may be at least partially the result of favorable summer conditions and resulting high survival rather than only favorable fall conditions and survival. The ratio of TNS to the FMWT of the previous year (Fig. 47) can be used as an indicator of successful recruitment of juveniles from the maturing adults sampled by the FMWT. This suggests that the increase in FMWT population index in 2011 resulted from a combination of favorable factors in the winter, spring, and summer preceding the fall. The data suggests that survival in the fall and preceding summer months was likely higher than other years, supporting the prediction for survival (Table 1*) [p. 61].

2. **<u>Effects of Fall Outflow on Abundance and Filtration Rates of Invasive Clam species (*Corbula* and *Corbicula*)</u>**

One hypothesis to explain why the Fall X_2 action should benefit Delta smelt is that it limits grazing pressure of invasive clams (*Corbula*) on phytoplankton abundance, thus contributing to more productive food webs for delta smelt. The adaptive management plan predicted that *corbula*[8] biomass in the LSZ would be higher at X_2=85 (2010) and lower at X_2=75 (2011). Brown (2012) concludes that this prediction was met:

> *Based on biomass, Potamocorbula were less abundant in Grizzly/Honker Bay and western Suisun Marsh during October 2011 compared to 2009 and 2010 (Fig. 43), supporting the prediction (Table 1). These differences were even more apparent in the turnover rate which normalizes the Potamocorbula grazing rates to the depth of the water column (Fig. 44).* [pp. 57-58].

More directly, the progress report submitted by the principal investigators to the Bureau of Reclamation for this study concludes that:

> "*Relative to the previous two dry years, the biomass of bivalves was decreased in the shallow portions of Grizzly and Honker Bays and in Western Suisun Marsh (including Montezuma and Suisun Slough) in 2011. The reduction in biomass was sufficient to limit the potential for bivalves to control phytoplankton biomass accumulation in fall.*" [Thompson 2012:1].

Thompson found that there was a statistically significant difference in biomass and grazing rate in the Grizzly/Honker Bay shallows and the Western Suisun March in 2011 as compared to 2010 and 2009. As the authors noted:

> "*The location of these decreased grazing rates is important as we might expect pelagic primary producers to do best in the shallows of Grizzly and Honker Bays and we might expect that marsh production would have a better chance of reaching other consumers when the bivalve grazers were greatly reduced as seen in 2011.*" [p. 5]

The principal investigators hypothesized that, "*the increasing salinity in fall that began in 1999 allows fall larvae to settle further upstream,*" in "*traditionally lower salinity areas,*" (Thompson et al. 2012:2-3). Once established in these areas, the authors hypothesize that the bivalves are more resistant to winter spring outflow and this results in higher grazing rates in the following spring. The authors found that, "*the fall grazing rates were sufficient to potentially limit phytoplankton biomass accumulation in 2009-2010 but not in 2011.*" (Thompson 2012:6).

The authors conclude that, "*the reduction in bivalve biomass and therefore grazing in 2011 could be due to recruitment losses in spring or fall and our ongoing work with the monitoring station samples should help delineate the cause,*" (Thompson 2012:6). However, the report also documents that *Potamocorbula* biomass was very high in the fall of 2006, despite the very wet spring that year. Further monitoring and studies are underway, including monitoring in 2012, to better identify the specific mechanism.

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[8] Thompson 2012 identifies the species as *Potamocorbula*, whereas Brown 2012 identifies them as *corbula*.

3. Effects of Fall Outflow on Phytoplankton Abundance

The adaptive management plan predicted that average phytoplankton biomass in the LSZ (excluding *Microcystis*) would be higher at X_2 is at 74 km, and measured concentrations of chlorophyll-a (a common surrogate for phytoplankton biomass) to test this hypothesis (Brown et al 2012). Although some monitoring suggested that the hypothesis was not met, the USGS data supported the hypothesis, and the draft report concluded:

> *Chlorophyll-a concentrations were highest in the LSZ during Sep-Oct compared to all the other years compared, with concentrations lowest in 2005 and 2006. Concentrations were greatest in Sep-Oct 2011 compared to other years across all salinity regions. High concentrations continued in the LSZ in Nov-Dec. In the other salinity regions, concentrations were more comparable across years. Although the EMP and USGS data are somewhat in conflict, we provisionally suggest that the prediction of higher phytoplankton biomass at low X_2 is supported, but the other part of the prediction at higher X_2s is uncertain. We give greater weight to the USGS data because of its slightly greater spatial coverage and observations made by experienced researchers during the EMP and USGS cruises.* [Brown et al. 2012:53].

In addition, investigators observed a significant phytoplankton bloom of long chained diatoms was observed in the fall of 2011, which had not occurred in recent years.

4. Effects of Fall Outflow on Delta Smelt Health and Growth Rates –

The majority of Delta smelt were caught in the low salinity zone in the fall, with a few fish caught in the Cache Slough area (Teh 2012; Baxter 2012). In general, analysis of Delta smelt health and condition showed they were generally in good condition, but there were not sufficient baseline data to compare with 2011 results (Teh 2012). In addition, the study found that:

> *"Otolith growth rates revealed that fish during the fall of 2011 were growing at a high rate, highest since 2000, however we did not observe a difference in growth among different habitats."* [Teh 2012:3].

Additional studies of otolith growth rates are ongoing.

***5.* Effects of Fall Outflow on Zooplankton Abundance**

DFG compared the results of zooplankton sampling in 2011 with earlier years. The results show that adult copepod densities (catch per unit effort, or CPUE) was generally higher in 2011 than in 2010, 2006, or 2005 for *P. forbesi* and *A. sinesis*, both in the low salinity zone and in freshwater. (Baxter 2012). Adult mysid densities were substantially higher in the low salinity zone in 2011 than in those prior years, whereas in freshwater mysid abundance was lower in 2011 in some months. (Baxter 2012) Brown 2012 found that, *"The prediction was that calanoid copepod biomass would be greater in the LSZ with low X_2 and the data did show that trend; however, given the high uncertainty in the data, a definite conclusion is not warranted."* (p. 55).

	Predictions for X2 scenarios		
	85 km	81 km	74 km
		Year used to test prediction	
Variable (Sep-Oct)	2010 (X2=85)	2005, 2006 (X=83,82)	2011 (X2=75)
Dynamic Abiotic Habitat Components			
Average Daily Net Delta Outflow	~5000 cfs?	~8000 cfs?	11400
Surface area of the fall LSZ	~ 4000 ha	~ 5000 ha	~ 9000 ha
Delta Smelt Abiotic Habitat Index	3523	4835	7261
San Joaquin River Contribution to Fall Outflow	0	Very Low	Low
Hydrodynamic Complexity in LSZ	Lower	Moderate	Higher
Average Wind Speed in the LSZ	Lower	Moderate	Higher
Average Turbidity in the LSZ	Lower	Moderate	Higher
Average Secchi Depth in the LSZ	Higher	Moderate	Lower
Average Ammonium Concentration in the LSZ	Higher	Moderate	Lower
Average Nitrate Concentration in the LSZ	Moderate	Moderate	Higher
Dynamic Biotic Habitat Components			
Average Phytoplankton Biomass in the LSZ (excluding *Microcystis*)	Lower	Moderate	Higher
Contribution of Diatoms to LSZ Phytoplankton Biomass	Lower	Moderate	Higher
Contribution of Other Algae to LSZ Phytoplankton biomass at X2	Higher	Moderate	Lower
Average Floating *Microcystis* Density in the LSZ	Higher	Moderate	Lower
Phytoplankton biomass variability across LSZ	Lower	Moderate	Higher
Calanoid copepod biomass in the LSZ	Lower	Moderate	Higher
Cyclopoid copepod biomass in the LSZ	Lower	Moderate	Moderate
Copepod biomass variability across LSZ	Lower	Moderate	Higher
Corbula biomass in the LSZ	Higher	Moderate	Lower
Predator Abundance in the LSZ	Lower	Moderate	Higher
Predation Rates in the LSZ	Lower	Moderate	Higher
Delta Smelt (DS) Responses			
DS caught at Suisun power plants	0	0	Some
DS in fall SWP & CVP salvage	Some?	0	0
DS center of distribution (km)	85 (77-93)	82 (75-90)	78 (70-85)
DS growth, survival, and fecundity in fall [a]	Lower	Moderate	Higher
DS health and condition in fall	Lower	Moderate	Higher
DS Recruitment the next year	Lower	Moderate	Higher
DS Population life history variability	Lower	Moderate	Higher

[a] Only survival from summer to fall as the ratio of FMWT population index to TNS population index was assessed.

Fig. 11 (Reprinted from Brown 2012:63-64). Predictions that were supported by the data are highlighted in green, and predictions that were not supported by the data are highlighted in red. Predictions highlighted in gray indicate that data are not yet available to support a conclusion, and predictions with no shading indicate that there is no data to assess whether the prediction was met or not.

III. LIMITATIONS ON THE USE OF THE BDCP EFFECTS ANALYSIS IN REVISING THE BAY-DELTA WATER QUALITY CONTROL PLAN

The State Board properly concluded that its 2010 Flow Criteria Report was based on the best available science. As detailed in this written submission, subsequent scientific reports and publications since 2010 overwhelmingly support the Board's conclusion. In contrast, as we noted above in the context of the hypothesis that physical habitat restoration can offset further flow reductions, both independent peer reviewers and state and federal fish and wildlife trustee agencies have repeatedly concluded that the Bay Delta Conservation Plan Effects Analysis fails to use the best available science. Because of its deeply flawed analytical approach, the Board should not rely on BDCP's effects analysis in this proceeding. In addition, the Board should consider independent scientific peer reviews of future iterations of the BDCP effects analysis (or require such review if it has not occurred) before relying on BDCP's scientific conclusions.

[National Research Council, 2011, A Review of the Use of Science and Adaptive Management in California's Draft Bay Delta Conservation Plan. The National Academies Press, Washington, DC. Available at: http://www.nap.edu/catalog.php?record_id=13148]

In 2011, the National Research Council (NRC 2011) reviewed the November 18, 2011 working draft chapters of the BDCP, and found critical gaps in the BDCP. The panel was not provided with the draft effects analysis. The NRC panel noted that the draft document:

> *"… creates the impression that the entire effort is little more than a post-hoc rationalization of a previously selected group of facilities, including an isolated [water] conveyance facility, and other measures for achieving goals and objectives that are not clearly specified."* [NRC 2011:43].

This independent review specifically commended the use of DRERIP conceptual models (such as Rosenfield 2010, described above), the IEP POD conceptual framework, specific goals and objectives, and the independent science advisor's adaptive management framework (which clearly identified uncertainties). The review concluded that, "*It is nearly impossible to evaluate the BDCP without a clear specification of the volume(s) of water to be diverted, whose negative impacts the BDCP is intended to mitigate.*" [p. 4].

[Red Flag memos (cited above)]: The Department of Fish and Game, U.S. Fish and Wildlife Service, and National Marine Fisheries Service identified substantial methodological flaws, stating that the Effects Analysis has a tendency to "*overstate Plan benefits*", "*turn the notion of uncertainty upside down*," made unjustified conclusions, relies on "*combat science*," "*deals with the critical concept of uncertainty inconsistently and does not effectively integrate, use, and report uncertainty in the Net Effects*," uses inadequate conceptual models, "*underemphasizes Bay-Delta water flows as a system-wide driver of ecosystem services to the San Francisco Estuary*," relies on selective use of data and models, ignores the best available models for splittail and longfin smelt, and "*continues to insist on an analytical approach to entrainment that does not reflect the best available science*." [*See esp*. pp. 1-2, 5, 8-10, 11-12, 15-17]

In particular, the agencies identified substantial flaws with the analysis of changes in spring outflow on longfin smelt, changes in fall outflow on delta smelt, and the analysis of impacts to the low salinity zone, with FWS concluding that:

> *"In summary, the current Effects Analysis does not appropriately deal with critical issues involving the role of the Low Salinity Zone as habitat for longfin smelt, delta smelt, and splittail. Until it addresses the right questions regarding flow, LSZ location, and turbidity, we are reluctant to rely on its conclusions."* [Red Flags 2012: 13-14; *see also* pp. 5-7, 12, 16]

[Parker, A., Simenstad, S., George, T., Monsen, N., Parker, T., Ruggerone, G., and Skalski, J. Bay Delta Conservation Plan (BDCP) Effects Analysis Phase 2 Partial Review, Review Panel Summary Report. Delta Science Program 2012. Available at: http://deltacouncil.ca.gov/sites/default/files/documents/files/BDCP_Effects_Analysis_Review_Panel_Final_Report_061112.pdf]

In June 2012 an independent peer review panel convened by the Delta Science Program issued its report reviewing the BDCP's draft effects analysis. The report states that:

> *…the Panel universally believes Chapter 5: Effects Analysis fails to achieve the fully integrated assessment that is needed to draw conclusions about such a momentous Plan. By missing or obscuring key concepts and specifics, it falls short of presenting an analytical framework for a compelling and rigorous analysis of whether and how the BDCP would achieve its biological and other objectives.* [p. 4].

The review panel concluded that, "*As it is currently written, the Effects Analysis is too inconsistent in its treatment of how effects are analyzed across listed species and the potential costs and benefits of the planned BDCP activities are too uncertain to provide an objective assessment of the BDCP on covered species*," (p. 5). The panel also found that:

- The effects analysis failed to adequately incorporate biological goals and objectives;
- The net effects assessment "*needs greater objectivity*," "*are substantially misleading*," and should be subject to independent peer review;
- The effects analysis ignores potential negative impacts of habitat restoration and other conservation measures;
- The effects analysis fails to address uncertainty systematically; and,
- The analysis of food resource availability and the effects of habitat restoration "*is grossly incomplete and overly simplistic*" [*see esp*. Parker 201:13-15, 21-25, 26-29, 34-36]

Question 2. How should the State Water Board address scientific uncertainty and changing circumstances, including climate change, invasive species and other issues? Specifically, what kind of adaptive management and collaboration (short, medium, and long-term), monitoring, and special studies programs should the State Water Board consider related to ecosystem changes and the low salinity zone as part of this update to the Bay-Delta Plan?

Response to Question 2: Recommendations to Address Scientific Uncertainty and Changing Circumstances

The basic questions to be addressed by adaptive management are: (1) how will the Delta ecosystem respond to implementation of the Bay-Delta Plan? and, (2) how should the Plan be modified as new information becomes available and/or new circumstances alter ecosystem conditions? These questions cannot be adequately answered until the Board has clearly defined desired ecosystem conditions and instituted a framework for designing, implementing, and modifying Bay-Delta Plan objectives over time to best achieve the desired response.

The foundation of that adaptive management framework is in the identification of biological outcomes for fish and wildlife species strongly influenced by flow and water quality parameters and bio-physical outcomes for the ecosystem as a whole. These outcomes (referred to here as "targets", but generally in other fora as "objectives") are defined such that they are specific, measureable, achievable, relevant to the particular goals that characterize the plan's overarching purpose (protecting the public trust values and beneficial uses of the Delta ecosystem) and time-bound (S.M.A.R.T.) – this level of specificity and detail being necessary for orienting, enforcing, measuring and evaluating progress of, and calibrating the adaptive management effort. The framework is completed by ensuring that information relevant to evaluating the Plan's performance is collected and evaluated, and future actions (ranging from accelerated review to large-scale modifications) and the conditions that trigger those actions are established as part of the program of implementation. In other words, adaptive management requires management targets (desired outcomes), a method of evaluating progress towards those targets (monitoring and data evaluation), and specific decision pathways that describe how, when, and under what circumstances new information is used to modify the implementation of the Plan. Recent insights into the impact of global climate change on the Bay-Delta ecosystem and its watershed (Null, S.E. and J.H. Viers 2012) emphasize the need for the Board to clearly articulate S.M.A.R.T. management targets and adaptive management decision-pathways in advance as this will establish the basis for recognizing when corrective action is required and how those corrective actions will be determined.

I. A LOGIC CHAIN ARCHITECTURE FOR PLANNING RESTORATION OF PUBLIC TRUST VALUES IN THE BAY DELTA

We propose using what we have termed a "Logic Chain" decision architecture for revising the Bay-Delta Plan and adaptively managing its implementation and subsequent modification. By posing a series of increasingly specific questions, the Logic Chain forces articulation of a Plan's desired outcomes, proposed actions to achieve those outcomes, and expected results of those actions. These questions are described in the attached "Logic Chain User's Guide" (Appendix

A), modified here from that which we developed for the Bay Delta Conservation Plan (BDCP). The orientation of different elements of the Logic Chain is displayed in the attached "Logic Chain Architecture" diagrams (Appendix A).

To apply the Logic Chain approach to revising the Plan, the Board must first identify in broad terms what the Plan is intended to accomplish in protecting public trust values and beneficial uses (i.e., 'Goals"). These should be short declarative statements that describe outcomes of a successful plan; there may be numerous different plan goals (e.g. at least one for each species or ecosystem characteristic of interest). Each Goal is associated with a S.M.A.R.T. (specific, measureable, achievable, relevant (to the goal), and time-bound) target (or "objective" in non-Clean Water Act usage) that describes unambiguously what attainment of the goal looks like.

A. Goals and SMART Targets

Goals and SMART targets are policy decisions informed by the best available science. Policy makers have set statutory and legal thresholds for restoration and recovery of native species, natural communities, and other public trust ecosystem values through enactment of the state and federal Clean Water Acts (CWA), state and federal Endangered Species Acts (CESA, ESA), the Central Valley Project Improvement Act (CVPIA), the California Natural Communities Planning Act (NCPA), and other laws. In implementing these legal mandates, the fish and wildlife trustee agencies have, in many cases, promulgated goals and targets related to achieving restoration and recovery thresholds. For example, NMFS and USFWS have developed draft recovery standards for Central Valley salmonids (NMFS 2009) and native pelagic species (FWS 1995). Further description of restoration targets for anadromous fish species are contained in the CVPIA's Anadromous Fish Restoration Program (AFRP) and products developed from implementation of the San Joaquin Settlement Act (USBR 2011). Furthermore, in adopting the flow criteria in the 2010 report the Board linked those criteria to the likely occurrence of specific desired outcomes for public trust resources. TBI et al (2010, exhibits 1-4) and CDFG (2010), in particular, provided the Board with detailed information regarding the population viability attributes of native estuarine species that are most strongly affected by flow conditions, and identified thresholds for viable populations that can and should be used to guide the setting of flow-related objectives in the Plan. These sources provide a surfeit of material from which the Board can develop its own set of goals and SMART targets calibrated to achieving its responsibilities under the Clean Water Act and the public trust and we incorporate them fully by reference.[9]

As we defined the term in our previous testimony (TBI et al. 2010, exhibits 1-4), "viability" means the maintenance of acceptable levels or conditions of four different biological characteristics that relate to the persistence of populations and estuarine ecosystems:

- Abundance
- Spatial distribution
- Diversity and
- Productivity

[9] We understand the Board's interest in having previous testimony cited referenced very specifically (by page, paragraph, etc.); however, given the volume of relevant information in TBI et al. (2010, exhibits 1-4) and CDFG (2010), such specific references would be inefficient and burdensome to the reader.

These terms were further defined by the National Marine Fisheries Service for "viable salmonid populations" (McElhany et al. 2000; Lindley et al 2007) and they are widely accepted as the relevant characteristics for gauging population viability in the field of conservation biology (e.g. Meffe and Carrol 1994).

Viable populations exhibit levels of each of these four characteristics that protect them from extirpation. The level of each of these attributes that represents protection of public trust values may equal or exceed those required to maintain viability, but the public trust cannot be maintained at values of these population attributes that represent non-viability of important species. Brief descriptions of the four viability attributes follow:

1. Abundance Targets

The number of organisms in a population is a common and obvious species conservation metric. More abundant populations are less vulnerable to environmental or human disturbances and risk of extinction and reflect a higher level of protection of public trust values. Populations or species with low abundance are less viable and at higher risk of extinction than large populations for reasons that include environmental variation, demographic stochasticity, genetic processes, and ecological interactions. Abundance is also correlated with and contributes to other viability characteristics including spatial extent, diversity, and productivity. Sufficient population abundance is necessary, but not sufficient alone, to guarantee viability into the future.

Example: In our 2010 testimony (TBI et al. exhibit #2), we suggested the Board adopt an abundance target for longfin smelt recommended by the US Fish and Wildlife Service in its draft Delta Native Fishes Recovery Plan (FWS 1995). That draft recovery plan indicated that longfin smelt would be considered recovered (with respect to abundance) when:

> *… its population dynamics and distribution pattern within the estuary are similar to those that existed in the 1967-1984 period. This period was chosen because it includes the earliest continuous data on longfin smelt abundances and was a period in which populations stayed reasonably high in most years…*[p. 56].

As discussed elsewhere in this submission and our previous testimony (TBI et al. 2010, Exhibit #2), longfin smelt population abundance is strongly correlated with Delta outflow; thus, population abundance targets can and should be scaled to prevailing annual hydrology. Unless the abundance target is corrected for hydrology, it may not be achievable during very dry periods and may not be relevant to the restoration goal during very wet periods (when attainment of the 1967-1984 average population could represent underperformance). We suggest that the abundance target for longfin smelt be defined as equaling the abundance *relative to unimpaired hydrology* seen during the 1967-1984 period and that the Board aim for attainment of this target within 6 years of implementation of any new water quality standards.

Note that this target does not suggest that actual Delta outflow approach unimpaired Delta outflow, only that abundance in the future equal or exceed that which occurred under similar hydrological conditions in the 1967-1984 period. Such an approach to a longfin smelt abundance target has the advantage of remaining agnostic on what actions (flow or other) are taken to achieve the target – if habitat restorations or modification of water diversion structures or operations are successful, then it is possible that less actual Delta outflow will be needed to achieve abundances documented in similar hydrological conditions in the past.

2. Spatial Distribution Targets

More widely distributed populations are less vulnerable to catastrophic events and risk of extinction (e.g., MacArthur and Wilson 1967; Meffe and Carrol 1994; Laurance et al. 2002). Therefore, maintaining or restoring spatial distribution of fish and wildlife species is a critical component of protecting these species and maintaining the public trust. Increased spatial distribution reduces susceptibility to localized catastrophes, predator aggregations, and disease outbreaks while simultaneously increasing the probability that at least some dispersing individuals will encounter habitat patches with favorable environmental conditions. The effect of geographic distribution on extinction risk is also apparent in the geographic attributes of extant freshwater fish species (Rosenfield 2002). The need to maintain adequate spatial distribution is regularly acknowledged in regulatory planning and decision-making regarding the Delta and its environs (e.g. FWS 1995; NMFS 2009).

Example: Delta smelt spend much or all of their life in the Delta's Low Salinity Zone. The size and geographic extent of this important habitat determines, to a large extent, the boundaries of the Delta smelt population during key parts of their life cycle. If at certain times of year, the LSZ is confined to a small geographic area (as when it is located to the east of Chipps Island where it is confined to narrow River Channels), then all or most of the Delta smelt *in the world* are also located in one area – all of their eggs are in a very small basket such that a catastrophic chemical spill (e.g. the Cantara Loop spill), disease outbreak (e.g. the Klamath salmon kill), predator aggregation, or entrainment event could eliminate the entire population very quickly. This is part of the rationale behind recommendations (ours and those in the FWS 2008 biological opinion) to expand habitat in the fall (e.g. the Fall X_2 action). The Board should describe, based on the best available science and linked to attributes of viability for key species, the desired spatial extent during relevant seasons and year types and then mandate that these spatial extent targets be met immediately upon promulgation of a new WQCP.

3. Diversity Targets

Species and populations that are both more genetically diverse and more diverse in life history patterns are more resilient to environmental change and less at risk of extinction. Natural diversity needs to be protected both within populations of native species in the Bay-Delta and in the habitats and processes of the ecosystem as a whole. Natural diversity (e.g. life history patterns) allows organisms to adapt to and benefit from environmental variability. This is an especially important characteristic in highly variable ecosystems such as the Delta. Flow criteria should also address the natural diversity of natural communities through specific targets for seasonality, frequency, and duration of freshwater Delta outflows. Variability among individuals in a population increases the likelihood that at least some members of the population will survive and reproduce regardless of natural variability in the environment.

Life history diversity provides the protection for species in time that spatial distribution provides in space – the risk and rewards are distributed across the population. The historic variability in the timing of the Delta's peak flows (e.g. Kimmerer 2004; Enright and Culberson 2010) is reflected in the life history of the Delta's native species; Delta smelt and longfin smelt display protracted spawning periods in this ecosystem, for example (Nobriga and Herbold 2010; Rosenfield 2010). The success of these different life history timings depended on the availability of resources (e.g. food, spawning conditions) in the next, unpredictable phase of the life cycle. By restricting "beneficial" flow periods to short time windows (e.g. VAMP), policy makers attempt to make native species' life histories uniform and predictable. But, if individual success still depends on future conditions that are unpredictable, then such artificial and rigid constraints on life history transformations that are triggered by flow may eventually lead to an entire population missing its "window of opportunity". Flow variability (frequency variation in magnitude, seasonality, and duration) within the Delta is a natural part of the ecosystem and flow criteria should insure both the maintenance of appropriate variability and the maintenance of the life history diversity that allows public trust resources to adjust to and thrive within that variability regime.

Example: The natural (historical) breadth of life history timing are known for several species that depend on the low salinity zone are well-documented. Just as the size and extent of the LSZ will be governed by the WQCP, the Board should ensure that the size and location of this critical habitat is maintained the approximate duration reflected in historical patterns of key species. In order for the target to be S.M.A.R.T., the Board will need to establish target dates for attainment of the desired duration of specific low salinity zone conditions.

Allowing for sufficient time for expression of life history diversity within populations will be absolutely essential as these organisms (and the Board) grapple with the changing seasonality of resource availability that is expected under global climate change scenarios. It is important to note here that the Board's formulation of flow criteria in its 2010 report based on a percentage of unimpaired as a multi-day running average, would by its nature tend to preserve the seasonality of peak flow events and flow troughs. Under this formulation, both the specific percentage of unimpaired flow and the averaging period for that flow that the Board requires will determine both the magnitude of peak flows *and their duration*.

4. Productivity Targets

A population's potential for population growth allows it to adjust to variable conditions in a dynamic estuary. The abundance, distribution and diversity of public trust resources cannot be adequately protected if human activities result in environmental conditions that regularly or chronically result in negative population growth (i.e., population decline), reduce the ability of depressed populations to recover, and/or cause the abundance, spatial extent, or diversity to fluctuate wildly. Species or populations with persistent negative population growth, as well as populations with limited ability to respond positively to favorable environmental conditions, are less viable and at higher risk of extinction. In general, extraordinary population variability increases the risk of extirpation (May 1971) and should be avoided (e.g., Thomas 1990). Rapid and large declines in species abundance produce "genetic bottlenecks" that may constrain viability of a species for many generations even after abundance has recovered. Similarly, actions that impede a small population's natural ability to capitalize on the return of beneficial environmental conditions (e.g. loss of unoccupied habitat, decreased reproductive potential, mortality inversely proportional to population size) represent significant challenges to that population's viability.

Example: As described above, current water management practices in the Central Valley result in (a) disproportionate impact to winter-spring Delta outflow and the Low Salinity Zone habitat when natural (unimpaired) hydrological conditions are dry (Figure 2) and (b) an extremely high frequency of naturally rare and devastating (super-critical) actual Delta outflow conditions (Figure 3). The frequency of extreme drought conditions and the truncation of the other, wetter end of the flow spectrum persistently reduce population and overall ecosystem productivity in the Bay-Delta – simply, the system is heavily impacted frequently and not allowed to recover when conditions would otherwise allow for recovery. The Board can improve ecosystem and population productivity of LSZ-dependent species by setting minimum flow frequency thresholds (e.g. super-critical years can occur at a maximum of 1 in 20 years and wet years must occur at least 3 of every 4 years in which hydrological conditions permit) and by insuring that water development impacts are more equitably distributed across water year types – we note that the Board's formulation of flow criteria in its 2010 report based on a percentage of unimpaired as a multi-day running average, would standardize the proportional effect of water development regardless of hydrological condition. Targets for restored productivity are necessarily defined as acceptable return frequencies for different conditions; the Board can and should establish a time-bound for attainment of these ecosystem productivity targets that allows for detection of natural patterns and deviations from those natural patterns (e.g. non-attainment).

B. Stressors and Stressor Reduction

Stressors are those forces that are believed to inhibit attainment of the Plan's goals and SMART targets. Uncertainty may manifest in different views regarding which forces limit recovery of public trust values or the relative strength of different stressors. One benefit of the Logic Chain Architecture is that it does not require an a priori ranking of different stressors; multiple stressors can be processed simultaneously. Instead, assumed stressors are treated as hypotheses about what limits attainment of goals and objectives so that stressor reduction targets (the next level of the Logic Chain) can be written in the following form:

> *"If* [stressor x] *is limiting attainment of* [target y]*, then we can attain the target by reducing* [stressor x]* by* ___ [insert specific, measureable reduction] *by* ___ [insert date]*"*

Examples of stressors that might be relevant to restoration of public trust values and beneficial uses of the Delta ecosystem include (but are not limited to):

- limited low salinity zone rearing "habitat" (characteristics of which would be specifically defined)
- inadequate transport/retention of larval fish (or food/nutrients)
- inadequate flushing of pollutants (toxins or nutrients)
- inadequate access/attraction to/from tributary habitats
- impaired migration due to physico-chemical blockages
- inadequate food production

Technical experts should be surveyed to determine whether the stressor reduction threshold is sufficient to produce measureable progress towards the related SMART target *if* (assuming) the stressor is actually operative.

Note that, during completion of the upper parts of the Logic Chain (description of goals, targets, stressors, and stressor reduction thresholds), it is undesirable to imply that a problem will or must be solved through a particular course of action; that is, we should not confuse the purpose of a plan (its goals and targets) with the means employed to attain those ends.

C. Actions (Flow Objectives)

Actions describe the measures that will be taken to reduce stressors and thus attain the SMART stressor reduction targets. Measures to reduce stressors should be implemented commensurate with (1) the magnitude of the action's effect, (2) the degree of scientific certainty regarding the action's effect and our ability to alleviate the stressor, (3) the speed with which remedies can be implemented, and (4) our ability to increase certainty and/or performance of the conservation strategy (i.e. our ability to learn from the action). Effort and resources expended addressing a stressor should also be inversely proportional to the risk of unintended and irreversible consequences from taking action. Actions to improve freshwater flow conditions in the Delta rate highly in each of these decision criteria because they are relatively certain to alleviate multiple stressors on multiple species, they can be implemented rapidly, and they are easy to modify (or undo) as we learn more and/or conditions change.

Implementing flow-related objectives is the main action that the Board will take in revising the Bay-Delta Plan to help ameliorate stressors (meet stressor reduction thresholds) in order to achieve ecosystem-wide goals and targets. More than one action may be required to fully address a particular stressor and individual actions may affect more than one stressor. The Board can and should describe the expected outcomes of each objective it promulgates – how much is each action expected to achieve (magnitude)? And how certain are we that the desired effect will be realized? Identification of the magnitude and certainty of an action's expected outcomes should not imply that the Board will only take actions with a high impact and high degree of certainty (although these are obviously preferred); rather, identifying the magnitude and certainty of outcomes for each action allows the Board to assess the potential for a suite of actions to attain sufficient stressor reduction and attainment of plan goals and targets. If numerous actions have relatively low certainty of producing positive impacts, then we would clearly want to identify additional measures that can "take up the slack" if expectations for one or more measures are not met.

II. The Logic Chain's Role in Adaptive Management

By clearly describing the Bay-Delta Plan's goals and targets with respect to desired outcomes for public trust values and beneficial uses of the Delta ecosystem and by articulating the pathways by which those outcomes will be attained, completion of the Logic Chain lays the groundwork for effective and transparent adaptive management of flow standards in the future. Well-articulated desired outcomes for biological and physical conditions of the future (S.M.A.R.T. targets) serve as beacons for management action; for instance, a management response is clearly required whenever targets are not attained by their specific time-frame. Thus, simply by defining relevant achievable targets in a specific and time-bound manner, the Board begins to define a decision pathway for adaptive management of the Plan. Similarly, the Logic Chain architecture forces planners to identify the key assumptions (which stressors are being addressed? how much do they need to be reduced to achieve the objective?) and uncertainties (will this action contribute more or less than expected to reducing its associated stressor? will it generate unforeseen negative outcomes?) that then become the focus of adaptive management monitoring and targeted research as detailed in the Plan's program of implementation.

Obviously, the nature of the performance monitoring and evaluation regime is critical to the success of the adaptive management framework we have described. At a minimum, in the program of implementation the Board should require implementation of monitoring and research activities equivalent to those in the existing monitoring and research program identified in the 2011 fall outflow adaptive management plan, with monitoring implemented in all water year types, as modified to ensure adequate evaluation of attainment of biocriteria and other SMART targets. The monitoring results and adaptive management decisions (implementation of decision tree framework) should be subject to independent peer review, and the results of the monitoring and adaptive management plan should be synthesized and peer reviewed every three to five years. At each subsequent review of this Bay Delta Plan, the Board should review the synthesis of results and peer review to consider changes to the Plan to better attain SMART targets and protect beneficial uses and the Public Trust.

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APPENDIX A

THE LOGIC CHAIN: TERMS AND APPLICATIONS
A User's Guide for the Bay-Delta Plan Update

BACKGROUND AND NEED

The San Francisco Bay-Delta and its watershed are home to numerous imperiled species, including (but not limited to) those that are officially protected by the federal or state Endangered Species Acts. The watershed is also the source for much of California's agricultural, municipal, and industrial water supply. Planning efforts to reconcile these two, often competing, demands are underway (e.g. BDCP, DSP, WQCP).

The process of developing and implementing a plan that would allocate sufficient water to meet these different needs is extremely complex. Restoration planning is complicated by the number and diversity of imperiled and/or recreationally/commercially valuable species, the physical complexity of the Delta, and uncertainty about the nature and strength of cause-effect relationships operating in this ecosystem. Furthermore, the ecosystem is changing in ways that are relatively well understood (e.g. sea level rise), incompletely understood (e.g. pelagic organism decline), and those that are unknown.

The Logic Chain architecture is designed to (1) standardize terminology used in the planning process, (2) increase clarity and specificity regarding expected outcomes of plan implementation (e.g. to allow evaluation of a conservation plan prior to its implementation), and (3) develop the inputs that will be necessary for adaptively managing plan implementation as efficacy of the plan is evaluated and actions are adjusted accordingly. This document serves to describe and define tiers of the Logic Chain so there is a shared understanding of its terms, the questions underlying different components of the architecture, and expectations of a comprehensive plan description.

The Logic Chain articulates a pathway from a plan's Goals and Targets, to the specific actions designed to achieve those aspirations, to the monitoring, research, and metrics that will capture the effects of the conservation actions, and through specific adaptive management decision pathways that adjust conservation effort in light of progress made towards Goals and Targets. The Logic Chain captures the underlying rationale and assumptions for the actions that comprise the overall conservation strategy ("the plan") and establishes benchmarks against which progress can be measured. This approach increases specificity and clarity regarding:

- desired outcomes and specific targets for recovery of covered species and ecosystem attributes;
- the stressors assumed to impede attainment of these outcomes;
- the plan's strategy for stressor-reduction;
- the conservation actions and their expected outcomes; and
- the metrics that will be monitored and studies performed to evaluate plan success.

The clarity and specificity of a Plan's articulation of the components of its Logic Chain affect our understanding of the data collection, analysis, synthesis, and evaluation processes that enable adaptive management. By articulating what the conservation strategy is trying to accomplish and how it intends to achieve its targets, the Logic Chain architecture facilitates both evaluation of the initial plan and assessment of its efficacy during implementation. Specific decision pathways (what decisions are necessary? when and how are they made? by who? how are the decisions implemented?) must be outlined in advance in order to maintain the Logic Chain's focus on attainment of desired outcomes.

THE LOGIC CHAIN – HOW IT WORKS

By capturing the answers to a standard set of questions, the Logic Chain architecture provides a means for explaining the challenges to ecosystem restoration and maintenance of public trust values and how a given conservation strategy intends to address those impediments. These questions and their position within the Logic Chain are described below. *The Logic Chain does not identify specific legal obligations (e.g. as spelled out in permit terms or water rights decisions); rather, it forms the basis for determining those obligations*. As our knowledge base grows (through initial evaluation and subsequent implementation of a plan and as a result of ongoing monitoring and research) management uncertainty can be reduced, allowing increased efficiency and efficacy in allocation of conservation effort.

LOGIC CHAIN QUESTIONS AND ASSOCIATED TERMINOLOGY

Below are examples of the questions that drive various levels of the Logic Chain. Each question calls for a particular type of information; labels for these Logic Chain components are indicated with underlining and italics and also appear on the associated schematic diagrams.

What's the problem? Numerous fish species in the Sacramento-San Joaquin Delta ecosystem are officially endangered or otherwise imperiled; collectively, they reflect a decline in various ecosystem functions and a diminution of public trust values. Ecosystem processes (such as floodplain inundation, primary and secondary productivity, and transport and retention of organisms, toxins, and nutrients) have been radically altered in this ecosystem. For each of several target species and for the ecosystem as a whole, *problem statements* provide a concise declaration of the ecological issues that a conservation plan will address. Problem statements are general and objective descriptions of the problem(s) and do not assume particular causes of, or solutions to, those problems.

What outcome(s) will solve the problem? The Logic Chain describes species and ecosystem attribute-specific *global goals* – general statements that disaggregate the problem statement into its various components. There may be more than one Goal associated with each problem statement. Goals represent desired outcomes that will solve the issue(s) identified in the problem statement. Again, these are simple, factual statements (that rely on trustee agencies' expert opinion) and do not pre-suppose a mechanism for solving the problem. The goals are "global" because they describe outcomes that may be partially or completely beyond the scope of any single plan. Still, identification of these global goals is important to create a context for the overall conservation strategy. Global goals and associated targets are delineated by the fish and wildlife trustee agencies (e.g., as identified in the various conservation/recovery plans).

How will we know then the global goal has been attained (what does solving the problem look like)? *Global targets* provide specificity to the desired biological or physical outcome (goal). Targets are specific, measureable, attainable, relevant to the goal, and time-bound (S.M.A.R.T.) statements of what level of restoration constitutes attainment of the goal. Global targets provide a clear standard for measuring progress towards a goal. As with global goals, global targets may be only partially relevant to the activities of a particular plan; their function is to define the magnitude of the problems so that investment in conservation activities is appropriate to the magnitude of the conservation challenge.

What currently prevents us from attaining the global targets? Physical, chemical, and biological attributes of the Delta have changed dramatically over the past several decades (and that change is expected to continue into the future). Some of these changes are *stressors* to covered species and important ecosystem processes. However, the precise contribution of each stressor to a species' population decline is uncertain and there is some disagreement over whether particular changes are stressors at all.

Our knowledge base (data, publications, conceptual and quantitative models) suggests which stressors are operating on particular species or ecosystem values. Describing the stressors (and assumptions about them) accurately and comprehensively is a key step in constructing a conservation plan and in managing adaptively as the plan is implemented. For example, clear statements regarding where a stressor occurs, which species/ecosystem attributes it impacts, and how certain we are that the stressor is important will help focus planning on the relevant stressors; ranking stressors is unnecessary at this stage because the logic chain elements that come after stressor identification provide insight into a stressor's potential magnitude and certainty as well as our ability to address the stressor – understanding these facets of assumed stressors is necessary in order to prioritize them.

Some stressors are beyond our control or beyond what we choose to control. For example, annual weather patterns (unimpaired hydrology) and ocean conditions cannot be impacted by local or regional conservation measures. Similarly, some problems may be beyond the geographical or legal scope of any given conservation plan. These *unmanaged stressors* are described in the planning process for two reasons: (1) so that it is clear that other stressors may affect ecosystem performance and (2) so that these stressors can be monitored/measured and used to more clearly reveal the true impacts of plan implementation (e.g. they may be used as covariates in an any analysis of ecosystem performance).

What will the plan do to reduce stressors? Stemming from the stressors identified for each species and the ecosystem, *Plan Targets* identify the scope of perceived problems that the plan will address. As with global goals and targets, stressor reduction targets are S.M.A.R.T. statements that clarify the plan's intentions; they articulate a desired outcome resulting from implementation of the conservation measures. These targets reveal the relative effort dedicated to alleviating each stressor and provide a basis for assessing whether the conservation measures will (cumulatively) achieve the plan's stressor reduction target (see *expected outcomes* below).

System-wide monitoring metrics and programs will be identified as a means of tracking progress towards stressor reduction (plan targets), global goals, and global objectives. Monitoring must also track unmanaged stressors because plan effectiveness will be judged after accounting for variance in these "background conditions" (because, for example, a spate of dry years would be expected to result in low abundance of many species and productive ocean conditions would be expected to contribute to higher returns of anadromous fishes). Data from monitoring plans will be collected, synthesized, and evaluated by an independent entity (to be defined) that is charged with evaluating plan effectiveness and advising policy-makers about ongoing adaptive management actions.

What actions will be taken reduce stressors (achieve the plan's targets)? The conservation strategy consists of a number of different actions that address one or more of the stressors identified above for one or more of the key species or ecosystem attributes). In order to estimate their value, importance, and overall contribution to plan success, these *conservation actions* must be described in terms of their expected contribution to stressor reduction. In addition, potential negative impacts and unintended consequences of the conservation measures should be described in the same detail as intended (positive) impacts. Furthermore, the logic chain requires an indication of the likelihood (certainty) that conservation measures will produce their anticipated effects (both positive and negative). Very few actions will have outcomes that are extremely certain; that is not a reason not to proceed with the action. The purpose of estimating an actions certainty of success is to (a) gauge its worth against other actions (high magnitude, high certainty actions being worth somewhat more than low magnitude, or low certainty actions) and (b) to enable evaluation of the certainty of the plan as a whole and the need for more or less aggressive action.

How will these actions achieve the goals and targets? In order to understand the value of each action (e.g. to prioritize implementation) and to assess the strength of the entire proposal, the planning process will convene teams of scientists and technical advisors to make detailed and, where possible, quantitative estimates of *expected outcomes* (positive and negative/unintended outcomes that are anticipated) from each conservation measure. Expected outcome magnitudes will be accompanied by estimates of the *uncertainty* surrounding the magnitude. In this way, the potential efficacy of the proposed plan can be evaluated prior to permit issuance and the plan's accomplishments can be assessed as implementation proceeds.

The magnitude of expected outcomes and uncertainties surrounding those outcomes will be based on explicit hypotheses about how we expect conservation measures to work. To the extent possible, conservation measures will be designed, implemented, and monitored in a way that allows testing the hypotheses upon which they are based. Information gathered from *compliance and performance monitoring* will be synthesized and evaluated to assess the validity of different hypotheses and the efficacy of the conservation actions and the overall plan; conservation effort and the array of conservation actions will be adjusted to make continuing progress towards the plan's stressor-reduction targets.

How will we know if it's working (and adjust if it's not)? Given the uncertainties inherent in managing such a large and complicated estuarine environment, a San Francisco Bay-Delta conservation strategy is expected to employ adaptive management – "learning to manage by managing in order to learn". Monitoring at various levels (system-wide, compliance, and measure performance) will capture physical, chemical, and biological changes in the ecosystem in order to determine the effectiveness of the overall plan and its component parts as well as ongoing changes in response to other drivers (e.g. climate change).

Data collection, analysis, synthesis, and evaluation are critical to plan success. Appropriate management structures for each of these processes should be established as part of an initial action plan. Furthermore, the means by which new information (e.g. lessons learned during early stage implementation) is incorporated into adaptive management decisions (decision pathways) should be described in detail prior to plan implementation as part of the BDCP governance process.

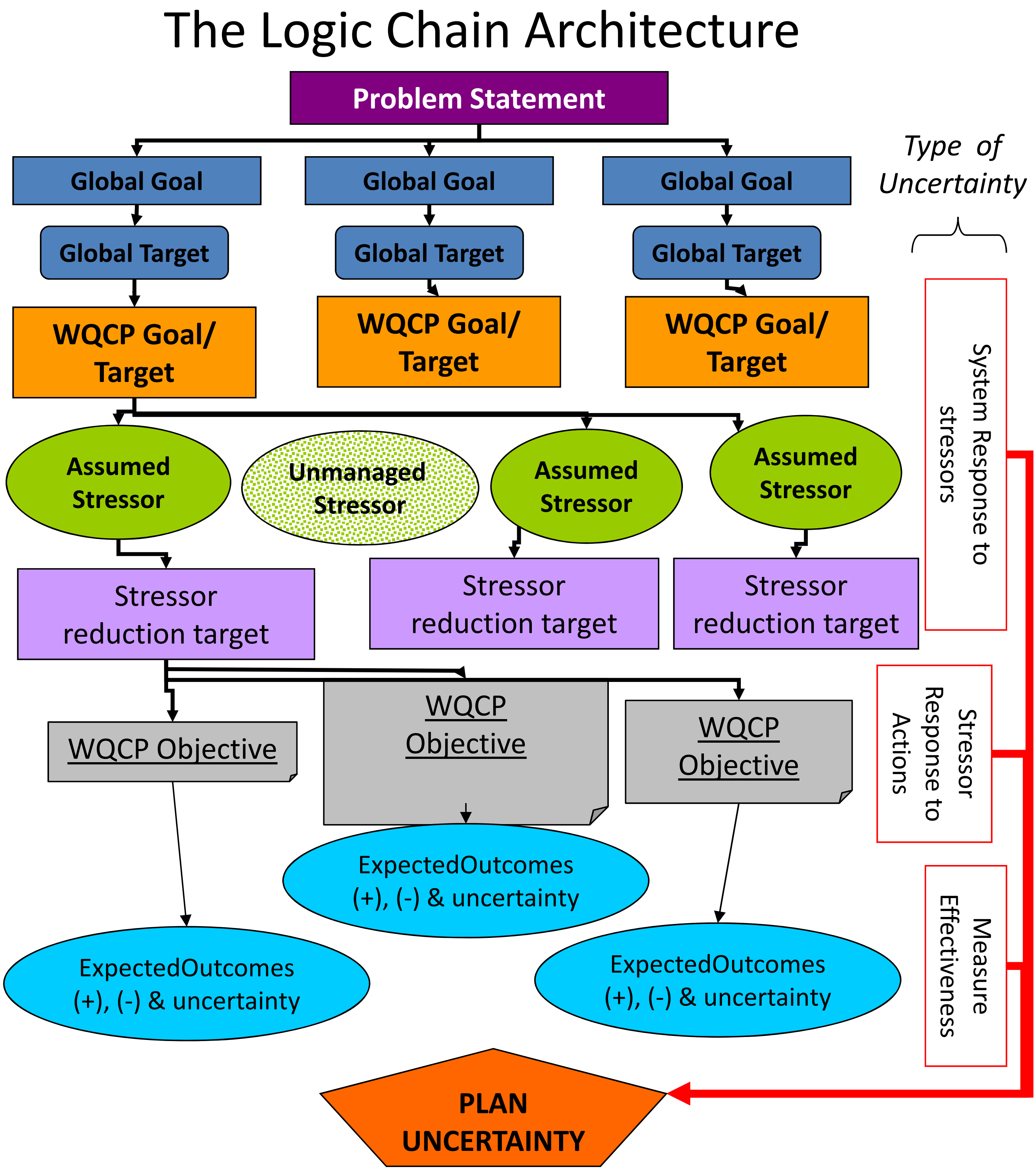
Adaptive management processes are characterized by dashed lines on the attached figure because they generally remain ill-defined; but, the details of how a plan responds to data, analysis, and emerging conditions should transparent from an early stage – their description cannot be delayed until plan implementation is under way. In particular, performance targets for conservation actions, stressor reduction (*stressor or plan targets*), and global targets must be S.M.A.R.T. Procedures for taking action when these targets are not being attained should be defined in advance. As one example, there should be pre-determined operating instructions that describe how will managers respond when, despite performance-as-expected of conservation measures, stressor reduction targets are not attained?

PRIORITIZATION PRINCIPLES

How should we choose between competing actions? Conservation actions must be prioritized to maximize the effect of limited resources, to provide rapid relief for the Bay-Delta's imperiled species, and to insure that the conservation strategy is based on the best available information and understanding of the target species and the Delta ecosystem. Factors that influence the prioritization of conservation measures include:

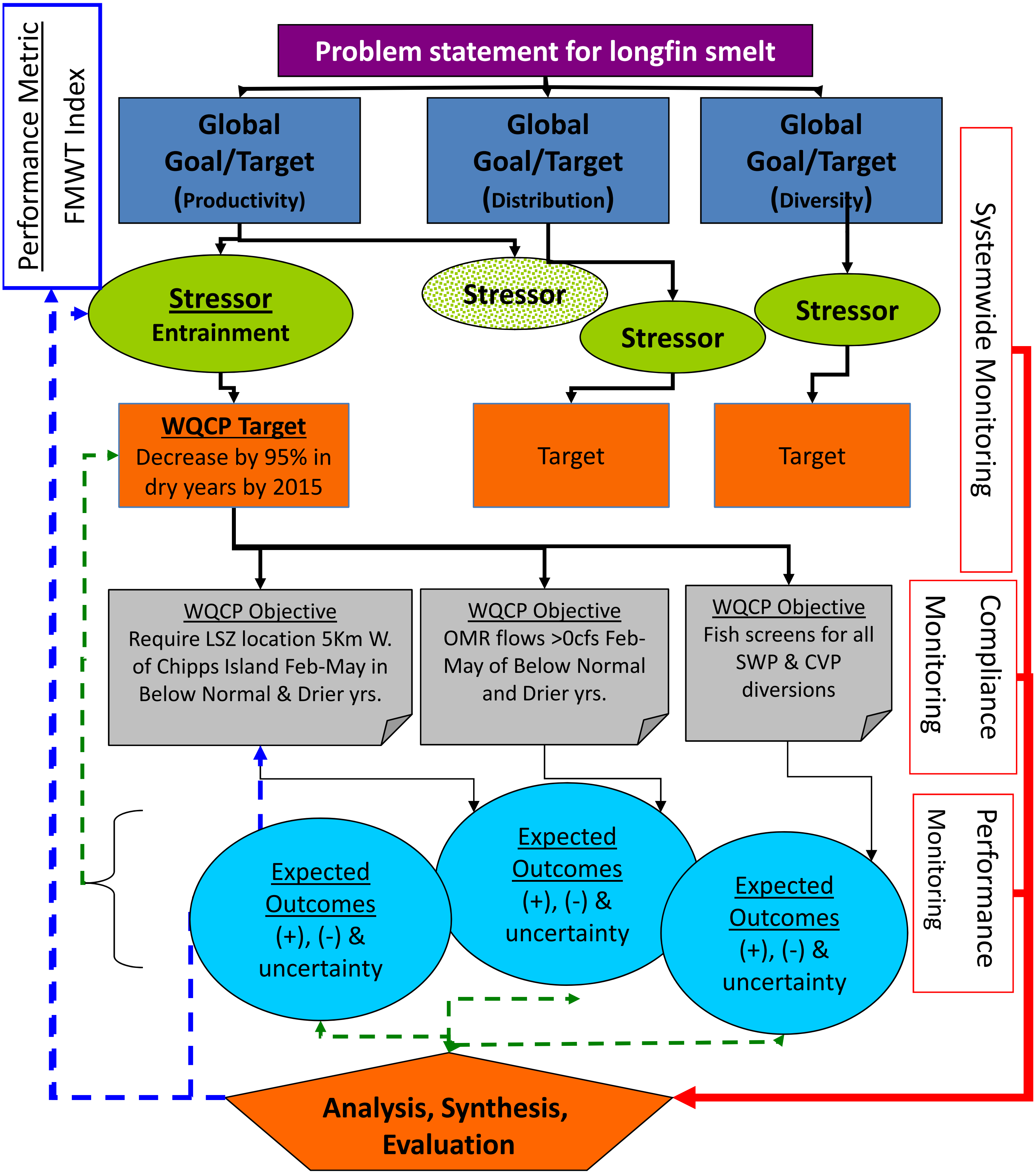
- Likelihood of positive and negative outcomes
- Magnitude and breadth (number of species affected) of positive and negative outcomes
- Time required to develop and document positive outcomes
- Ability to implement the action (e.g. financial, legal, and logistical constraints).
- Reversibility

These principles should guide description of actions in the plan and be addressed explicitly as part of the justification for each plan element (conservation action).



Relationship of Logic Chain elements to each other and to different types of uncertainty faced by the planning process. Ideally, each "problem" that the Plan hopes to address has its own logic chain. The inclusion of "Global" Goals and Objectives into the logic chain is a recognition that the WQCP, nor any other plan, is intended to address all aspects of ecosystem or population restoration in the Central Valley – WQCP Goals and Targets represent this Plan's contribution to overarching restoration outcomes.

Incorporating Flow into WQCP Planning



Schematic showing how hypothetical State Board actions are integrated by the Logic Chain into conservation planning for a native fish species of the Low Salinity Zone. Monitoring (red boxes) have been inserted where the Logic Chain identified assumptions/uncertainties. Black lines indicate the connection between Logic Chain elements in the planning process. Green dashed lines suggest some of the necessary pre-project evaluations. Blue lines represent post-implementation adaptive management decision pathways that must be specified in advance.

APPENDIX B

(Reports and Publications Unavailable Online)

Going with the flow: the distribution, biomass and grazing rate of *Potamocorbula* and *Corbicula* with varying freshwater flow (May and October 2009-2011).

J. Thompson[1], K. Gehrts[2], F. Parchaso[1], H. Fuller[2]

[1] U.S. Geological Survey, Menlo Park, CA; [2] Division of Environmental Services, Department of Water Resources , West Sacramento, CA

Summary of findings:
Spatially intensive benthic samples from >200 stations were analyzed for bivalve biomass, filtration rate, grazing rate, and water column turnover rate for three Octobers (2009-2011) to determine if the increased freshwater flow in fall 2011 would decrease the bivalve grazing in the low salinity zone in fall. Relative to the previous two dry years, the biomass of bivalves was decreased in the shallow portions of Grizzly and Honker Bays and in Western Suisun Marsh (including Montezuma and Suisun Slough) in 2011. The reduction in biomass was sufficient to limit the potential for bivalves to control phytoplankton biomass accumulation in fall. It is likely they could decrease the phytoplankton biomass by their feeding, but they did not have a sufficient grazing rate to exceed the phytoplankton growth rate during fall 2011, if the phytoplankton growth rate is assumed to be similar to that observed by Kimmerer et al. (2012) in 2006-2007.

Introduction

The POD conceptual model recognizes that food limitation may be contributing to the decline of Delta Smelt (Baxter et al. 2008). The questions of how food has changed during the POD years and the factors responsible for those changes have not been resolved. We know that the variability in salinity decreased in late summer and fall during the POD and that Delta Smelt are mostly in the low salinity zone (LSZ) during this period. There are several components of the LSZ food web that might be affected by this change in salinity. We discuss here the response of the benthic bivalves and how their change in biomass in space and time might reduce phytoplankton, copepods, bacteria, and possibly microzooplankton.

The distributions of *Potamocorbula amurensis* (*Potamocorbula* hereafter) and *Corbicula fluminea* (*Corbicula* hereafter) are dependent on the salinity distribution at the time their larvae are available for settlement, the number of adults present in the area of settlement, and the environmental stresses on the population after settlement. Field data shows that these bivalves overlap within the LSZ region which is consistent with laboratory studies on the juvenile/larval salinity tolerances for both species (Nicolini and Penry 2000, McMahon 1999). Based on data collected for the Environmental Monitoring Program Benthic Program we know that *Potamocorbula* is more persistent and is a larger presence in the LSZ than is *Corbicula*. We have also observed that the pattern is reversed upriver of the LSZ where the freshwater clam, *Corbicula*, becomes the dominant form. It is important to understand the dynamics of both clams as previous field (Thompson et al 2008, Lopez et al. 2006) and modeling (Lucas et al 2002, Lucas et al 2009) work has shown that both bivalves can limit phytoplankton biomass in the bay and delta. In addition, experimental work has shown zooplankton nauplii and ciliates can be filtered out of the water column by *Potamocorbula* in the bay (Kimmerer et al 1994, Greene et al 2011). *Corbicula* can filter fast-moving ciliates (Scherwass et al 2001) and glochidia (Scherwass et al 2005) but there have been no experiments on their ability to filter copepod nauplii. Thus, *Potamocorbula* may limit food supplies in the LSZ and both *Potamocorbula* and *Corbicula* may consume phytoplankton and zooplankton as it is transported towards the LSZ although *Corbicula* are likely to dominate in this upstream habitat in most years.

Because Delta Smelt feed on zooplankton (mostly calanoid copepods, Nobriga 2002) throughout their lives, any direct reduction in zooplankton through filtration by bivalves or indirect reduction in zooplankton due to food limitation needs to be examined. Thus, this project concentrated on the magnitude of bivalve grazing within the LSZ, within the tidal dispersion zone of the LSZ, and upstream of the LSZ during the fall periods.

Bivalve conceptual models

The distribution and dynamics of *Potamocorbula* and *Corbicula* are based on their physiological salinity limits and their life history characteristics. As explained below, *Potamocorbula* is the dominant grazer within the LSZ and *Corbicula* is the dominant grazer upstream of X2. As X2 and the LSZ moves up- and down-bay, the overlapping region of *Corbicula* and *Potamocorbula* moves with it so we will always have to consider both species when we examine foodweb dynamics in the LSZ. In addition, declines in phytoplankton biomass can not be assumed to be due to local grazing due to the tidal dispersion of pelagic particles and thus grazing must be assessed in regions within the tidal dispersion sphere of influence. The major difference in *Potamocorbula* and *Corbicula* other than their salinity tolerance is their method and season of reproduction that determines their distribution within their salinity range and their response to the fall increase in salinity intrusion.

Potamocorbula

Potamocorbula is a dioecious (sexes are separate), fecund (45,000-220,000 oocytes), broadcast spawning bivalve with external fertilization, a short lived non swimming trochophore larvae and a motile suspension feeding veliger larvae. Both larval stages have a broad salinity tolerance (2-30). The larvae settle at day 17-19 and thus can be moved by the currents for substantial distances before settling.

Potamocorbula recruitment usually occurs in the western Delta in fall and in the northern estuary in early spring through fall (Parchaso and Thompson 2002). Thus larvae have been available to respond to the recent fall periods of increasing salinity. We observed an increase in the biomass and abundance of *Potamocorbula* at Chipps Island in late 1999 and early 2000 (USGS unpublished data). We hypothesize that the increasing salinity in fall that began in 1999 allows fall larvae to settle further upstream. The high salinity may also allow *Potamocorbula* that settles in previously marginal salinity zones to persist, because individuals have grown sufficiently large in fall to become more tolerant of environmental stresses during the following winter.

The antidote to this fall incursion of bivalves is a large outflow event such as was seen in spring 2006. The mass mortality in spring 2006, observed as a drop in abundance and biomass of *Potamocorbula* to near zero at a Chipps Island station (USGS unpublished data), was short lived. The recruitment and subsequent biomass was very high in the fall of 2006 at that location because there were no adults to interfere with the larvae, and the salinity was high enough for a long enough period to allow the recruits to grow and persist. The elevated fall 2006 biomass then carried into the spring of the following year when Delta outflow was again low. We hypothesize that the effect of the recent increases in fall salinity was an increase in recruitment of *Potamocorbula* in traditionally lower salinity areas. The corollary to this hypothesis is that if these animals are given sufficient time to grow they become more resistant to osmotic and physical stresses during the winter peaks in Delta outflow which results in higher grazing rates in the following spring than we might expect with normal fall salinity distributions.

Corbicula

Corbicula is a simultaneous hermaphrodite (Kraemer and Galloway 1986) thereby making it possible for one individual to establish a population. Adults hold unfertilized eggs until there is sufficient food at which time they produce sperm and the eggs are fertilized. The larvae (pediveligers) develop in 3-5 days, are brooded in the gills of the adult before release, cannot swim but are found in the plankton for their first 48 hours, and are limited to salinities ≤2. They depend on their small size (200 µm) and mass (0.1 mg dry weight) to allow currents to re-suspend and transport them after settling (Aldridge and McMahon 1978). As a freshwater bivalve, this strategy is good for moving larvae downstream with the currents but may be less effective at widening their distribution throughout the system. It is not surprising that *Corbicula*, as a freshwater bivalve, would have an opposite reproductive seasonality to that of *Potamocorbula*. Eng (1979) and Heinsohn (1958) found a large spawning peak in the spring followed by a smaller fall peak in the Delta. If this reproductive seasonality persists today then *Corbicula* is most likely to expand down river and down-bay in the spring but its expansion into new down bay areas is likely to be limited in fall by the increasing salinity.

Methods

The DWR EMP program sampled 175 benthic stations (single sample at each location with a $0.05m^2$ bottom grab) throughout the Delta and northern bay in one week in May and October from 2007-2011 (Figure 1). The sampling design (generalized random tessellated stratified design) allows for a random selection of stations in various strata which DWR defined as habitat type (lake, large river, river, slough, bay, large bay). The station locations changed each year for all but 50 stations (the annual panal) which were sampled throughout the program.   Twenty two additional stations were added beginning in October 2009 to establish channel-shoal pairings at some locations to determine if shallow locations had significantly different bivalve populations than their adjacent channel stations. In order to focus on the low salinity zone and it's nearby habitat, we further parsed the strata into the following regions (Figure 2): Grizzly/Honker Bays (≤4m), Shallow Suisun Bay (not in channel and <7m), Channel Suisun Bay, Lake (Big Break and Sherman Lake with adjoining sloughs), Western Suisun Marsh (Suisun Slough, Montezuma Slough west of Nurse Slough), Eastern Suisun Marsh (Montezuma Slough east of Nurse Slough), and Confluence (Sacramento River up to Browns Island, San Joaquin River to False River out of Franks Tract).

Samples were sieved through 0.5mm screens, preserved in 10% formalin in the field, and changed to 70% alcohol at 1-2 weeks.   Samples of live bivalves were collected at annual panel stations to estimate weight as a function of length; clams were measured, dried, weighed, ashed, and reweighed to determine ash-free dry weight (AFDW). Samples were sorted by a contractor (Hydrozoology) and returned to DWR. Bivalves from all samples were measured using an image analyzer or hand calipers and length of each animal in each sample was converted to AFDW using the live animal length to weight conversions calculated at the annual panel stations.   Biomass at a station was estimated by summing these values.

Consumption rate was estimated two ways. The first rate, the filtration rate, is the highest consumption rate that we would expect. Filtration rate is the product of bivalve biomass and species specific pumping rates (PR's) which were adjusted for temperature. *Potamocorbula* pumping rates have been estimated at two temperatures to be ≈400 L $(gAFDW)^{-1}d^{-1}$ at temperatures ≥15°C and 270 L $(gAFDW)^{-1}d^{-1}$ at temperatures <15°C (Cole et al. 1992). *Corbicula* pumping rate was determined at four temperatures by Foe and Knight 1986) and data were fitted to an exponential model which was then used to determine temperature specific pumping rates. Filtration rates assume no depletion boundary layer (the local reduction in food concentration when vertical mixing rate is too low to compensate for the loss due to consumption at the bed) and that animals filter all of the time. The second rate, the grazing rate, incorporates a concentration boundary layer and is smaller than the filtration rate when there are large populations. Filtration rates were converted to grazing rates by reducing the pumping rates to adjust for the presence of a concentration boundary layer. This adjustment was based on O'Riordan's (1995, Figure 7b) refiltration relationship, $n_{max} = F_c/(s/d_o)$, where n_{max} is the maximum refiltration proportion (ie the proportion of water previously filtered), F_c is a species specific refiltration factor determined in the laboratory for *Potamocorbula* (2.5) and *Venerupis* (3.0, similar to *Corbicula* in size and habit), s is the distance between siphon pairs, and d_0 is the diameter of the excurrent siphon. The diameter of the excurrent siphon was changed throughout each year to reflect the change in average size of animals as the year progressed, and the distance between siphon pairs was based on density of animals observed in our benthic sampling assuming equidistant spacing within the 0.05 m^2 grab. The use of maximum refiltration proportion maximizes the effect of the concentration boundary layer resulting in a conservative grazing rate estimate. The combined use of filtration rate and grazing rate should give a reasonable range of possible consumption rates. We assumed all bivalves grazed continuously.

Data and Approach

Biomass, filtration rate, grazing rate and grazing rate water column turnover rate have been calculated for each region and are summarized in Tables 1-4. Water column turnover rate is a method of normalizing grazing and filtration rates by depth of the water column. The resulting number is more intuitive of the bivalves effect on pelagic particles (biologic and refractory) than grazing rate because it reflects the number of times in a day that a population could filter the overlying water column if the water was stationary. With this value, the importance of water depth becomes apparent; if it is assumed that the same population lived on the bottom of a 1m vs a 10m water column, the bivalves would filter the 1 m water column ten times the rate at which they filter the deeper water column.

The data are not normally distributed and regions have unequal number of samples so non-parametric measures of statistical significance (Kruskal-Wallis) have been used to compare regions and time periods. As with most benthic data, the median value is shown in plots because it is the best way to eliminate the influence of one very high or very low value in a region.

Findings

General Distribution Patterns

When the entire sampling domain with the data from all three years is combined, there are several observations that can be made about persistent patterns that don't seem to be affected by water year type (Figures 3 and 4a). First *Potamocorbula* has a larger presence, and thus larger filtration rate in fall than spring, and the opposite is true of *Corbicula*. Second, *Potamocorbula* have very low filtration rates in the spring in the shallows of Grizzly and Honker Bays for all three years. Third, filtration rates for both bivalves in the lower reaches of Sacramento and San Joaquin Rivers (just upstream of confluence) are consistently lower than the surrounding areas and there appears to be less seasonality in this region than in the rest of the system.

These persistent distribution patterns become even more apparent when we narrow the focus to the LSZ (Figure 4b). We can also see that the area where the two bivalve species overlaps can be described as within and just upstream of the confluence and on the eastern end of Montezuma Slough (east of Nurse Slough). When the distributions are plotted separately for each year (Figure 5a) and compared for May and October we see that the zone of overlap in May is within the range of X2 over the previous 6 months with a few exceptions in 2009-2010. In 2011 *Potamocorbula* were consistently upstream of the maximum X2 in the previous 6 months. This pattern persists into fall 2011 with *Potamocorbula* being observed upstream of the X2 maximum in all years (Figure 5b). Unlike May 2011, the October 2011 distribution showed some *Corbicula* within the X2 range.

Differences between years in regions (Fall 2009-2011)

When the filtration rates, grazing rates, and water column turnover rates are compared between years within the regions, only the values in the Grizzly/Honker Bay shallows and the Western Suisun Marsh showed a statistically significant difference between years (Kruskal-Wallis, $p<0.05$). Grizzly/Honker bay biomass, filtration, grazing, and turnover rates were all similar in 2009 and 2010 but were significantly less in 2011 than in 2010 (Figure 6a, 6b). The western Suisun Slough rates were similar in 2009 and 2010 but the 2011 rates were different from both the 2009 and 2010 rates (Figure 7a, 7b). The location of these decreased grazing rates is important as we might expect pelagic primary producers to do best in the shallows of Grizzly and Honker Bays and we might expect that marsh production would have a better chance of reaching other consumers when the bivalve grazers were greatly reduced as seen in 2011.

Differences between areas in years (Fall 2009-2011)

Because we are most interested in the effect that the bivalve grazers have on the system, we will show grazing turnover rates in this section (data for other parameters are in tables 1-4). The pattern and values for grazing turnover rate were similar in 2009 and 2010 with the shallow regions, Grizzly/Honker Bay, Suisun Bay Shallow, and West Suisun Marsh, having much higher values than the remaining areas that are mostly upstream or deeper than these stations (Figures 8 and 9). The bimodal distribution of values highlights the significant differences in these groups. The Confluence region had significantly lower turnover rates than those observed in Grizzly/Honker Bay and in the West Suisun Marsh in both 2009 and 2010. The West Suisun Marsh also had significantly higher rates than were observed in Suisun Channel in 2009 and 2010. In addition the Confluence rates were significantly lower than the Grizzly/Honker Bay rates and the West Suisun Marsh rates were significantly higher than the rates in the Lakes region in 2010 Figure 9).

Grazing turnover rates in 2011were lower and the bimodal distribution of values was less pronounced. There were no significant differences between the regions with the median values fell between 0.1 and 0.5 d^{-1} (Figure 10).

Time Series in Grizzly/Honker Bay Shallows

Figures 11 and 12 show the full time series (May 2009-October 2011) for all parameters for the Grizzly/Honker Bay region. Because the shallow areas are the presumed source of locally grown phytoplankton, grazing in this region is the most likely to have an effect on net phytoplankton growth.

Biomass, filtration rate, grazing rate, and grazing rate turnover rate all show the same strong seasonal pattern which is expected since all values are derived from biomass. In this region, where the bivalves are almost all *Potamocorbula*, filtration rate is derived from biomass with one conversion factor. It should be noted that in other regions, where *Corbicula* and *Potamocorbula* occur together the conversions are less linearly related to biomass.

Spring filtration rates (medians of 0.2-0.3 m d^{-1}) are about an order of magnitude less than fall filtration rates (2, 4, and 1 m d^{-1}). Grazing rates showed a similar pattern with spring rates (0.2, 0.3, and 0.1 m d^{-1}) an order of magnitude less than fall rates (2, 3, 1 m d^{-1}). Grazing water column turnover rate was very low with populations needing 10-20 days to totally turnover the water column in spring (0.1, 0.1, 0.05 d^{-1}). Fall grazing turnover rates were much higher with populations turning over the water column every 1-2 days (0.6, 1, 0.4 d^{-1}). If we assume a spring phytoplankton growth rate of 0.5-0.6 d^{-1} (Kimmerer et al in press) we can state that the bivalves were unlikely to be a controlling factor on spring phytoplankton biomass accumulation in any year. Fall phytoplankton growth rates have not been recently measured but summer rates (0.7-1.0 d^{-1}) would be about equivalent to the loss rates by bivalves in 2009-2010 but not in 2011 when bivalve turnover rates (0.4 d^{-1}) were unlikely to limit a bloom from developing in the shallow water.

Significance of Findings

We saw a decline in bivalve biomass and therefore grazing rate during and following the increased freshwater flow in spring and fall 2011. In examining the shallow Grizzly and Honker Bay data we found that bivalve grazing was unlikely to have an impact on net phytoplankton growth in spring during any of the years examined (2009-2011). We also found that the fall grazing rates were sufficient to potentially limit phytoplankton biomass accumulation in 2009-2010 but not in 2011.

The reduction in bivalve biomass and therefore grazing in 2011 could be due to recruitment losses in spring or fall and our ongoing work with the monitoring station samples should help delineate the cause. We were surprised by the persistence of *Potamocorbula* in the confluence area in 2011 despite the down bay position of X2. Our present working hypothesis is that it is the salinity gradient and therefore change in salinity over short periods of time that is important in determining the distribution of both species rather than the absolute salinity at a location. If true, this hypothesis would support the presence of *Potamocorbula* upstream of X2 in spring 2011.

Next Steps

Fall Study: We will measure bivalves and calculate biomass, filtration rate and grazing rate of the bivalves in the May and October 2012 GRTS samples when the samples have been sorted. We are presently measuring bivalves in the monitoring stations to better determine the seasonality of recruitment of both species and to determine if there are interannual and spatial differences in recruitment. Recruitment patterns are a critical component in our understanding of why bivalves have limited success in some areas and during some periods. We are submitting abstracts for two posters for the Bay-Delta conference that will highlight what we learn about recruitment for each species.

HSG Study: We are finishing the analyses of the May 2011 data and when that is complete we will repeat the analysis done here on the samples from throughout the study domain. The values reported will include biomass, grazing and filtration rates, and recruit abundance and the analysis will include the effect of depth on these rates for each species.

The combination of analyses in both studies will give us an opportunity to examine if and when populations that settle in the fall are still present in spring and if these "carry-over" populations are adding a new dimension to the bivalve community seasonal patterns.

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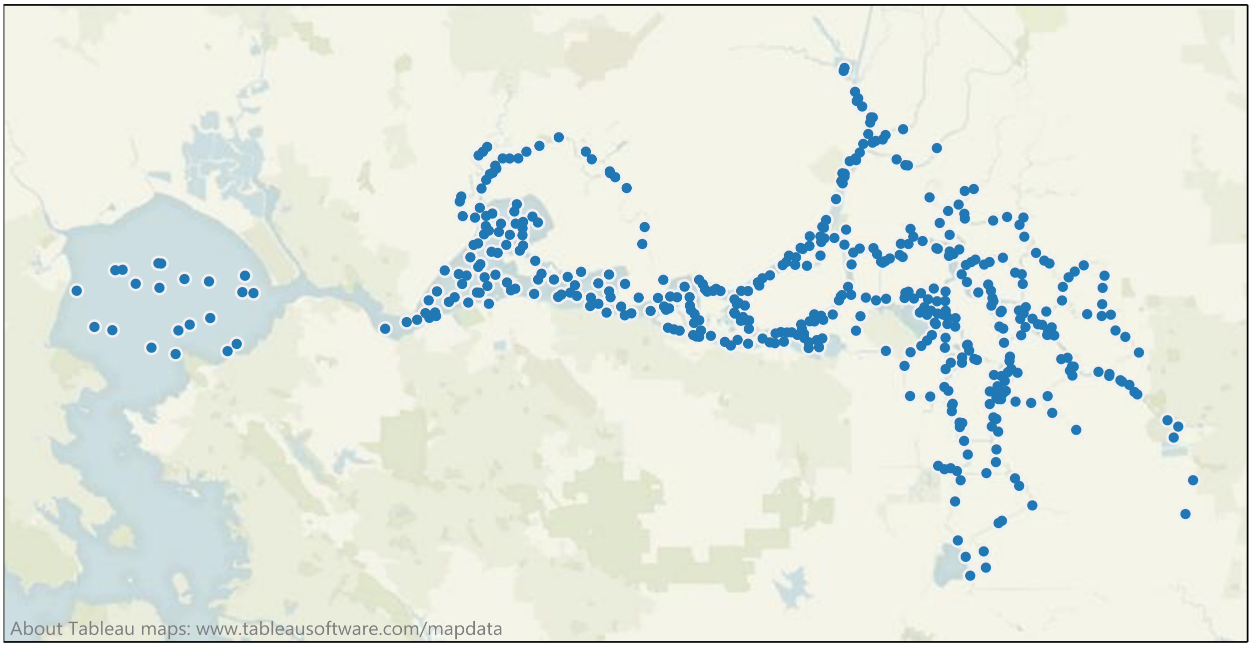


Map based on average of long and lat. The data is filtered on year, Panel and Strata. The year filter has multiple members selected. The Panel filte has multiple members selected. The Strata filter has multiple members selected

Figure 1. Composite (2007-2011) of all stations sampled by DWR in the GRTS benthic study.

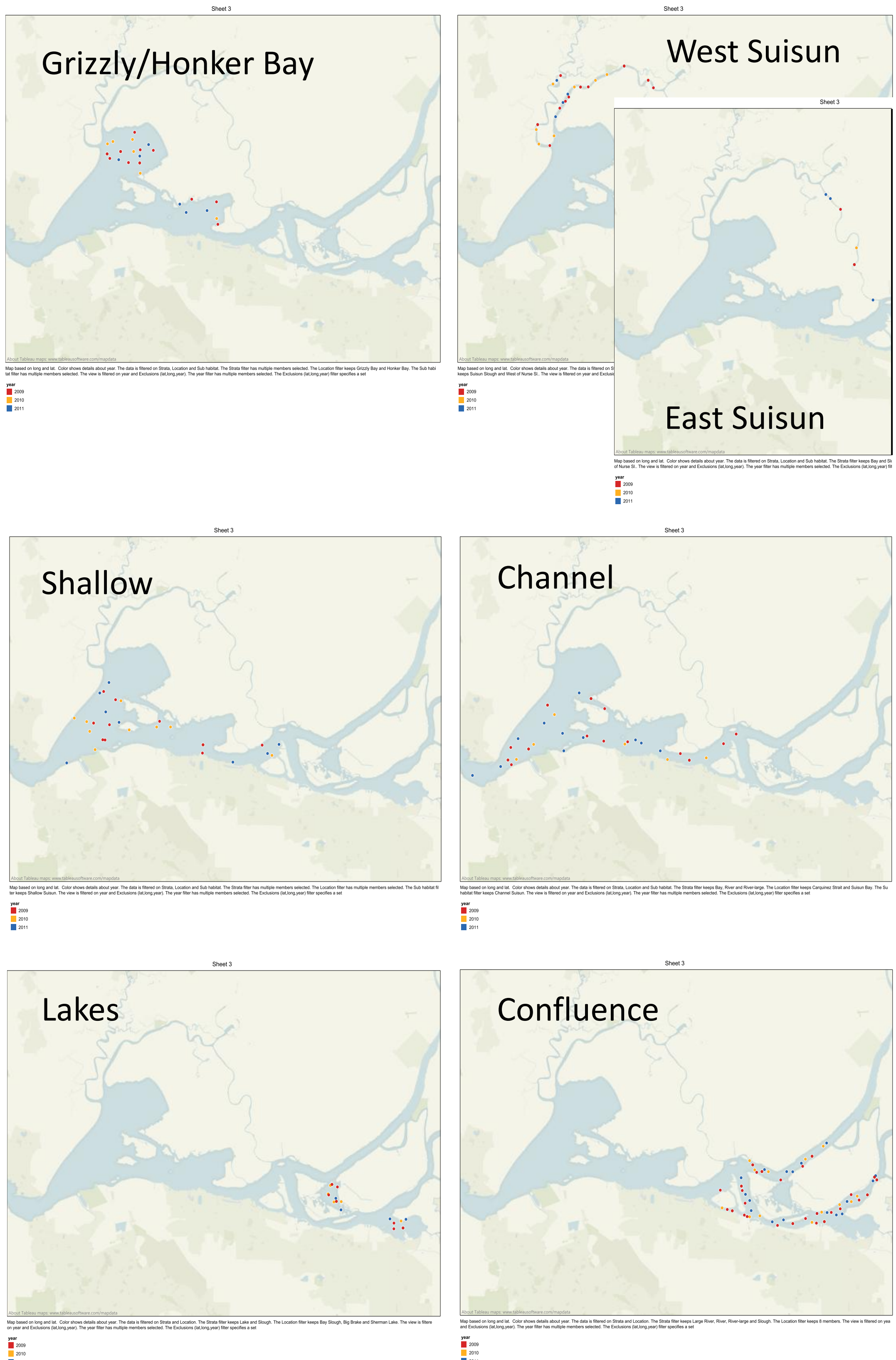


Figure 2. Regions established for this study.

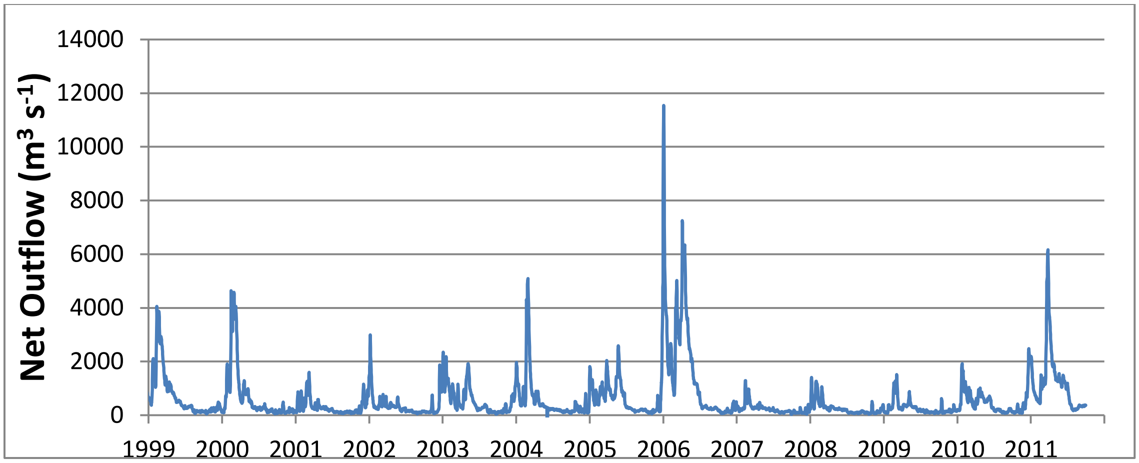


Figure 3. Net Delta Outflow for pelagic organism decline (1999- present). Note the years of the benthic study encompass a dry-below normal year (2009), a dry-above normal year (2010), and a wet year(2011).

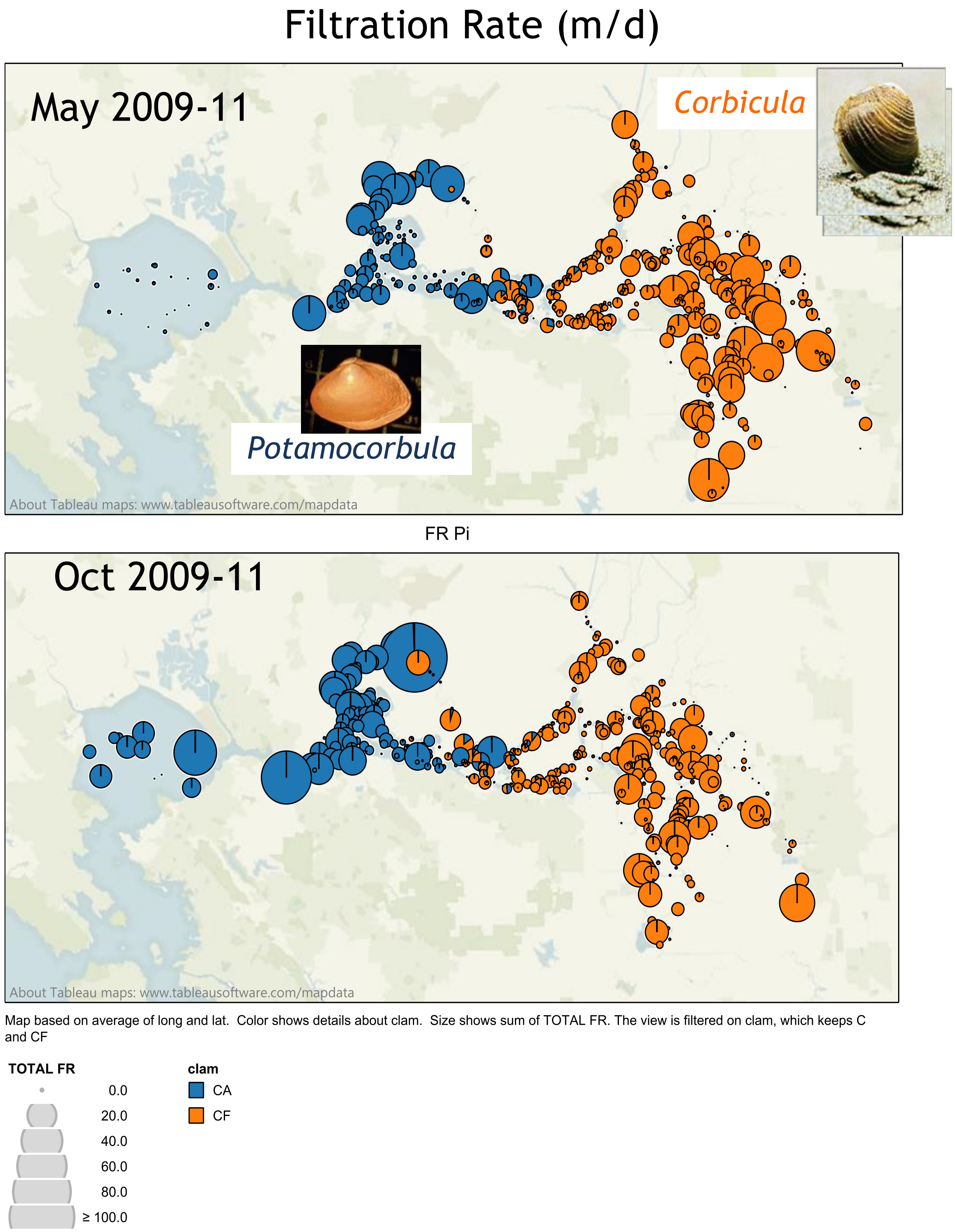


Figure 4a. Filtration rate for *Potamocorbula* (blue) and *Corbicula* for May and October of 2009-2011. The combination of data sets allows us to see persistent patterns that were not influenced by freshwater inflow during these three years.

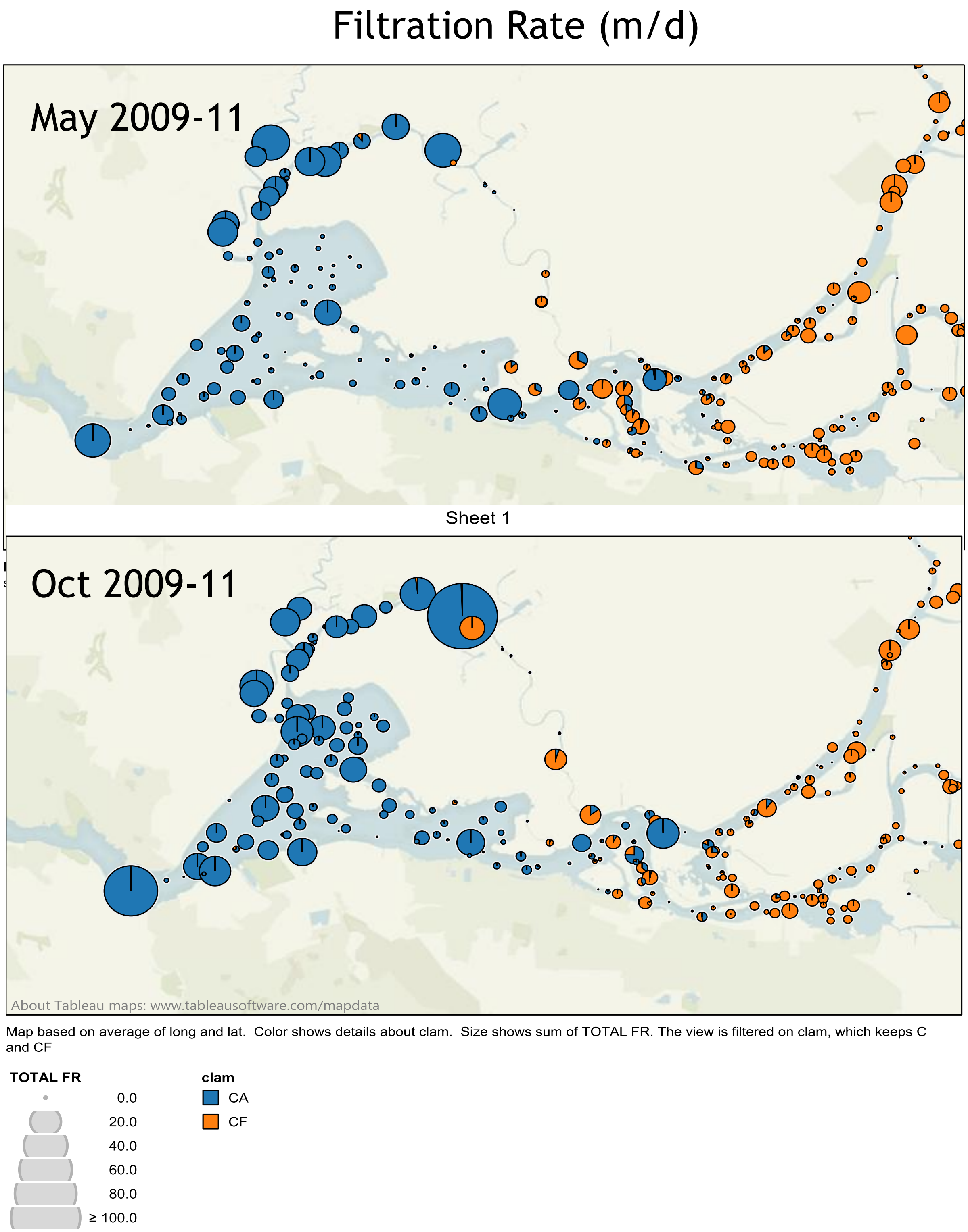


Figure 4b A close-up of the LSZ region in Figure 4a.

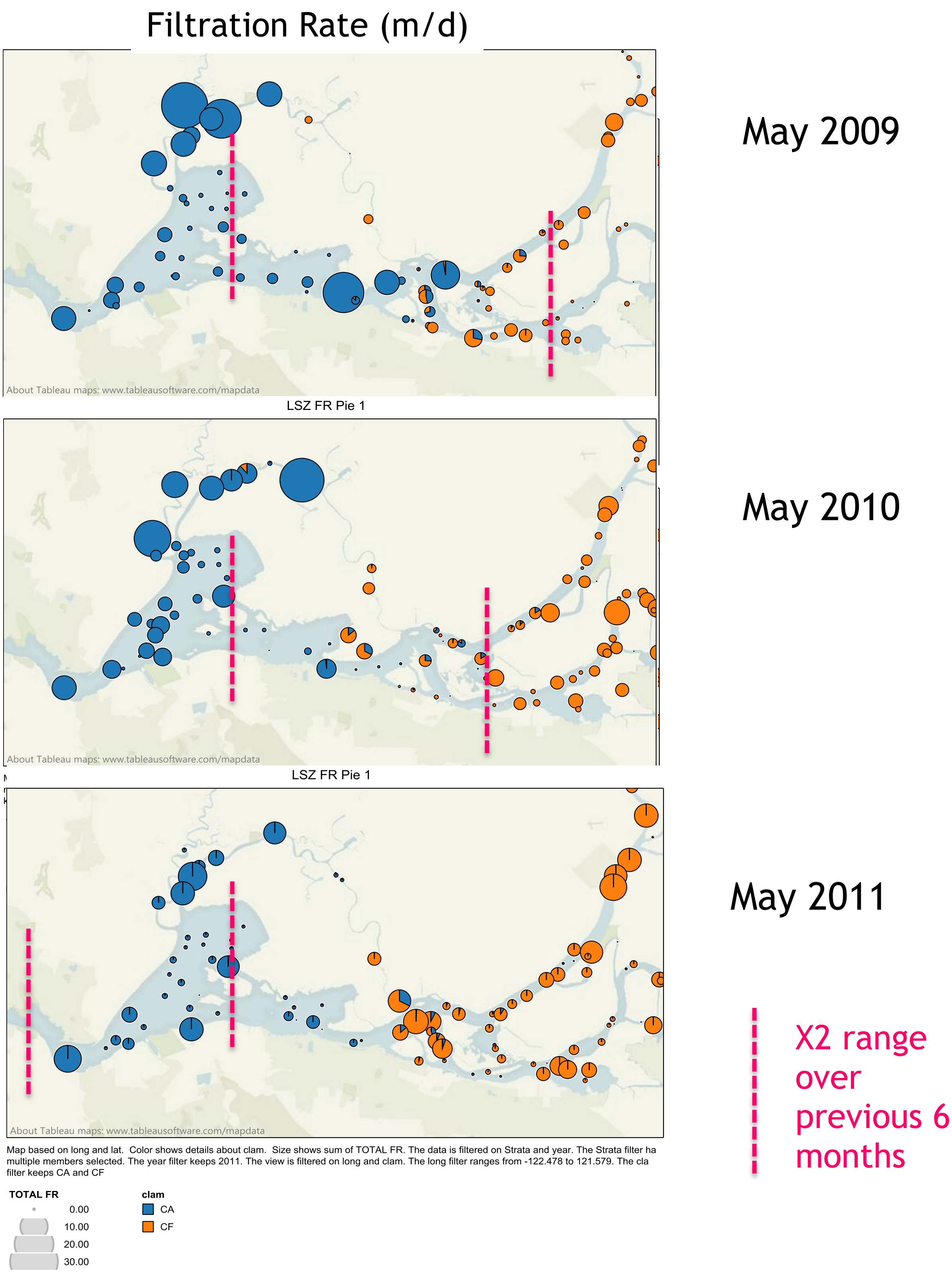


Figure 5a. Filtration rate for both bivalves in May 2009, 2010, and 2011. Range of X2 over previous 6 months shown on map as range where bivalves were expected to overlap.

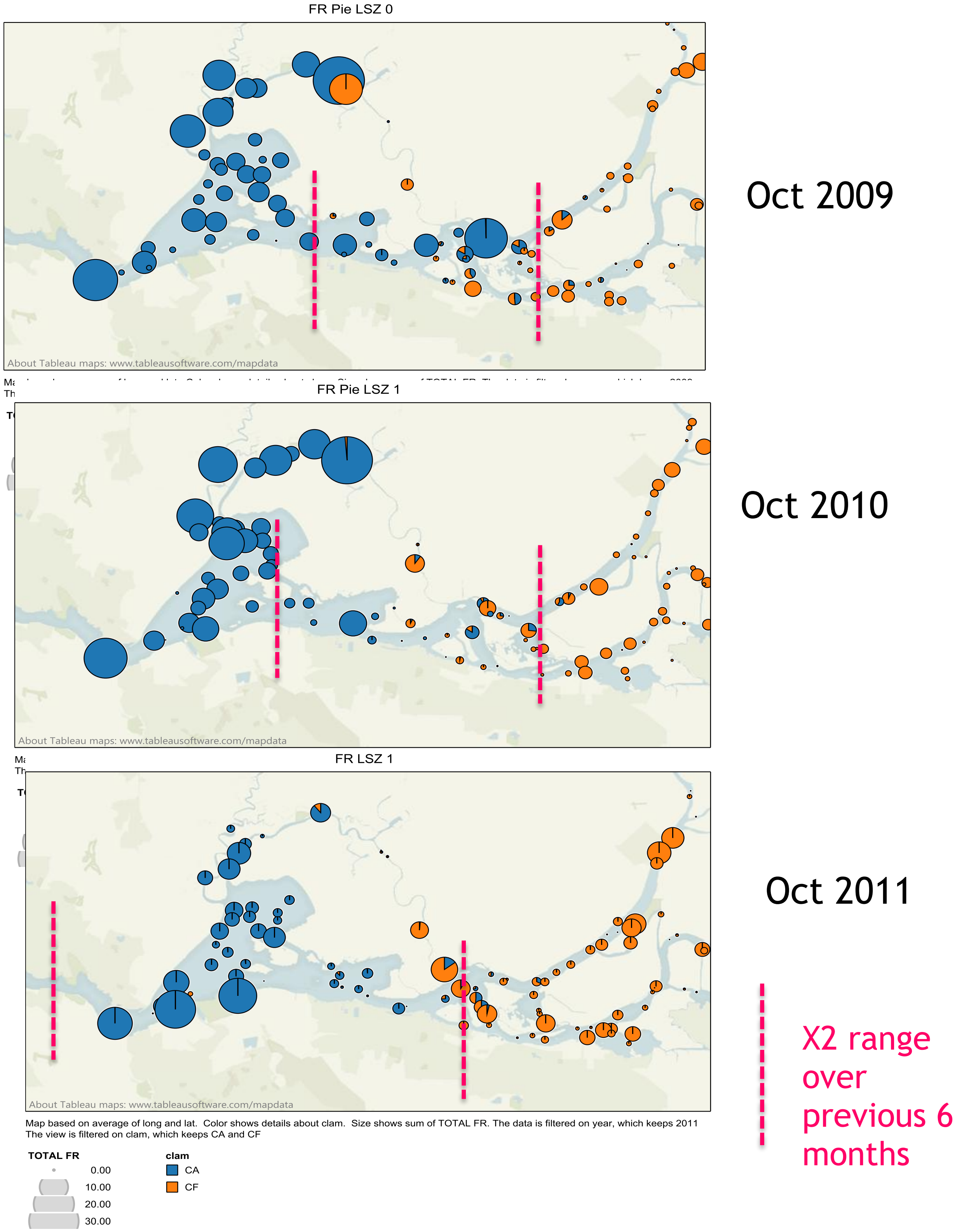


Figure 5b. Filtrattion rate for both bivalves in October 2009,2010, and 2011. Range of X2 over previous 6 months shown on map as range where bivalves were expected to overlap.

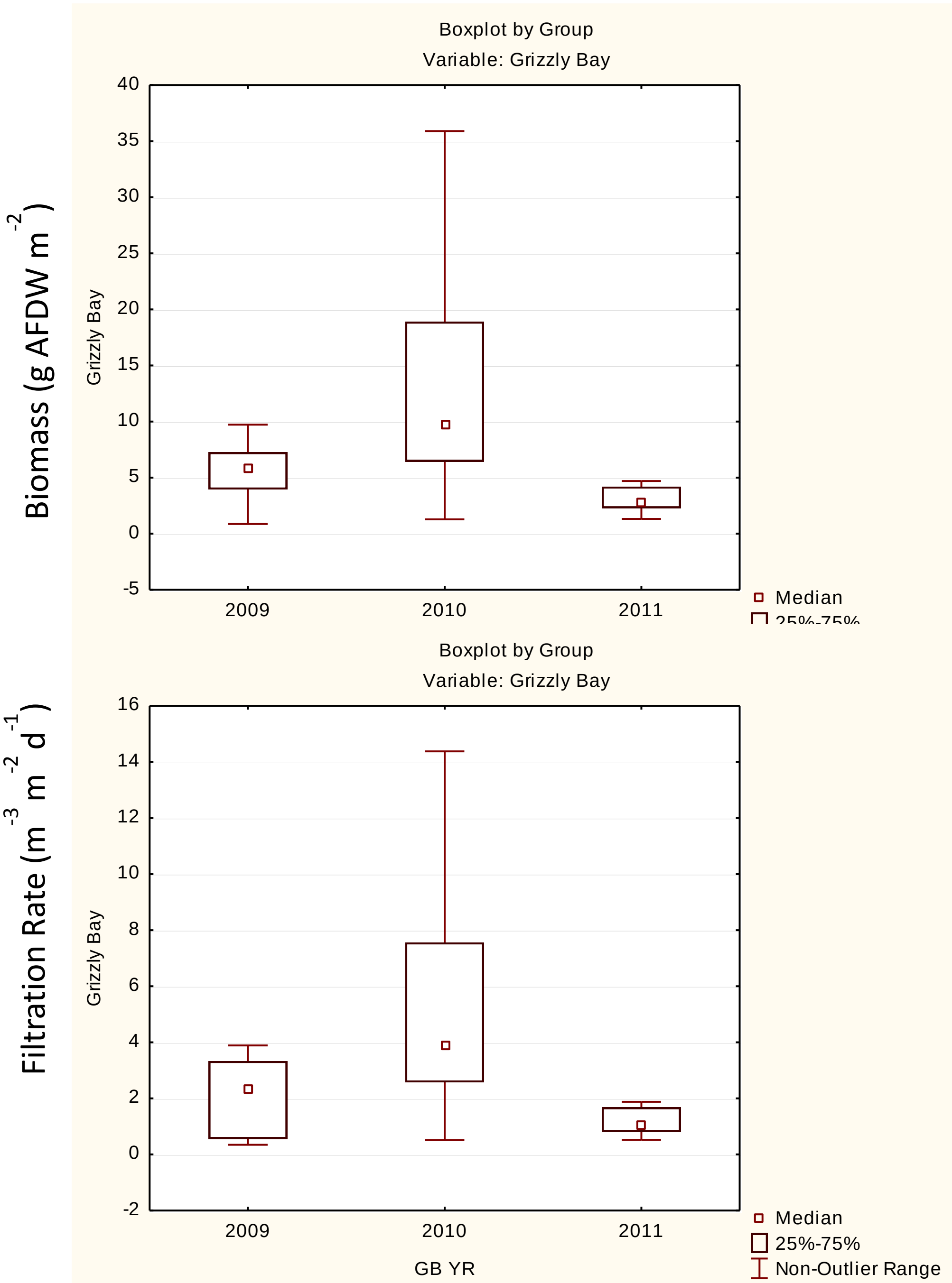


Figure 6a. Biomass and filtration rate during the October sampling periods in Grizzly/Honker Bay shallows. All values (biomass, filtration rate, grazing rate, and turnover rate) for Grizzly/Honker Bay clams were not significantly different between 2009 and 2010 but were significantly different for 2010 and 2011.

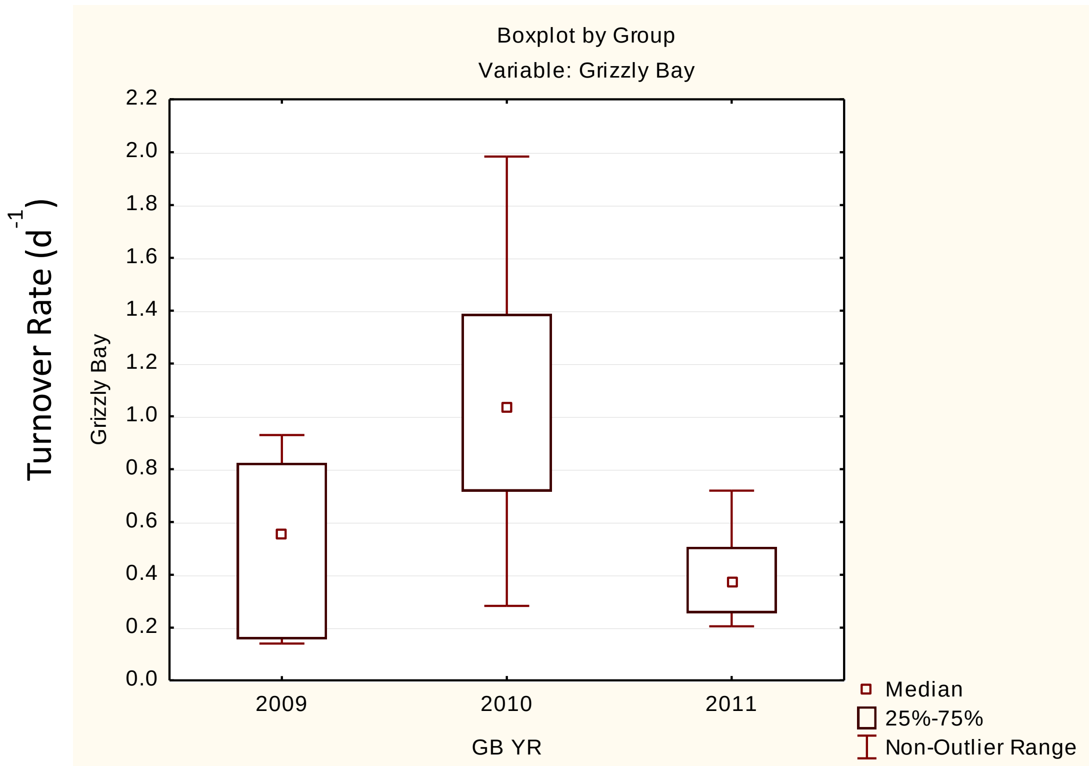


Figure 6b. Grazing rate normalized by water depth in Grizzly/Honker Bay shallows estimates water column turnover rate, the more conservative of the two calculated turnover rates.

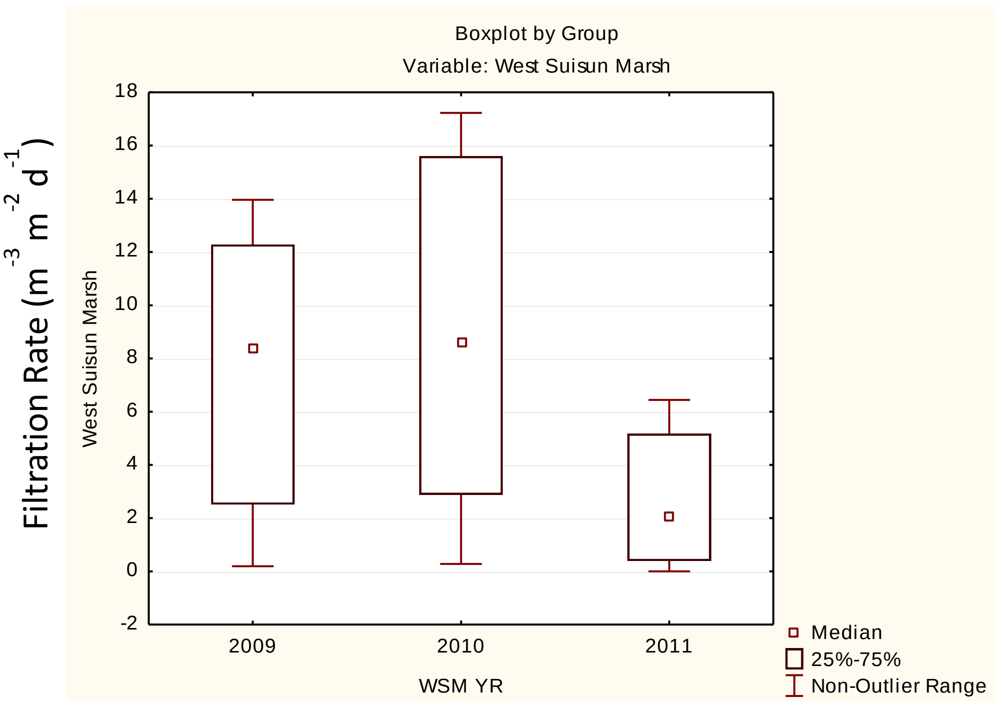
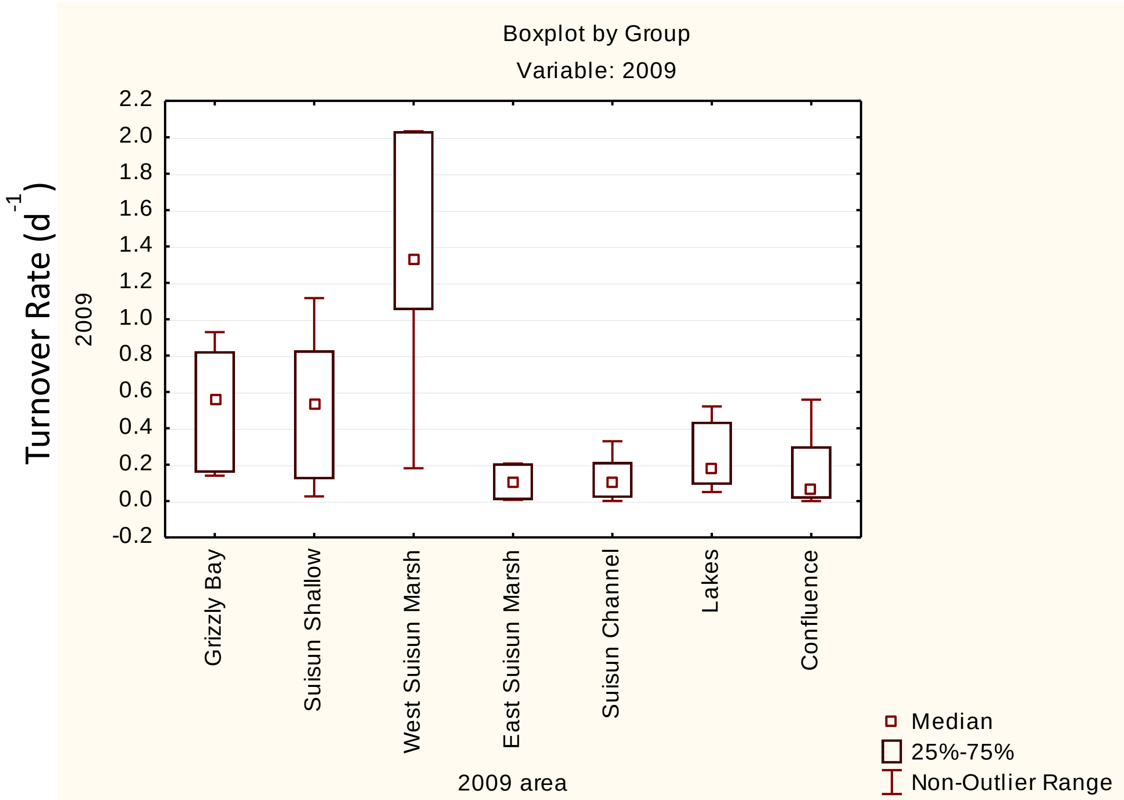


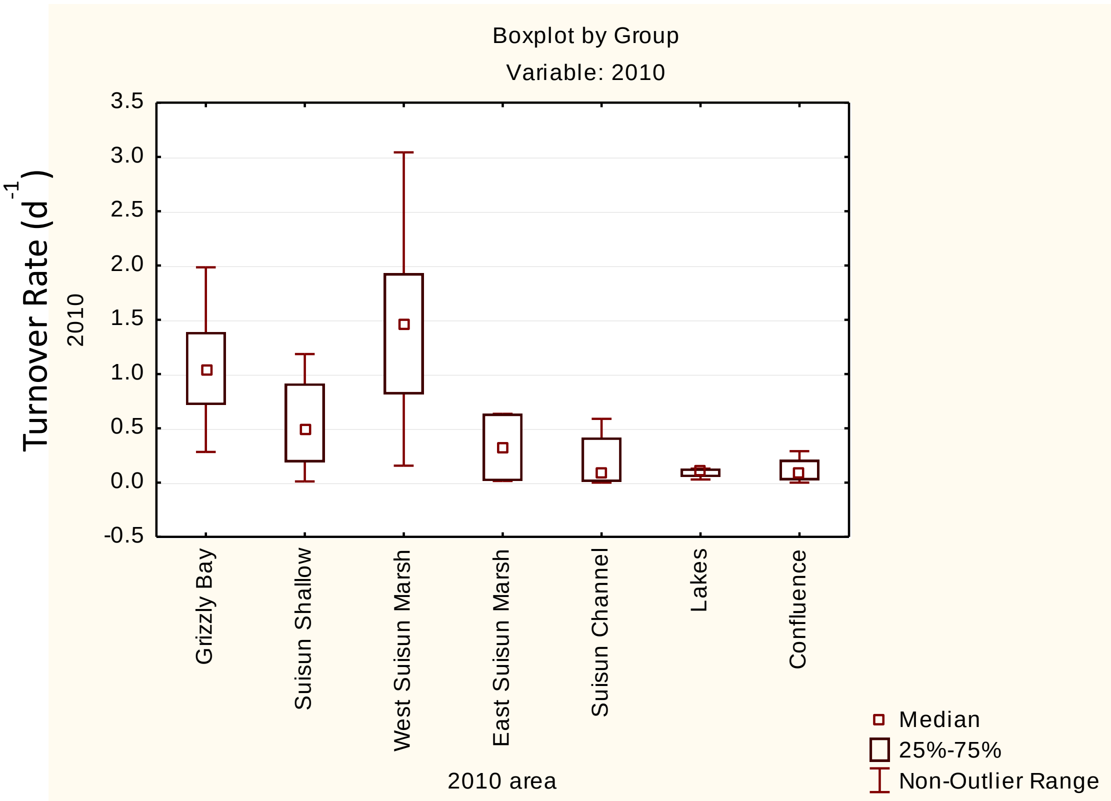
Figure 7a. Biomass and filtration rate during the October sampling periods in West Suisun Marsh region. All values (biomass, filtration rate, grazing rate, and turnover rate) for West Suisun Marsh clams were not significantly different between 2009 and 2010 but were significantly different for 2010 and 2011.

Figure 7b. Grazing rate normalized by water depth in West Suisun Marsh estimates water column turnover rate, the more conservative of the two calculated turnover rates.



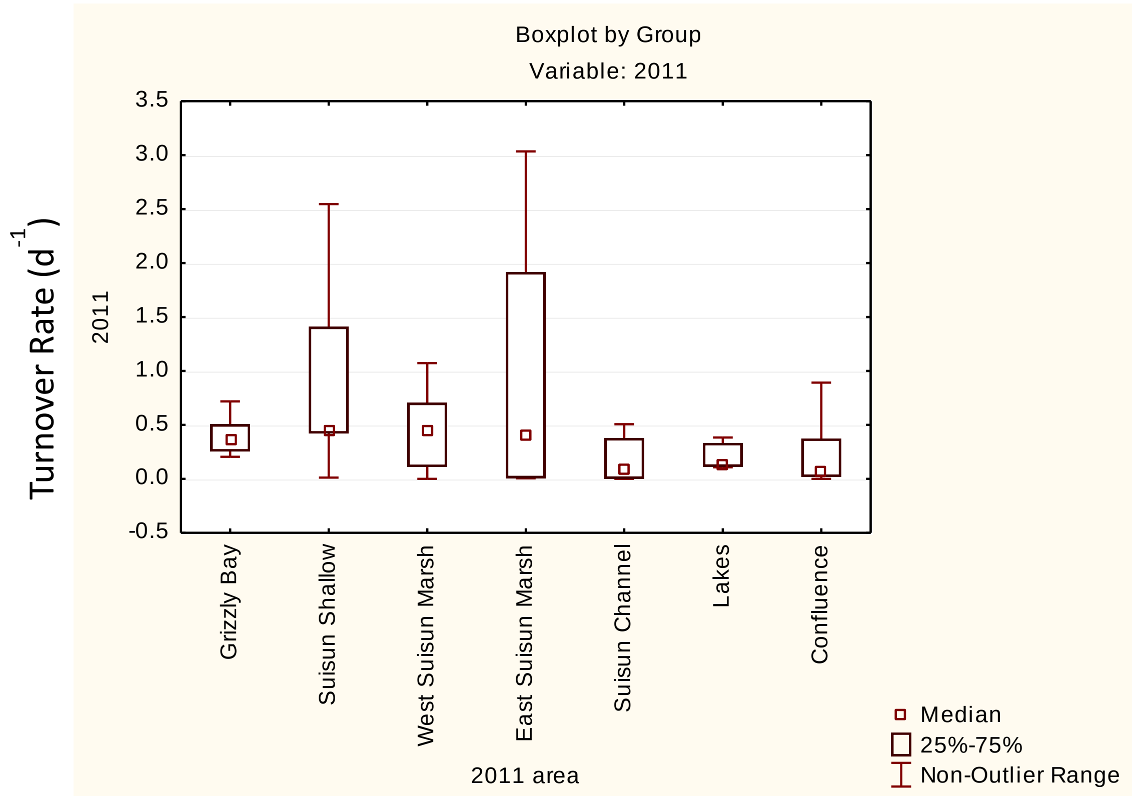
2009	Grizzly/ Honker Bay	Suisun Shallow	West Suisun Marsh	East Suisun Marsh	Suisun Channel	Lakes	Confluence
Grizzly/Honker Bay							0.049
Suisun Shallow							
West Suisun Marsh					0.00006		0.000004
East Suisun Marsh							
Suisun Channel			0.00006				
Lakes							
Confluence	0.0492		0.000004				

Figure 8. Grazing turnover rates for all regions in 2009. Table shows regions that had similar values (line) and those significantly different at $p \leq 0.05$ (Kruskal-Wallis test).



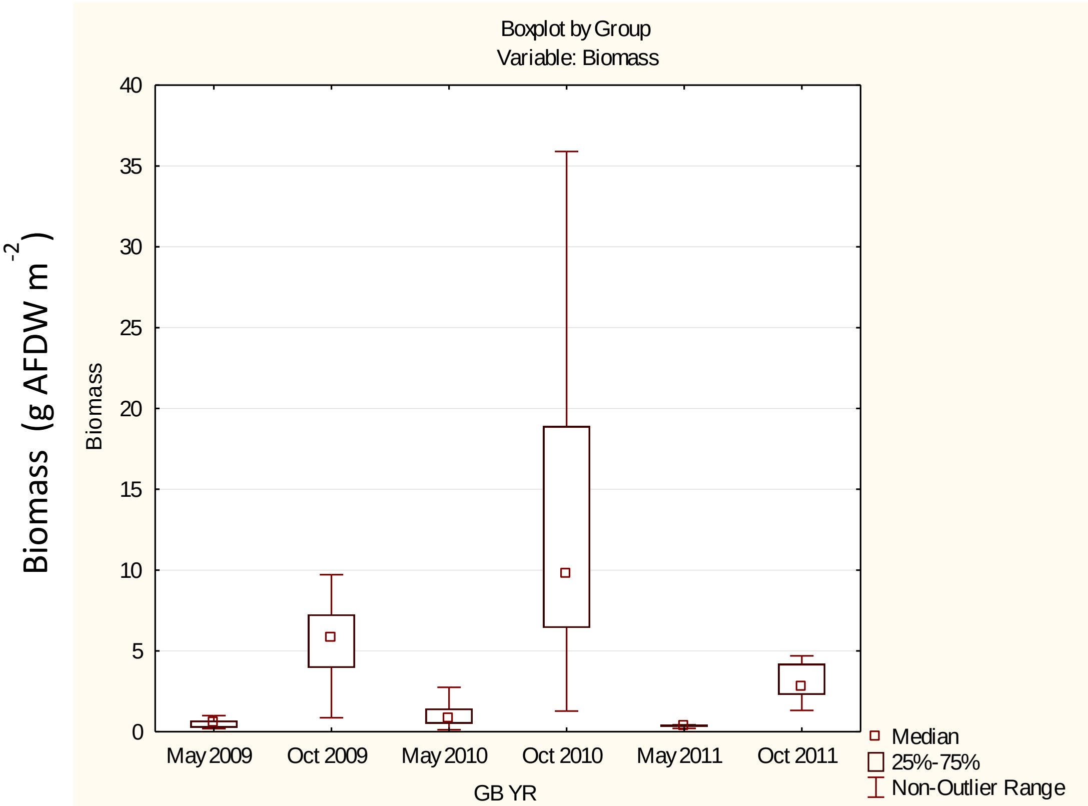
2010	Grizzly/ Honker Bay	Suisun Shallow	West Suisun Marsh	East Suisun Marsh	Suisun Channel	Lakes	Confluence
Grizzly/Honker Bay					0.006		0.0004
Suisun							
West Suisun Marsh					0.0006	0.025	0.00002
East Suisun Marsh							
Suisun Channel	0.006		0.0006				
Lakes			0.025				
Confluence	0.0004		0.00002				

Figure 9. Grazing turnover rates for all regions in 2010. Table shows regions that had similar values (line) and those significantly different at p≤0.05 (Kruskal-Wallis test).



2011	Grizzly/ Honker Bay	Suisun Shallow	West Suisun Marsh	East Suisun Marsh	Suisun Channel	Lakes	Confluence
Grizzly/Honker Bay							
Suisun Shallow							
West Suisun Marsh							
East Suisun Marsh							
Suisun Channel							
Lakes							
Confluence							

Figure 10. Grazing turnover rates for all regions in 2011. Table shows all regions had statistically similar values (line) at p≤0.05 (Kruskal-Wallis test).



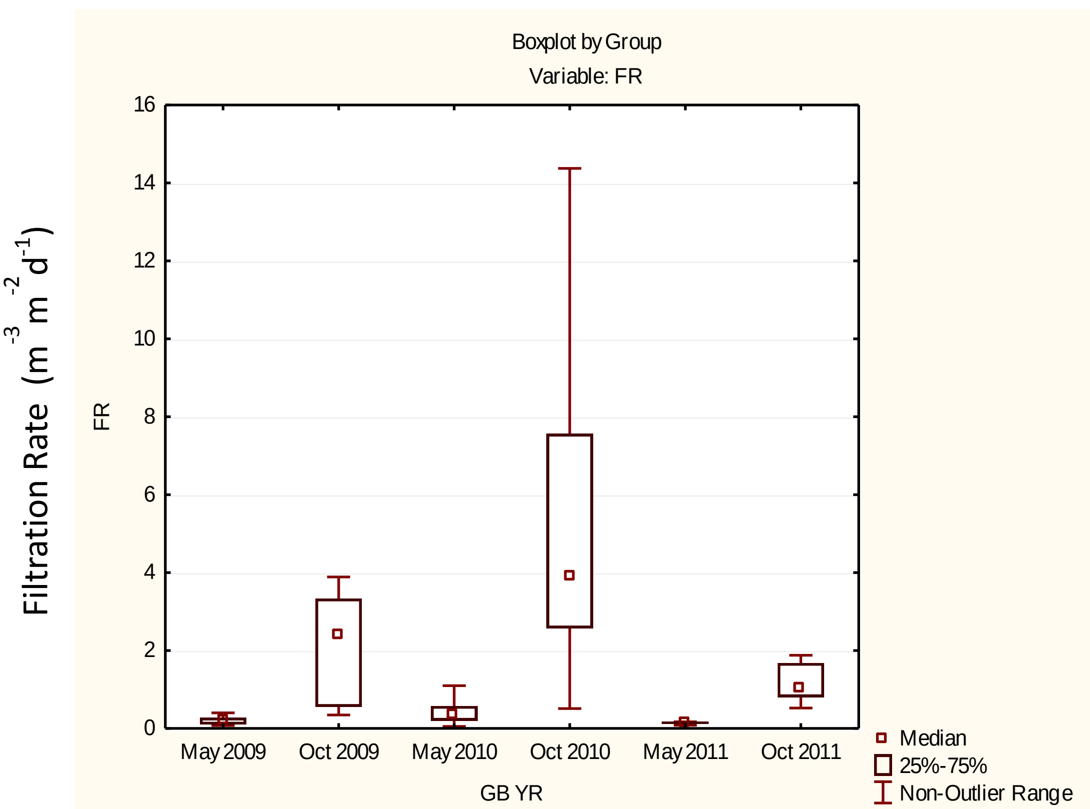
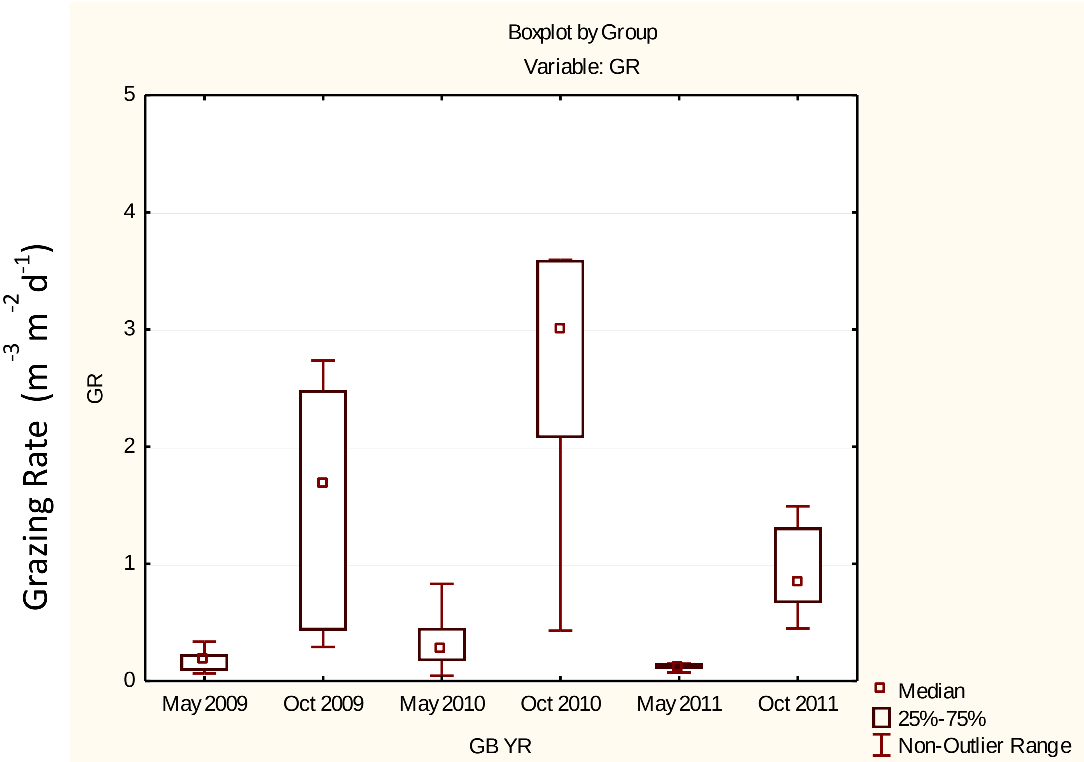


Figure 11. Biomass and filtration rate of bivalves in the shallow habitat of Grizzly and Honker Bays in May 2009-October 2011.



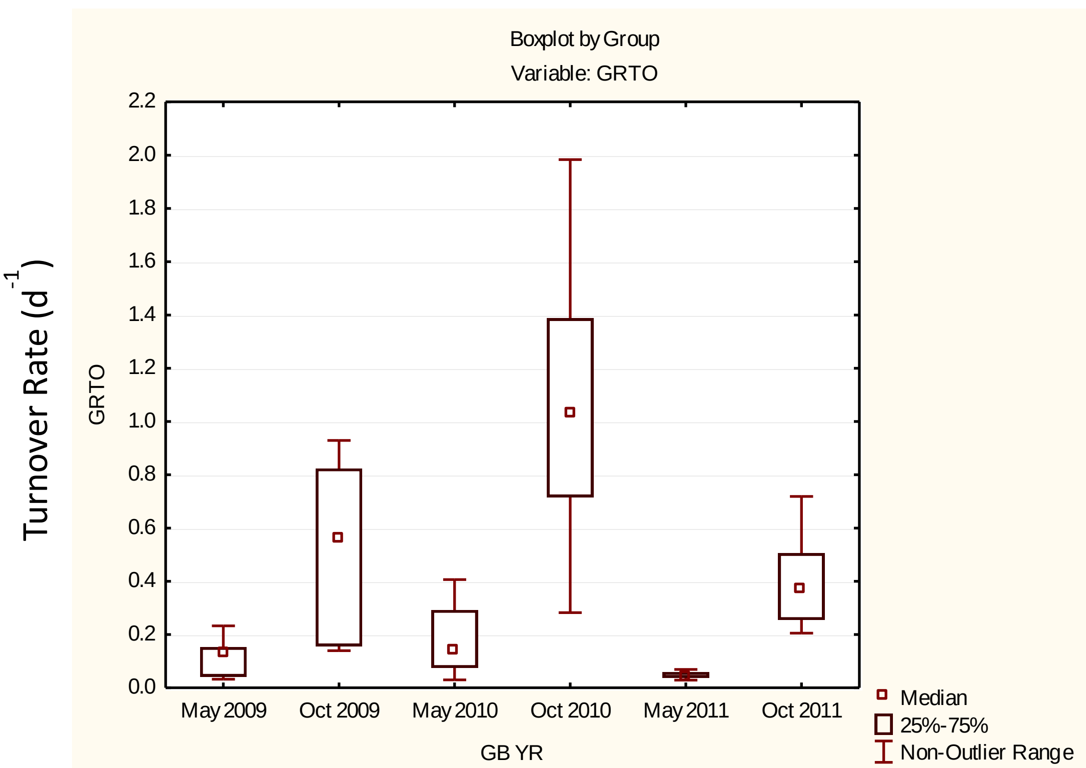


Figure 12. Grazing rate and water column turnover rate of bivalves in the shallow habitat of Grizzly and Honker Bays in May 2009-October 2011.

Table 1. Biomass (g AFDW m^{-2}) (N: sample number, CL: confidence limit)

Region	N	Mean	-95% CL	+95%CL	Median	Min	Max
2009							
Grizzly/Honker Bay	11	5.3	3.5	7.1	5.8	0.9	9.7
Suisun Shallows	10	8.4	3.6	13.2	8.4	0.3	17.2
Suisun Channel	16	7.3	0.1	14.6	3.3	0.0	56.4
East Suisun Marsh	2	11.7	-135.8	159.2	11.7	0.1	23.3
West Suisun Marsh	11	16.0	7.3	24.6	12.9	0.0	34.9
Confluence	28	11.9	5.8	17.9	5.9	0.0	57.4
Lakes	7	8.1	4.2	12.0	7.9	2.0	12.8
2010							
Grizzly/Honker Bay	9	13.6	4.8	22.4	9.8	1.3	35.9
Suisun Shallows	11	7.2	2.0	12.4	4.2	0.0	21.1
Suisun Channel	12	9.0	-0.5	18.5	3.3	0.0	53.5
East Suisun Marsh	2	27.5	-310.2	365.3	27.5	0.9	54.1
West Suisun Marsh	11	25.6	8.2	42.9	14.3	0.7	90.6
Confluence	25	10.4	5.4	15.3	5.5	0.0	43.9
Lakes	6	7.2	-4.5	19.0	3.3	0.9	30.0
2011							
Grizzly/Honker Bay	9	3.6	1.8	5.3	2.8	1.3	9.1
Suisun Shallows	9	13.3	-0.7	27.3	4.0	1.7	49.2
Suisun Channel	16	9.0	2.0	16.0	3.4	0.0	42.6
East Suisun Marsh	4	28.9	-29.1	87.0	19.3	0.4	76.7
West Suisun Marsh	8	7.3	1.5	13.1	5.2	0.0	16.1
Confluence	30	12.1	6.9	17.2	7.7	0.0	50.7
Lakes	5	4.9	1.6	8.3	3.1	3.0	8.7

Table 2. Filtration Rate ($m^{-3}m^{-2}d^{-1}$)

Region	N	Mean	-95% CL	+95%CL	Median	Min	Max
2009							
Grizzly/Honker Bay	11	2.2	1.3	3.0	2.4	0.4	3.9
Suisun Shallows	10	3.4	1.4	5.3	3.4	0.1	6.9
Suisun Channel	16	3.1	0.2	6.1	1.3	0.0	22.5
East Suisun Marsh	2	0.8			0.8	0.0	1.6
West Suisun Marsh	11	11.6	0.9	22.2	8.4	0.2	57.2
Confluence	28	1.6	0.1	3.2	0.4	0.0	20.7
Lakes	7	0.6	0.3	0.8	0.5	0.2	0.9
2010							
Grizzly/Honker Bay	9	5.4	1.9	9.0	3.9	0.5	14.4
Suisun Shallows	11	2.9	0.8	5.0	1.7	0.0	8.4
Suisun Channel	14	3.2	-0.1	6.4	0.8	0.0	21.4
East Suisun Marsh	2	2.1			2.1	0.1	4.0
West Suisun Marsh	10	13.0	1.0	25.1	8.6	0.3	58.0
Confluence	25	0.9	0.5	1.2	0.6	0.0	3.0
Lakes	6	0.6	-0.4	1.6	0.2	0.1	2.6
2011							
Grizzly/Honker Bay	9	1.4	0.6	2.1	1.0	0.5	3.6
Suisun Shallows	9	3.9	-0.8	8.6	1.6	0.6	19.7
Suisun Channel	16	3.6	0.8	6.4	1.4	0.0	17.0
East Suisun Marsh	4	3.0	-3.2	9.2	1.9	0.0	8.3
West Suisun Marsh	8	2.7	0.6	4.9	2.1	0.0	6.4
Confluence	30	1.1	0.6	1.6	0.6	0.0	4.5
Lakes	5	0.4	0.1	0.7	0.3	0.2	0.7

Table 3. Grazing Rate ($m^{-3}m^{-2}d^{-1}$)

Region	N	Mean	-95% CL	+95%CL	Median	Min	Max
2009							
Grizzly/Honker Bay	11	1.6	0.9	2.2	1.7	0.3	2.7
Suisun Shallows	10	2.4	1.1	3.8	2.4	0.1	4.8
Suisun Channel	16	2.1	0.5	3.6	1.1	0.0	11.7
East Suisun Marsh	2	0.6			0.6	0.0	1.3
West Suisun Marsh	11	8.0	1.2	14.7	6.5	0.2	36.5
Confluence	28	1.2	0.2	2.3	0.4	0.0	13.8
Lakes	7	0.5	0.3	0.7	0.5	0.1	0.7
2010							
Grizzly/Honker Bay	9	3.6	1.5	5.6	3.0	0.4	8.7
Suisun Shallows	11	2.1	0.7	3.6	1.4	0.0	6.4
Suisun Channel	14	2.1	0.2	3.9	0.7	0.0	11.9
East Suisun Marsh	2	1.7			1.7	0.1	3.3
West Suisun Marsh	11	8.4	1.5	15.4	4.3	0.2	37.1
Confluence	26	0.7	0.4	0.9	0.5	0.0	2.1
Lakes	6	0.4	-0.1	0.9	0.2	0.1	1.3
2011							
Grizzly/Honker Bay	9	1.1	0.6	1.6	0.8	0.4	2.7
Suisun Shallows	8	3.1	-0.4	6.6	1.6	0.6	13.2
Suisun Channel	16	2.6	0.7	4.6	1.1	0. 0	11.8
East Suisun Marsh	4	2.1	-2.1	6.4	1.5	0.0	5.6
West Suisun Marsh	9	1.9	0.5	3.3	1.3	0.0	4.9
Confluence	30	0.9	0.5	1.2	0.6	0.0	3.3
Lakes	5	0.4	0.1	0.6	0.3	0.2	0.6

Table 4. Grazing Turnover Rate (d^{-1})

Region	N	Mean	-95% CL	+95%CL	Median	Min	Max
2009							
Grizzly/Honker Bay	11	0.5	0.3	0.7	0.6	0.1	0.9
Suisun Shallows	10	0.5	0.2	0.8	0.5	0.0	1.1
Suisun Channel	16	0.2	0.1	0.3	0.1	0.0	0.6
East Suisun Marsh	2	0.1			0.1	0.0	0.2
West Suisun Marsh	11	2.1	0.6	3.6	1.3	0.2	8.2
Confluence	28	0.3	0.0	0.5	0.1	0.0	2.7
Lakes	7	0.3	0.1	0.4	0.2	0.0	0.5
2010							
Grizzly/Honker Bay	9	1.1	0.6	1.5	1.0	0.3	2.0
Suisun Shallows	11	0.5	0.2	0.8	0.5	0.0	1.2
Suisun Channel	14	0.2	0.1	0.3	0.1	0.0	0.6
East Suisun Marsh	2	0.3			0.3	0.0	0.6
West Suisun Marsh	11	1.5	0.9	2.1	1.5	0.2	3.0
Confluence	25	0.1	0.1	0.2	0.1	0.0	0.6
Lakes	6	0.1	0.0	0.3	0.1	0.0	0.4
2011							
Grizzly/Honker Bay	9	0.4	0.3	0.6	0.4	0.2	0.7
Suisun Shallows	9	0.9	0.2	1.6	0.5	0.0	2.6
Suisun Channel	16	0.2	0.1	0.4	0.1	0.0	1.0
East Suisun Marsh	4	1.0	0	3.2	0.4	0.0	3.0
West Suisun Marsh	8	0.4	0.1	0.8	0.4	0.0	1.1
Confluence	30	0.3	0.1	0.5	0.1	0.0	1.9
Lakes	5	0.2	0.0	0.4	0.1	0.1	0.4

DFG ERP Progress Report I

Program Manager	Carol Atkins	Phone 916-445-0074
Project Manager	Swee Teh	Phone: (530) 754-8183
ERP Grant #	E1183004	

Project Title: Fall X2 Fish Health Study: Contrasts in Health Indices, Growth and Reproductive Fitness of Delta Smelt and Other Pelagic Fishes Rearing in the Low Salinity Zone and Cache Slough Regions

Table of contents

<u>**Executive Summary**</u>

The goal of this project is to establish a conceptual and analytical framework for investigating the relationships among environmental stressors, ecosystem variables, and health indices of at-risk fish species. Because the measurement of these indices will be determined for the first time in this study, results will be used to establish baselines for fish health indices that will provide critical data for current and future comparisons. A comprehensive suite of measurements generated from sufficient numbers of each fish species collected during a relatively "good" year should provide for an important assessment of contaminant effect, pathogens/disease, and nutritional status (body condition, lipid content) and whether they are related to region of capture (i.e. proximity to sources) and growth of the different fish species, including delta smelt.

Results from the analyses will provide measures of fall growth (otolith daily increments and RNA/DNA ratio), fish fitness condition (condition factor, triglyceride (TAG), and histopathology), and indicators of environmental stressors (Acetylcholinesterase (AChE), Na^+/K^+-ATPase, histopathology, and pathogens) in delta smelt and other at-risk species across the three contrasting regions in the estuary. We will also be able to examine delta smelt 'first spawn' fecundity that can be related to fall growth and condition. Furthermore otolith microchemistry can determine the migratory life history type, which may influence growth, condition and reproductive fitness. This study will advance our current understanding of cause-effect relationships of the fall outflow and salinity-habitat manipulations on delta smelt in the next three years (2011-2013) and will provide a baseline health index for delta smelt and the other species included in these studies.

This first progress report provides a description of initial results from delta smelt and water quality data that were collected (in collaboration with DFG and USGS) in the San Francisco Estuary from August to December 2011. Since we do not have long term (multiple years) datasets for many of the indices, we focused our initial analyses on comparing different habitats defined in the current study as the Cache slough-Sacramento Deepwater Ship Channel area, <1psu, 1to 6psu>, >6psu and/or by regions (Cache slough, Confluence, Honker, and Suisun Bay). Initial findings contained in this report are summarized as follows:

- For the fall survey, greater than 50% of all delta smelt were collected from fresh to 2 psu habitats. Moreover, catch distributions varied significantly from month to month among the different habitat and region scales, such that conducting statistical analysis was difficult.

- Otolith growth rates revealed that fish during the fall of 2011 were growing at a high rate, highest since 2000, however we did not observe a difference in growth among different habitats. A majority of the fish collected during the FMWT survey were of the migratory life history type with only a few individuals being resident freshwater fish, however for

the August TNS survey a large number of resident fish were observed in the Cache Slough-Sacramento River Deep Water Ship Channel.

- Morphometric condition of the fish appeared relatively ‘good’ for 2011. Indices of CF and HSI were relatively similar from August to December and among habitats and regions suggesting that a majority of the fish were in relatively similar health throughout the fall. Nutritional biomarker (TAG) also exhibited similar indices from August to December. Both RNA/DNA and GSI increased with time and subsequently at decreased temperatures, suggesting fish surviving during the fall had improved indices through the fall.

- Enzymatic biomarkers AChE and $Na^{+}K^{+}$-ATPase among the delta smelt sub-sampled from August-December 2011 indicate that approximately 60% were found within a normal “healthy: range. However, some fish showed lower enzyme activity indicating that contaminants may also play a role in the health of Delta smelt.

- Pathogens are present in most water bodies and only become problematic for fish when exposed to stressful environmental conditions. The current study showed that the presence of bacterial organisms among delta smelt is influenced by the synergistic interactions of three environmental stressors: turbidity, salinity, and water temperature. Delta smelt carrying *Mycobacterium* DNA are more pronounced in locations with significant interaction of conductivity and water temperature.

- Histopathological analysis indicates fish surviving to December month in the <1 psu salinity are healthier than fish sampled in September–November months.

- Correlation, canonical and clustering analysis indicated that combinations of salinity, turbidity, and temperature significantly affect the outcome of most health parameters except TAG and ATPase

- The optimal mean turbidity and temperature for over 50% of the total fish sampled at 0-2 psu salinity with threshold condition factor of 0.6-0.8 were 59.15 NTU and 13.25°C.

A. Project Description

This project was initiated in collaboration with the Interagency Ecological Program (IEP) long-term fish monitoring surveys and studies to investigate the health of four fish species occupying the low salinity zone during the critical fall period. The purpose of this study is to examine the potential effects of stressors (e.g. contaminants, pathogens/diseases, and poor feeding success) on the "health" of delta smelt, striped bass, threadfin shad, and American shad collected from three regions in the upper San Francisco Bay Delta Estuary (SFE), namely the Cache Slough complex (Cache Slough and Sacramento Deepwater Ship Channel), the Sacramento/San Joaquin river confluence and Suisun Bay. Comprehensive indicators of health as used in the current study include various key measurements of fish fitness such as growth, fecundity, histopathological changes, nutritional status, and other stressor induced impairments. The position of the low salinity zone has been hypothesized to benefit delta smelt by enhancing factors leading to better recruitment the following year. To assess the role of health on survival of fish through the fall, we will use a "characteristics of survivors" approach in which health measurements of fish from each sampling period (month) can be compared to previous periods to determine the characteristics (health factors measured in this study) of fish that survived each period. This approach will employ a rigorous and comprehensive examination of multiple stressors and physicochemical factors affecting the growth, reproduction, and general health of fish through the critical fall period.

B. Measures of Fish Health

Fish are robust indicators of the ecological conditions in aquatic habitats. As such, fish health monitoring is an important and ecologically relevant approach for determining the effect of stressors to fundamental fish functions such as growth, reproduction, and recruitment. Reproductive and developmental parameters are among the most important sentinel endpoints for assessing exposure to contaminants (Anderson et al. 2007). While thousands of chemical contaminants are continuously being introduced into the San Francisco Estuary (SFE) (Kuivila and Hladik 2008, Werner et al. 2008, Johnson et al. 2010), few studies to date have focused on determining the biological effects of contaminants particularly among fish experiencing population declines in the SFE.

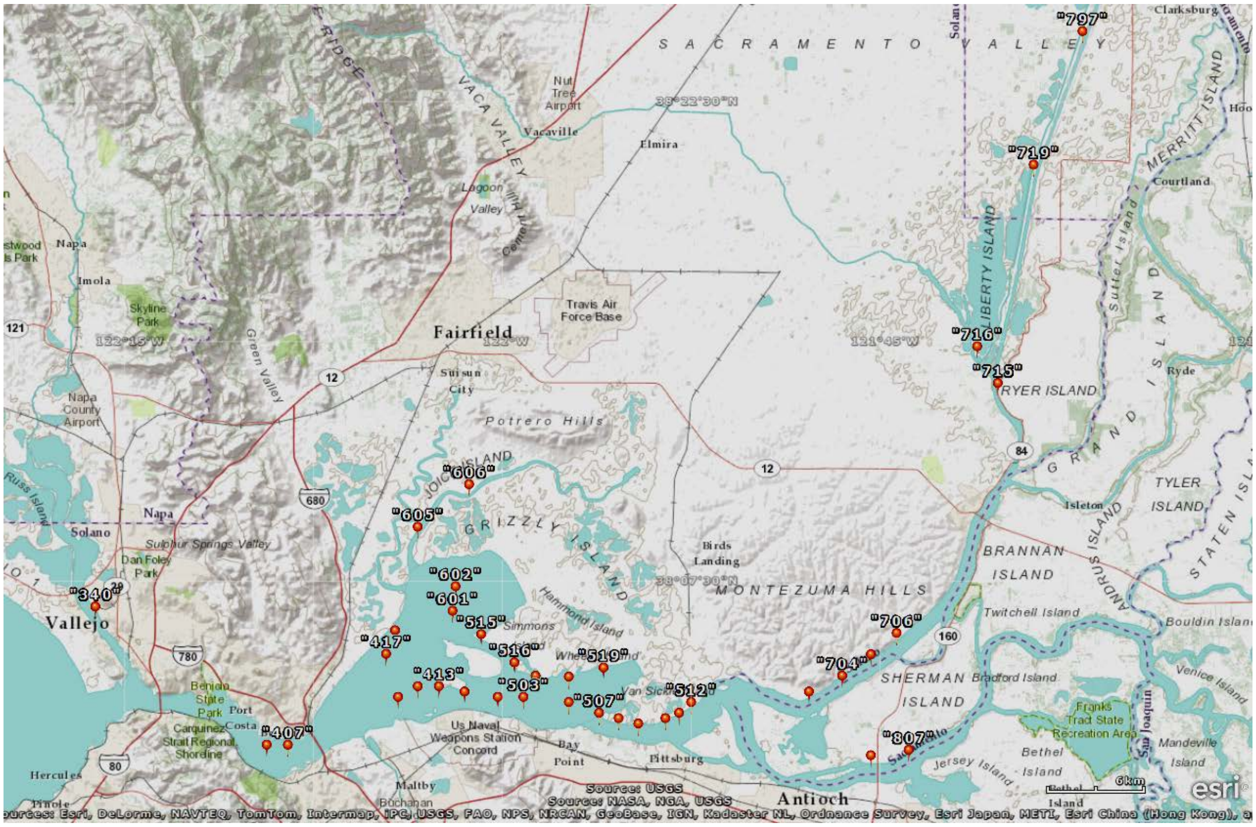
Although it is impossible to determine the precise factors contributing to the health of a free-ranging species caught at a certain site, the use of an integrated approach incorporating fish condition indices in concert with assessments of nutritional status, disease, enzymatic and histopathologic biomarkers, growth and reproduction are good indicators of the general health of the species (Adams et al., 1989). The nutritional status of fish can mediate impacts of stress in susceptible species (Brinkmeyer and Holt, 1998; Gaspasin et al., 1998; Ashraf et al., 1993). Fish nutrition, in part, may reflect shifts in food quality and availability, which was identified as an important factor in fish declines in the estuary (POD Report 2007). The presence of disease in wild fish populations is a significant health indicator because it represents the cumulative effects of multiple stressors and variables in the aquatic environment, many of which are unknown or poorly defined (Hedrick 1998). Curiously, the role of disease to fish populations in the SFE has received less attention than many other potential causes. Enzymatic and histopathologic biomarkers in fish may indicate elevated stress due to changes in the environment or from contaminant exposure (Adams et al., 1989, Teh et al., 1997). Specific lesions and changes in enzyme activity suggest exposure to specific stressors. This Project will attempt to understand these relationships by utilizing a comprehensive approach of measuring the effects of multiple stressors that could profoundly impact the health of pelagic species and the SFE ecosystem.

The compounding impacts of multiple stressors elicit significant physiological and behavioral responses that may create significant changes on reproduction, survival, and growth of fish (Jarvi 1990). Although it is notoriously difficult to separate the combined effects of multiple stressors acting simultaneously, the additive effects of physicochemical stressors likely exacerbate negative effects. Stressors may render both sub-lethal and lethal effects to animals, but in an ecological context the sub-lethal effects are usually more relevant than direct mortality. Ecological consequences may affect individual fitness (reproduction, survival, and growth) potentially leading to population-level effects (Mesa 1994).

C. <u>Project Task.</u>

Delta smelt and water quality data were collected with the cooperation of DFG and USGS at different sites in the San Francisco Estuary over 5 months during two different surveys, Summer Tow Net Survey (STN) and Fall Midwater Trawl (FMWT) in 2011 (Fig C.1). At each sampling site, delta smelt were counted and measured for fork length by DFG. The fish were flash frozen in liquid nitrogen with individual ID numbers. Up to 40 fish from each sampling site were stored in liquid nitrogen and sent to the Aquatic Health Program (AHP) at UC Davis for health analysis (UCD). Necropsies were performed on 331 delta smelt caught between August and December 2011.

Figure C.1. Station locations in the upper Sacramento-San Joaquin Estuary sampled by the Department of Fish and Game for delta smelt and other fish species used in the Fall X2 fish health study.



C-1. Necropsy

Delta smelt (n=331) received from the fish surveys were removed from liquid nitrogen. Fork lengths (FL) and body weights (BW) were measured while frozen to determine condition factor (CF). Samples were allowed to thaw briefly to allow the skin to appear natural then pictures were taken with a ruler and ID tag. Otoliths were removed for age, growth, and isotopic microchemistry determinations. The brain and left gill were removed and frozen in liquid nitrogen then stored at -80^{o}C for enzyme analysis while the right gill was removed and preserved in 10% buffered formalin for histopathology. A finclip of the caudal fin was preserved in 90% ethanol and stored at -20^{o}C for potential genetic analysis. For visceral organs, the gastrointestinal (GI) tract was first removed from the fish. Then gonads and liver were carefully separated from the GI tract. The GI tract was preserved in 95% ethanol at room temperature and sent to Randy Baxter (Co-PI) at DFG for gut content analysis. Liver and gonads were weighed to determine Hepatosomatic (HSI) and gonadosomatic (GSI) indices, respectively. When livers and/or gonad weights were greater than 0.005 g the samples were split into two portions. Un-split liver and gonads were placed in 10% buffered formalin. If the liver or gonad were split than the first portion was placed in 10% buffered formalin at room temperature for histopathology. The second portion was frozen in liquid nitrogen and stored at -80^{o}C for hormonal and enzyme analysis (liver) or fatty acid analysis (gonad). The kidney and spleen were removed and stored in culture media for pathogen analysis. The muscle along the dorsal section of the fish was separated with the skin carefully removed, then frozen in liquid nitrogen and stored at -80^{o}C for Triglyceride (TAG) and RNA/DNA determinations. The remaining carcass was frozen in liquid nitrogen and stored at -80^{o}C.

C-2. Otolith Growth and Microchemistry Analysis

Otoliths were mounted onto glass slides with Crystal Bond thermoplastic resin in the sagittal plane, grounded to the core on both sides with wet-dry sandpaper and polished with a polishing cloth and 0.3-micron polishing alumina. Otoliths were digitized with a digital camera at a magnification of 100X. Otolith increments were enumerated and the distance from the core to each daily ring was measured using Image-J NIH software. Growth rates were quantified using several approaches. Total growth rate was quantified simply by dividing the length of the fish, minus an initial hatch length of 5-mm, by the age estimate derived from daily otolith increment enumeration. Next the size at each daily increment was estimated using the "Biological Intercept Model" (BIM) method previously developed for delta smelt (Hobbs et al. 2007). Month-specific growth rates were quantified by counting back from the day of capture to the beginning and end of each month and determining the length for those dates with the BIM. The estimated length at the beginning of the month was subtracted by the end length and divided by the number of calendar days in the month. Otolith strontium isotope ratios were quantified using methods previously developed (Hobbs et al 2007; 2010). Briefly, the strontium isotope profile from the core to the edge along a similar path used for aging, was scanned using a laser beam of 55-microns moving at a speed of 10-microns per second. Laser profiles began at 100-micron in the core to be sure to encompass the natal chemistry.

We determined age and otolith growth rates of 297 delta smelt collected monthly from August to December of 2011 and spatially among four habitats defined by collection within the region north of the confluence of the main stem Sacramento River with Cache Slough region up the deep water ship channel (Cache-SDWSC) and three salinity zones (<1 psu, 1-6 psu and >6 psu) (Table 2). Overall the distribution of the growth rates varied from 0.23 to 0.54 mm-day and exhibited a bi-modal distribution, however this was the result of a temporal effect with growth rates being significantly reduced in November and December survey months (Fig C.2.1). The strong temporal trend precluded a simple statistical analysis of the spatial variation. To account for the temporal pattern in growth rates we standardized growth rate for each individual to the mean growth rate for the month the fish was collected (Fig C.2.2). Even after accounting for the temporal trend, growth rates varied considerably within each habitat and thus no statistical difference was found between habitats, however the distribution for fish in the >6psu salinity zone was narrowed, relative to the other habitats suggesting high salinity habitats may not provide for high growth rates and fish growing slowly may either move to other habitats or not survive (Fig C.2.3).

The studies main objective was to evaluate growth during the fall period, therefore we estimated growth rates specific to the months of July, August, September and October (Month Specific Growth Rate) using the BIM model to back-calculate the size at the beginning and end of each month from the otolith record. Month specific growth rates were examined for each survey month in the 2011 study period(August-December) and we considered fish collected during the December Survey to be fully recruited to the adult stage, and thus compare the December survey to the previous survey months. Month Specific Growth Rate distributions varied between survey months (Figure C.2.4). Month Specific Growth Rates for July did not appear to be different between the December survey and previous months, although the distribution for the November survey did appear to vary less than other survey months. Note that sample size was smaller for the November survey. Month Specific Growth Rates for August did differ with the December survey fish exhibiting faster growth during the month of August compared to August-October, although November was similar to December survey fish. Month Specific Growth Rates for September and October overall was lower than previous months and no trends were observed among survey months.

Otolith microchemistry was performed to determine the migratory history of 280 delta smelt collected monthly from August to December of 2011 and spatially among four habitats defined by collection within the region north of the confluence of the main stem Sacramento River with Cache Slough region up the deep water ship canal (Cache-SDWSC) and three salinity zones (<1 psu, 1-6p psu and >6 psu) (Table C.2.1). We found the migratory life history to be the dominant life history type with 231 migratory individuals overall. A majority of the resident life history type were found in the Cache-SDWSC sites, however we found four resident fish collected at the <1psu and one resident in the 1-6 and >6psu respectively. For the 1-6 and >6 individuals, they were collected in August when fish could potentially be continuing to migrate to the low salinity zone from high salinity zone. The residents fish collected at <1psu occurred in October and December suggesting resident fish could move into low salinity water later in the fall when the low salinity zone is in closer proximity to freshwater habitats. We examined the total growth rates of resident and migratory individuals, however no significant differences were found.

Table C.2.1. Otolith analysis: Estimated growth rates (mm/day) of delta smelt from August to December 2011 from 4 regions of the San Francisco Estuary, Cache-SDWSC, <1 psu, 1-6 psu, and >6 psu.

Region	Month	Necropsied (n)	Aged (n)	Growth rate (mm/day)		
Cache-SDWSC	August	42	37	0.41	±	0.06
< 1 psu	August	0	0			
1-6 psu	August	24	24	0.46	±	0.05
> 6 psu	August	4	4	0.44	±	0.04
Cache-SDWSC	September	2	2	0.39	±	0.05
< 1 psu	September	7	5	0.42	±	0.04
1-6 psu	September	29	25	0.38	±	0.04
> 6 psu	September	5	5	0.41	±	0.02
Cache-SDWSC	October	3	3	0.29	±	0.01
< 1 psu	October	33	32	0.36	±	0.04
1-6 psu	October	8	8	0.39	±	0.03
> 6 psu	October	2	2	0.37	±	0.01
Cache-SDWSC	November	18	16	0.28	±	0.01
< 1 psu	November	2	1	0.27	±	
1-6 psu	November	5	5	0.28	±	0.01
> 6 psu	November	17	3	0.28	±	0.01
Cache-SDWSC	December	8	7	0.28	±	0.03
< 1 psu	December	69	69	0.29	±	0.02
1-6 psu	December	15	15	0.28	±	0.02
> 6 psu	December	35	31	0.28	±	0.02

Figure C.2.1. Count distribution of total growth rates (mm/day) for all 297 delta smelt collected from August to December 2011.

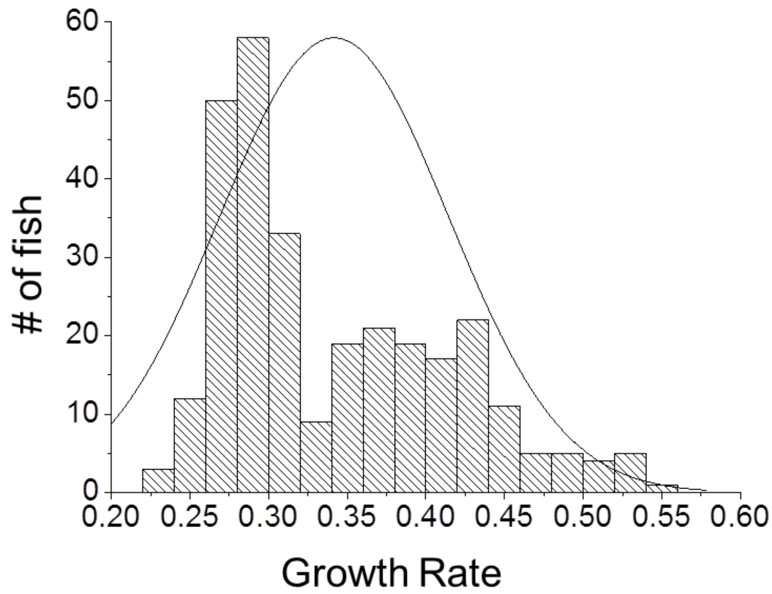


Figure C.2.2. Growth rates of delta smelt for each month of the fall study period. The dark horizontal bars represent the mean for each survey month. Open circles represent the growth of individual fish.

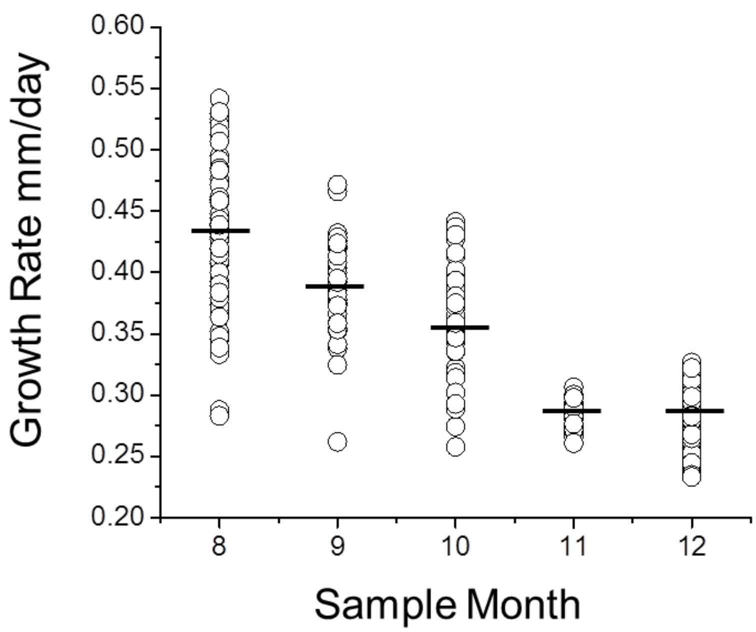


Figure C.2.3. Growth rates standardized by the mean growth rate for each survey month by the four habitats groups, CDS- Cache Slough to Deep Water Ship Channel sites, <1 psu, 1 to 6psu and >6 psu salinity zones.

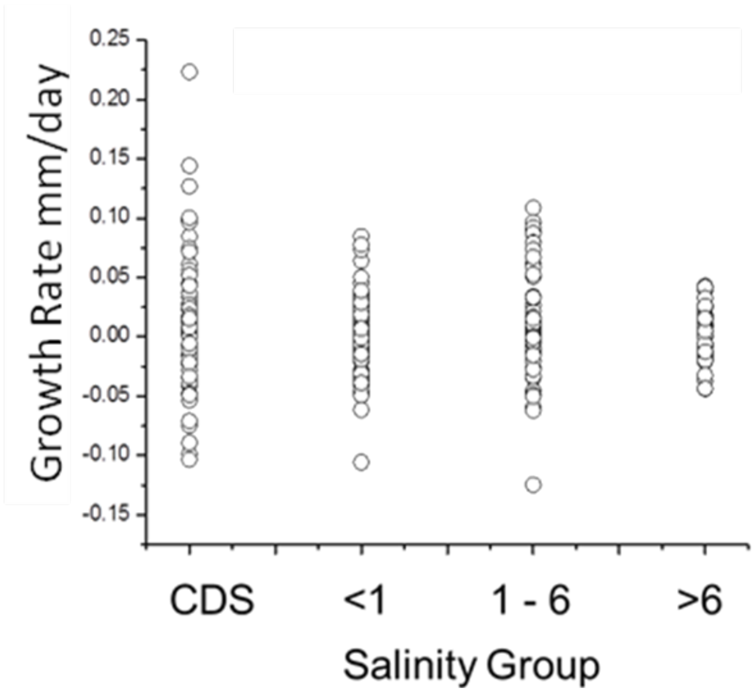
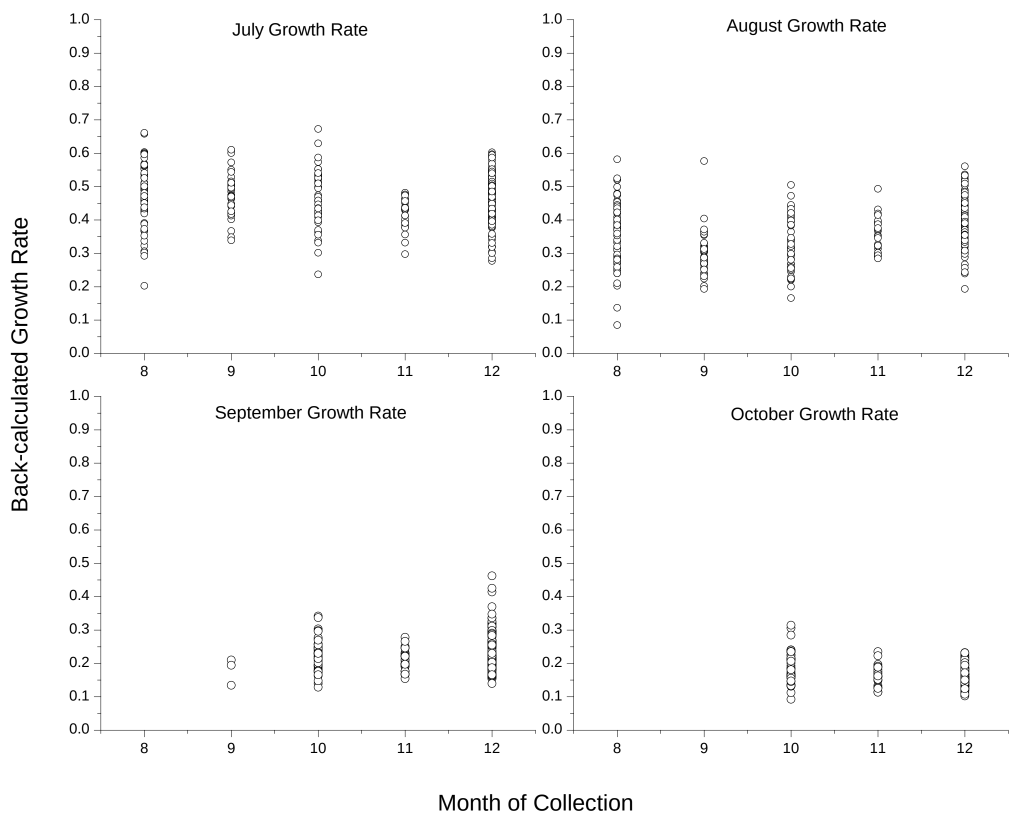


Figure C.2.4. Otolith analysis: Back-calculated Month Specific Growth Rates for fish collected from August (8) Summer Tow-net Survey, and September (9) to December (12) Fall Midwater Trawl
Survey.



C-3. Morphometry

The morphometric analysis is summarized in Table C.3.1-5. Most trawl stations had no more than 10 fish collected during any time period, while some sites had a lot of samples (>10) such as 602 and 715 (Aug), 507 (Oct), and 705 and 706 (Dec). Some sites had more than 40 fish such as 716 (Aug) but only 36 fish were retained for analysis. Samples were not consistently collected at each site. For example, site 519 had delta smelt collected each month except November, while several sites only had 1 sample in only one month (e.g. 340, 512, and 802). Comparisons for univariate analysis were conducted on temperature (^{o}C), salinity (psu), turbidity (NTU), secchi (m) separately by two region specifications: 1) Cache Slough Complex, <1 psu, 1-6 psu and >6 psu; and 2) Suisun Bay, Honker Bay, Confluence, and Cache Slough Complex. Due to the unbalanced sample size a multivariate statistical approach was necessary to examine the data.

Considerable variation of each morphometric analysis was too high to determine a significant difference in relationship to the environment and by region. The FL and BW increased with time (Fig C.3.1 and C.3.4) but did not appear to be influenced by salinity (Fig C.3.2 and C.3.5). Condition Factor (CF) indicates the relative health of a fish. The CFs were similar over temperature (Fig. C.3.6), between regions (Fig. C.3.7 and 8) and in relation to salinity. The Hepatosomatic index (HSI) indicates health and reproductive status. High HSI values can indicate good growth, maturity and reproductive status for females. The HSI was similar in relation to salinity (Fig. C.3.10) and between and within regions (Fig. C.3.11 and 12). The relative similarities of the CF and HSI suggest the relative health status of the delta smelt. The gonadosomatic index (GSI) represents the reproductive status of the fish. There was no relationship with salinity (Fig. C.3.14). There was a weak (R^2 =0.236) relationship between time (Fig. C.3.15) and GSI but there was no relationship with other factors, suggesting that GSI was influenced more by time than any other factor. As a 1-2 year spawner, delta smelt mature during November/December in order to spawn in the late winter to early spring (Moyle, 2002). As expected, the GSI values began to increase in November (Fig C.3.15). When examining GSI by region, Cache slough exhibited larger values in November then the other regions and showed similar response in December (Fig. C.3.15 and 16).

Using the canonical analysis, (Fig. C.3. 3, 9, 13, and 18), it was determined that a combination of temperature, salinity and turbidity had a significant effect on morphometric indices. This suggests that not a single factor but a combination of at least three factors significantly drives the health status of delta smelt. Since GSI was also affected by a combination of temperature, salinity and turbidity, the reproductive status is affected. Further analysis is needed to determine the magnitude of the effect of the three factors and at what range is optimal for delta smelt. Threshold limits for general health status based on morphometric analysis can be determined in the future with additional yearly sampling.

Table C.3.1. Morphometric data of delta smelt collected from the Summer Tow Net Survey in August 2011. Indices of Fork Length (FL), Body Weight (BW), Condition Factor (CF), Hepatosomatic Index (HSI), and Gonadosomatic Index (GSI) are reported as Means ± Standard Deviation. N/A represents no data was available.

Site	**n**	**Date**	**FL (mm)**	**BW (g)**	**CF**	**HSI**	**GSI**
340	1	8/26/11	47.00	0.79	0.77	0.66 ± 0.00	N/A
418	3	8/25/11	50.33 ± 7.23	1.00 ± 0.48	0.73 ± 0.11	0.74 ± 0.09	1.70
501	3	8/24/11	51.33 ± 4.04	0.80 ± 0.35	0.60 ± 0.24	0.95 ± 0.29	0.31
519	1	8/24/11	41.00	0.43	0.62	1.01	N/A
602	20	8/25/11	42.70 ± 4.61	0.51 ± 0.15	0.65 ± 0.06	0.69 ± 0.22	0.29
716	38	8/23/11	43.95 ± 6.16	0.63 ± 0.27	0.71 ± 0.06	0.93 ± 0.50	0.40 ± 0.13
797	4	8/23/11	46.00 ± 5.89	0.74 ± 0.27	0.73 ± 0.06	0.80 ± 0.05	0.39 ± 0.06

Table C.3.2. Morphometric data of delta smelt collected from the Fall Midwater Trawl in September 2011. Indices of Fork Length (FL), Body Weight, Condition Factor (CF), Hepatosomatic Index (HSI), and Gonadosomatic Index (GSI) are reported as Means ± Standard Deviation. N/A represents no data was available.

Site	**n**	**Date**	**FL (mm)**	**BW (g)**	**CF**	**HSI**	**GSI**
405*	5	9/8/11	51.00 ± 2.35	N/A	N/A	N/A	N/A
411*	2	9/8/11	54.50 ± 1.00	N/A	N/A	N/A	N/A
413	3	9/8/11	51.33 ± 2.08	1.08 ± 0.11	0.79 ± 0.04	0.82 ± 0.05	0.39
501*	1	9/8/11	59.00	N/A	N/A	N/A	N/A
502	4	9/8/11	52.25 ± 5.12	1.10 ± 0.63	0.78 ± 0.08	0.74 ± 0.13	0.19
503	3	9/8/11	49.67 ± 9.29	0.90 ± 0.48	0.67 ± 0.05	0.73 ± 0.03	0.42 ± 0.01
507	5	9/15/11	65.20 ± 7.50	2.26 ± 1.21	0.76 ± 0.11	0.74 ± 0.18	1.05 ± 1.46
512	1	9/13/11	59.00	1.40	0.68	0.33	N/A
515	2	9/15/11	44.00 ± 2.83	0.71 ± 0.02	0.84 ± 0.13	0.68 ± 0.06	N/A
516	1	9/13/11	42.00	0.60	0.81	N/A	N/A
518	4	9/13/11	52.50 ± 6.03	1.28 ± 0.56	0.85 ± 0.21	0.71 ± 0.23	0.43
519	9	9/13/11	47.33 ± 7.55	0.82 ± 0.38	0.73 ± 0.06	0.54 ± 0.24	0.20
715	2	9/14/11	56.00 ± 1.41	1.51 ± 0.48	0.85 ± 0.21	0.65 ± 0.36	0.38 ± 0.31
802	1	9/12/11	56.00	1.15	0.65	0.77	0.39

* Fish measured on the boat therefore body weight could not be obtained.

Table C.3.3. Morphometric data of delta smelt collected from the Fall Midwater Trawl in October 2011. Indices of Fork Length (FL), Body Weight (BW), Condition Factor (CF), Hepatosomatic Index (HSI), and Gonadosomatic Index (GSI) are reported as Means ± Standard Deviation. N/A represents no data was available.

Site	n	Date	FL (mm)	BW (g)	CF	HSI	GSI
411	1	10/5/11	59.00	1.59	0.77	0.56	0.31
505	1	10/5/11	51.00	0.94	0.71	0.71	N/A
507	24	10/7/11	55.38 ± 5.14	1.26 ± 0.35	0.74 ± 0.07	0.70 ± 0.15	0.40 ± 0.07
508	2	10/7/11	60.00 ± 8.49	1.44 ± 0.51	0.66 ± 0.04	0.67 ± 0.11	N/A
509	3	10/7/11	48.00 ± 6.24	0.84 ± 0.37	0.72 ± 0.10	0.68 ± 0.12	0.26 ± 0.08
510	1	10/7/11	63.00	1.66	0.66	0.77	0.49
511	1	10/7/11	59.00	1.68	0.82	0.71	0.39
517	1	10/7/11	62.00	1.72	0.72	0.67	0.62
518	7	10/7/11	59.71 ± 4.42	1.60 ± 0.37	0.74 ± 0.07	0.72 ± 0.15	0.47 ± 0.14
519	2	10/7/11	51.50 ± 2.12	0.94 ± 0.09	0.69 ± 0.02	0.54 ± 0.05	0.23
721	3	10/11/11	49.00 ± 4.48	1.09 ± 0.33	0.91 ± 0.07	0.49 ± 0.16	N/A

Table C.3.4. Morphometric data of delta smelt collected from the Fall Midwater Trawl in November 2011. Indices of Fork Length (FL), Body Weight (BW), Condition Factor (CF), Hepatosomatic Index (HSI), and Gonadosomatic Index (GSI) are reported as Means ± Standard Deviation. N/A represents no data was available.

Site	n	Date	FL (mm)	BW (g)	CF	HSI	GSI
407	1	11/9/11	51.00	0.84	0.64	1.69	N/A
412	2	11/9/11	58.50 ± 4.95	1.39 ± 0.37	0.68 ± 0.01	0.68 ± 0.05	0.96 ± 0.56
517	2	11/14/11	50.50 ± 2.02	1.19 ± 0.09	0.93 ± 0.05	0.80 ± 0.08	0.57
518	1	11/14/11	56.00	1.60	0.91	0.66	1.25
601	1	11/10/11	57.00	1.16	0.63	0.30	0.78
606	10	11/10/11	58.60 ± 5.19	1.56 ± 0.35	0.76 ± 0.07	0.73 ± 0.17	0.52 ± 0.17
609	1	11/10/11	53.00	1.19	0.80	1.24	0.70
703	1	11/15/11	51.00	1.10	0.83	0.62	0.05
704	4	11/15/11	55.25 ±0.96	1.54 ± 0.16	0.91 ± 0.05	0.72 ± 0.23	0.59 ± 0.11
705	1	11/15/11	57.00	1.72	0.93	0.73	0.65
719	10	11/21/11	56.60 ± 3.50	1.71 ± 0.34	0.93 ± 0.06	1.23 ± 0.40	1.11 ± 0.58
797	8	11/21/11	66.00 ± 13.51	3.04 ± 1.99	0.95 ± 0.05	0.77 ± 0.13	1.71 ± 1.07

Table C.3.5. Morphometric data of delta smelt collected from the Fall Midwater Trawl in December 2011. Indices of Fork Length (FL), Body Weight (BW), Condition Factor (CF), Hepatosomatic Index (HSI), and Gonadosomatic Index (GSI) are reported as Means ± Standard Deviation.

Site	**n**	**Date**	**FL (mm)**	**BW (g)**	**CF**	**HSI**	**GSI**
417	1	12/8/11	57.00	1.30	0.70	0.59	0.58
502	1	12/7/11	50.00	0.90	0.72	0.69	0.84
507	2	12/9/11	58.00 ± 7.07	1.52 ± 0.78	0.74 ± 0.12	0.53 ± 0.20	2.32
509	1	12/9/11	63.00	1.93	0.77	0.85	1.93
515	1	12/9/11	63.00	2.04	0.82	0.38	1.92
516	1	12/9/11	66.00	2.50	0.87	0.79	1.11
517	3	12/9/11	61.33 ± 5.03	1.85 ± 0.41	0.80 ± 0.06	0.53 ± 0.10	1.58 ± 0.36
518	6	12/9/11	58.67 ± 2.34	1.51 ± 0.20	0.74 ± 0.04	0.64 ± 0.12	0.73 ± 0.39
519	6	12/9/11	61.17 ± 3.06	1.74 ± 0.25	0.76 ± 0.05	0.54 ± 0.23	1.45 ± 1.07
605	1	12/8/11	54.00	1.08	0.69	0.75	0.08
606	13	12/8/11	58.09 ± 4.41	1.65 ± 0.36	0.84 ± 0.12	0.73 ± 0.26	0.92 ± 0.56
703	12	12/12/11	56.58 ± 3.15	1.29 ± 0.21	0.71 ± 0.05	0.62 ± 0.15	0.68 ± 0.37
704	7	12/12/11	60.71 ± 4.89	1.67 ± 0.36	0.73 ± 0.04	0.59 ± 0.22	1.01 ± 0.29
705	22	12/12/11	61.27 ± 4.27	1.67 ± 0.41	0.72 ± 0.07	0.66 ± 0.21	1.21 ± 0.99
706	42	12/12/11	63.12 ± 3.85	1.90 ± 0.32	0.75 ± 0.10	0.72 ± 0.21	1.59 ± 0.71
719	6	12/16/11	61.33 ± 6.62	1.85 ± 0.47	0.79 ± 0.07	0.63 ± 0.16	1.61 ± 1.25
797	2	12/16/11	67.50 ± 0.71	2.52 ± 0.10	0.82 ± 0.06	0.53 ± 0.03	2.32 ± 0.58
806	1	12/13/11	57.00 ± 0.00	1.41 ± 0.00	0.76 ± 0.00	0.65 ± 0.00	0.84 ± 0.00
807	2	12/13/11	63.00 ± 7.07	1.84 ± 0.49	0.73 ± 0.05	1.11 ± 0.53	2.26 ± 1.64

Figure C.3.1. Fork length (FL) of delta smelt collected from August to December 2011.

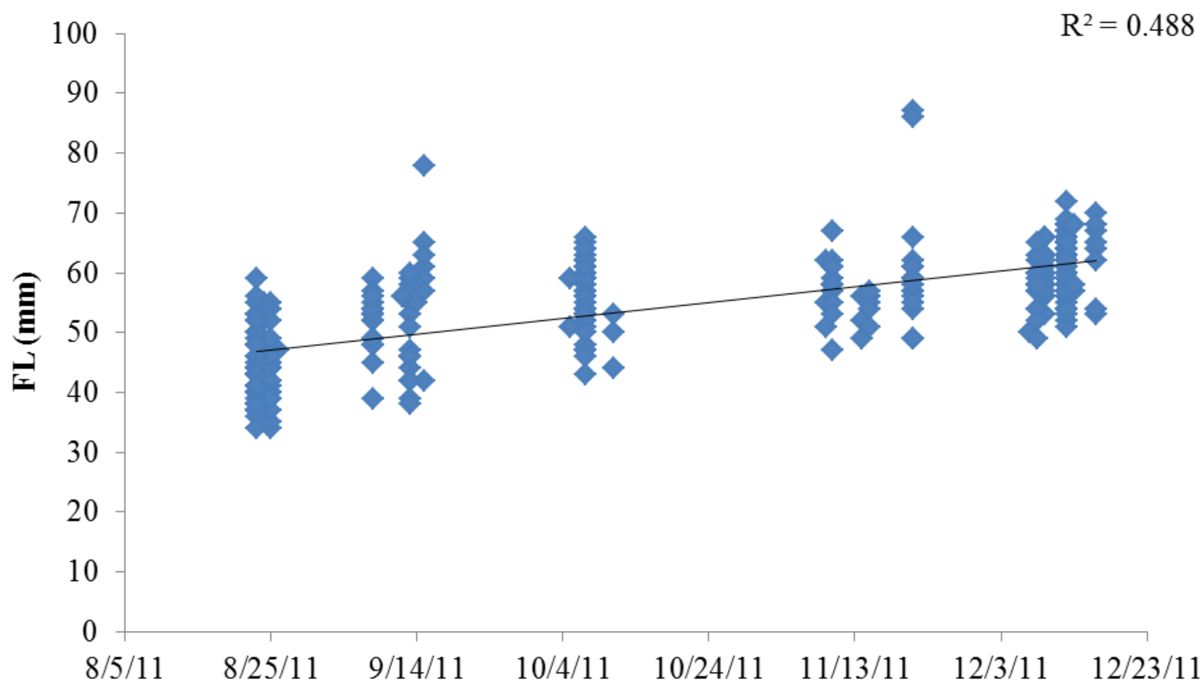


Figure C.3.2. Fork length (FL) over salinity (psu) of delta smelt collected from August to December 2011.

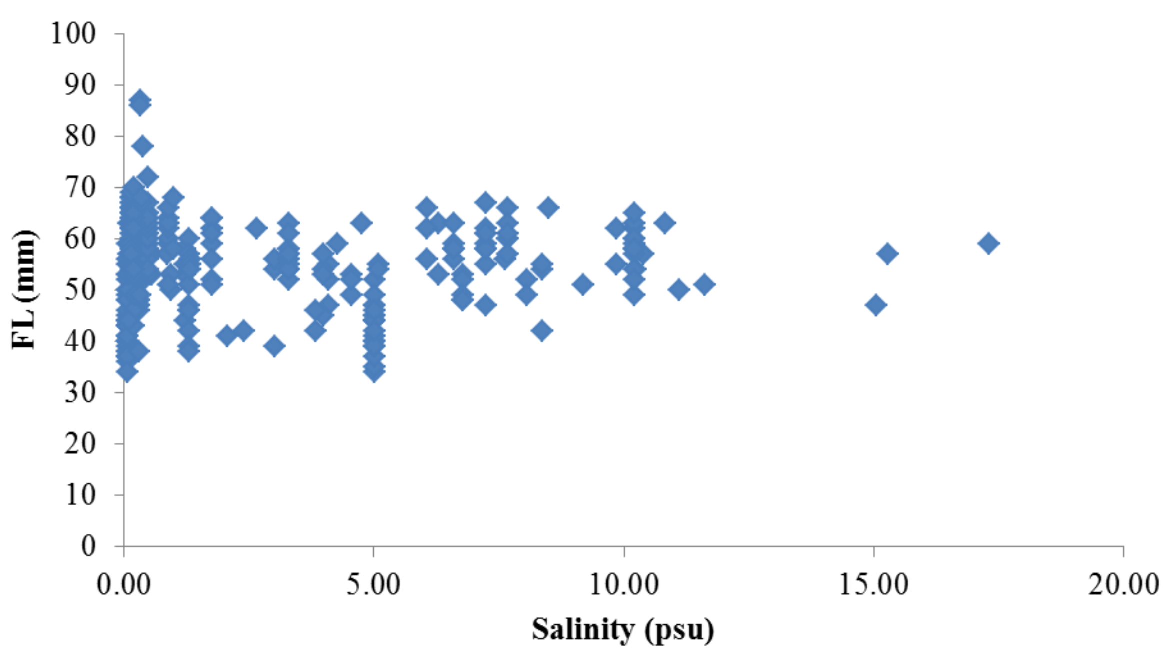
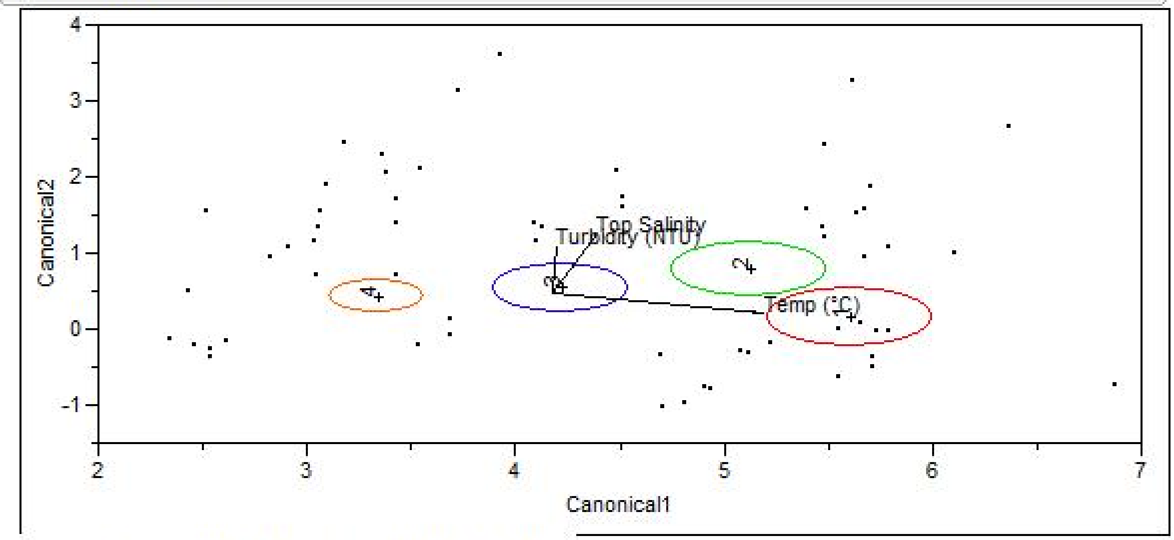


Figure C.3.3. Canonical analysis of Fork Length by temperature, salinity and turbidity of delta smelt collected from August to December 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.79153681	95.9171	95.9171	0.66469543
0.03365994	4.0789	99.9960	0.18045454
0.00003331	0.0040	100.0000	0.00577117

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.5399855	24.8601	9	776.51	<.0001
Pillai's Trace	0.4744172	20.0994	9	963	<.0001
Hotelling-Lawley	0.8252301	29.1276	9	953	<.0001
Roy's Max Root	0.7915368	84.6944	3	321	<.0001

Within Matrix
Between Matrix
Eigenvectors
Scoring Coefs

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Figure C.3.4. Weight (g) of delta smelt collected from August to December 2011.

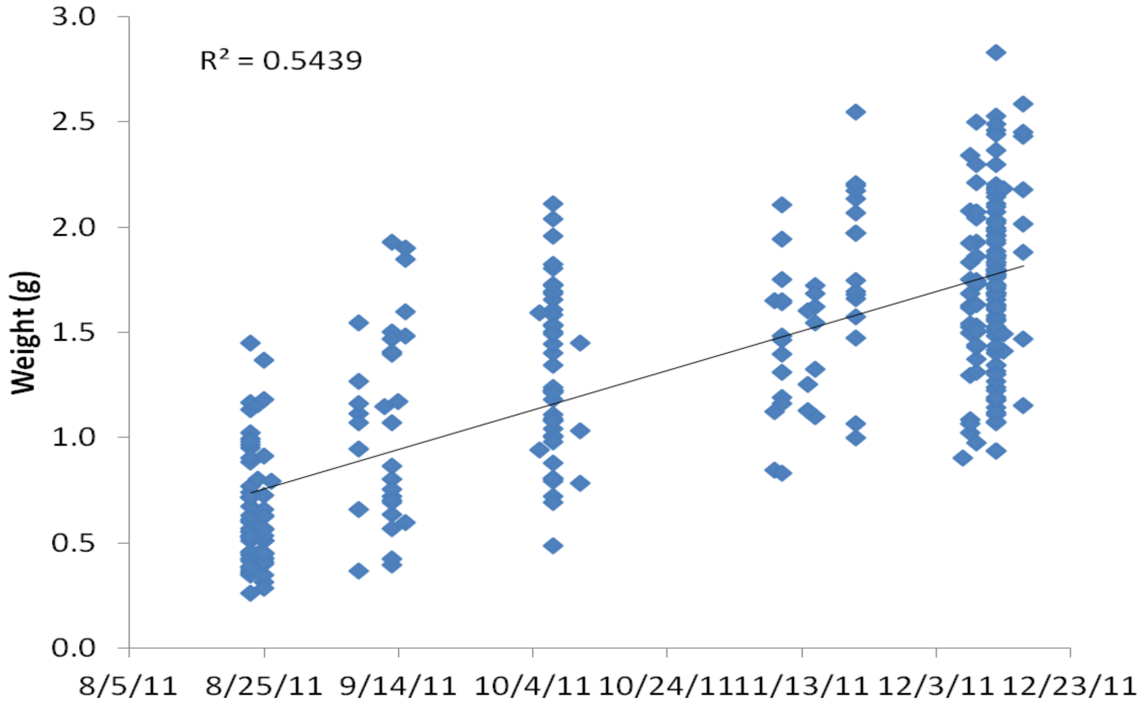


Figure C.3.5. Weight (g) over salinity (psu) of delta smelt collected from August to December 2011.

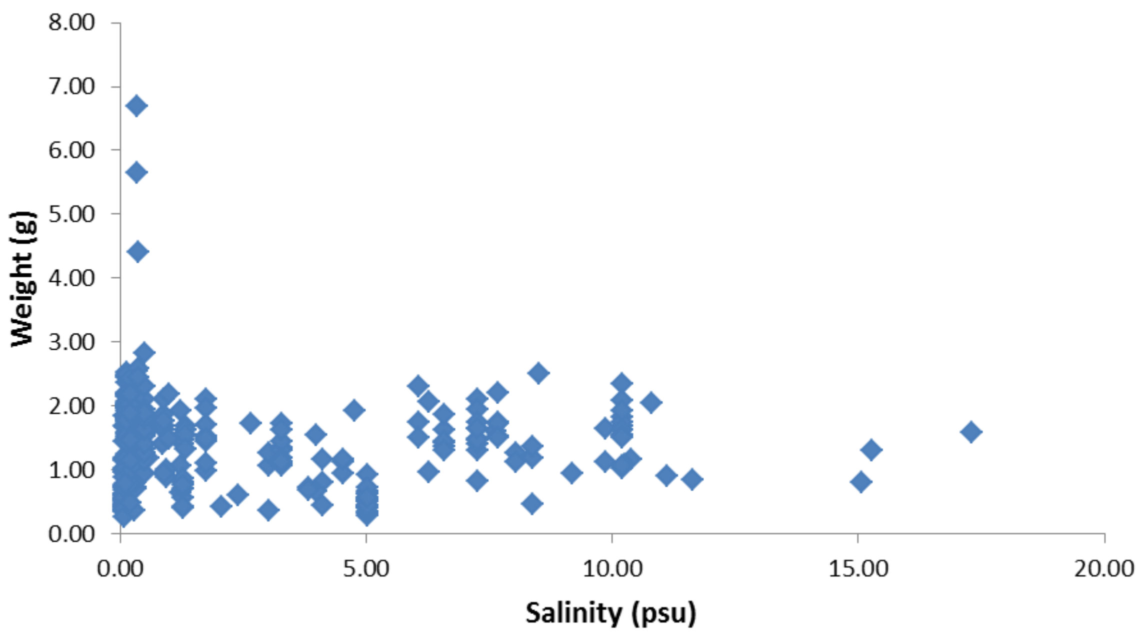


Figure C.3.6. Condition factor (CF) over salinity (psu) of delta smelt collected from August to December 2011.

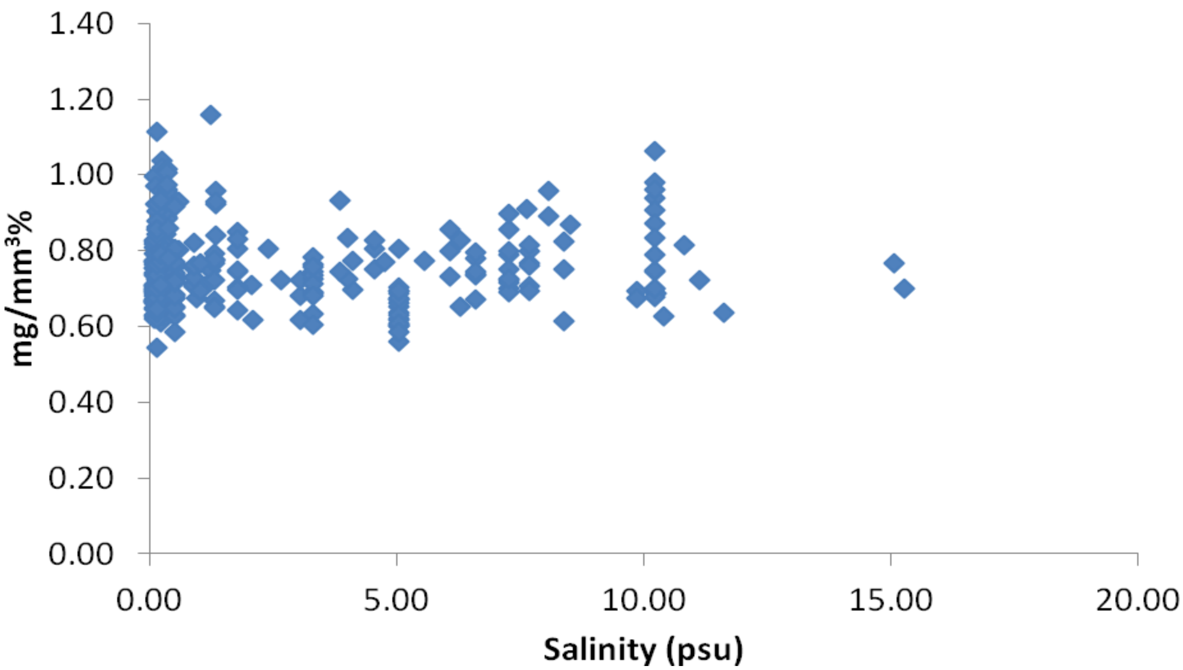


Figure C.3.7. Condition Factor (CF) for delta smelt from Cache/SDWSC (C/S), <1 psu, 1-6 psu, and >6 psu from August to December 2011.

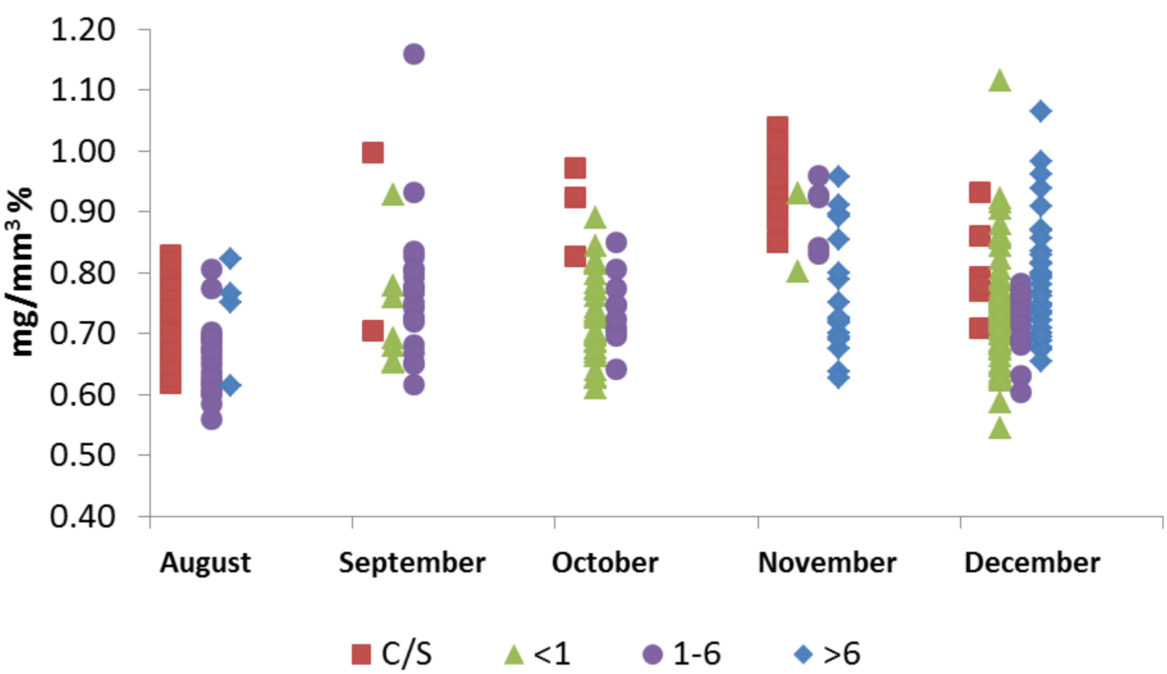


Figure C.3.8. Condition factor (CF) by region of delta smelt collected from August to December 2011. Each bar represents the mean ± standard deviation.

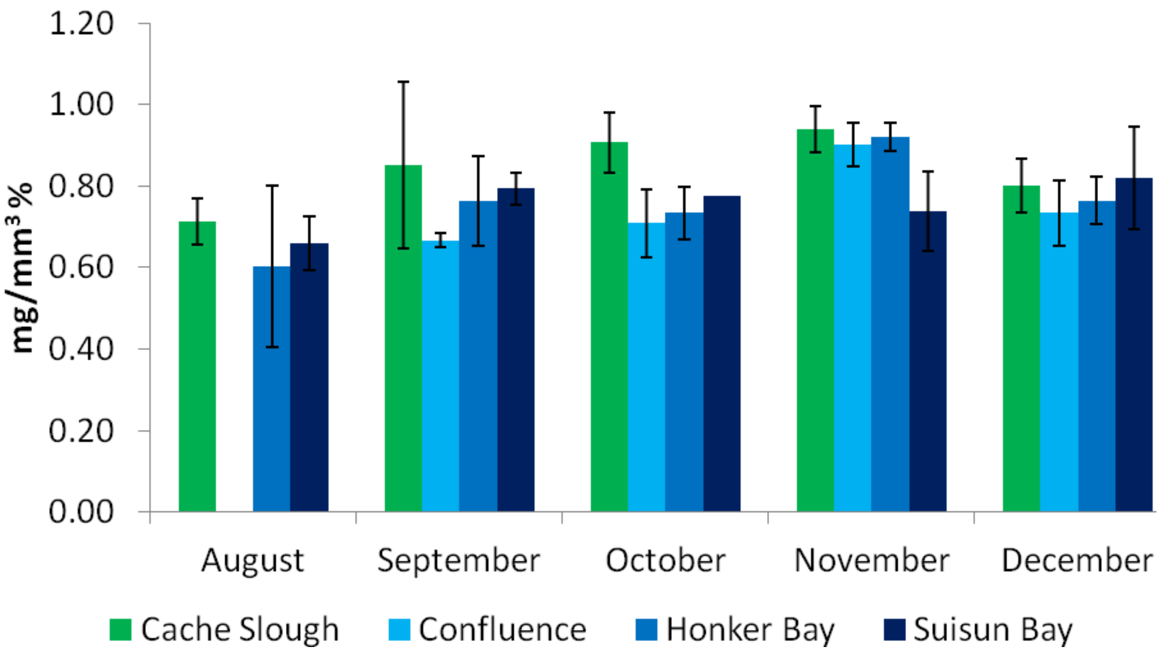
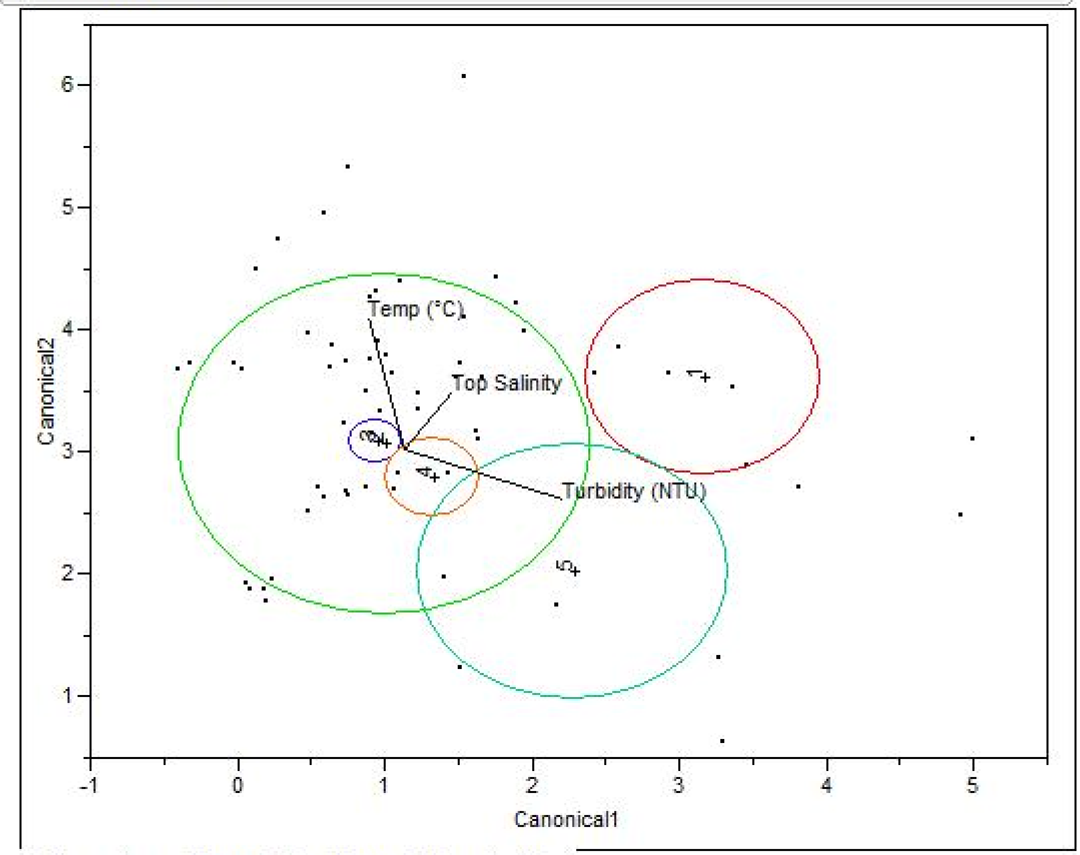


Figure C.3.9. Canonical analysis for Condition Factor by temperature, salinity and turbidity of delta smelt collected from August to December 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.21869784	80.7771	80.7771	0.42361782
0.04953578	18.2963	99.0734	0.21725055
0.00250861	0.9266	100.0000	0.05002329

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.7798635	6.9108	12	841.64	<.0001
Pillai's Trace	0.2291522	6.6161	12	960	<.0001
Hotelling-Lawley	0.2707422	7.1446	12	950	<.0001
Roy's Max Root	0.2186978	17.4958	4	320	<.0001

Within Matrix

Between Matrix

Eigenvectors

Scoring Coefs

Figure C.3.10. Hepatosomatic index (HSI) over salinity (psu) of delta smelt collected from August to December 2011.

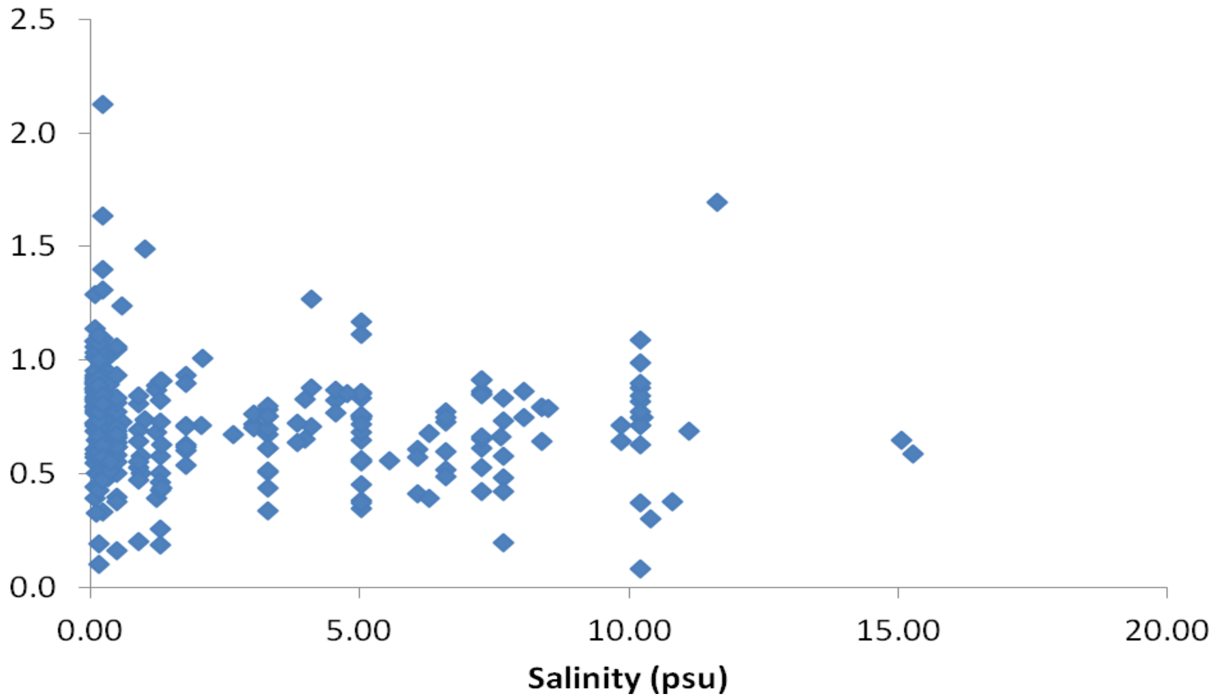


Figure C.3.11. Histosomatic index (HSI) for Cache/SDWSC (C/S), <1 psu, 1-6 psu, and >6 psu for delta smelt from August to December.

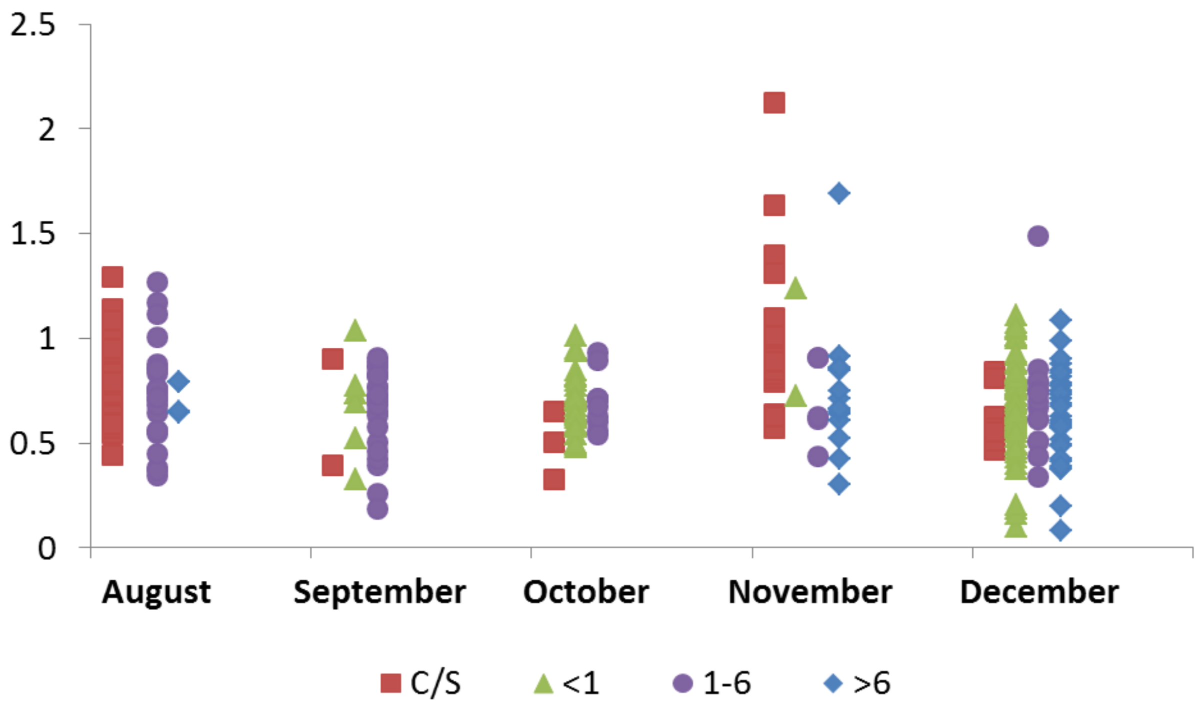


Figure C.3.12. Hepatosomatic index (HSI) by region of delta smelt collected from August to December 2011. Each bar represents the Mean ± Standard Deviation.

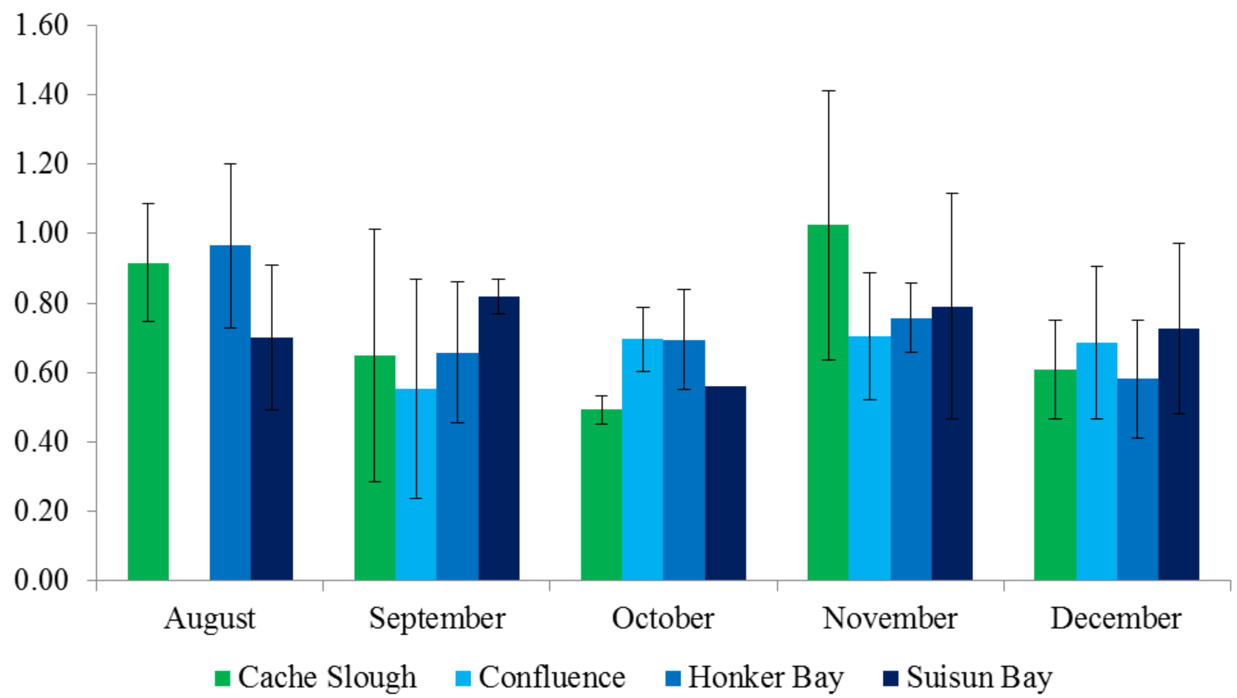
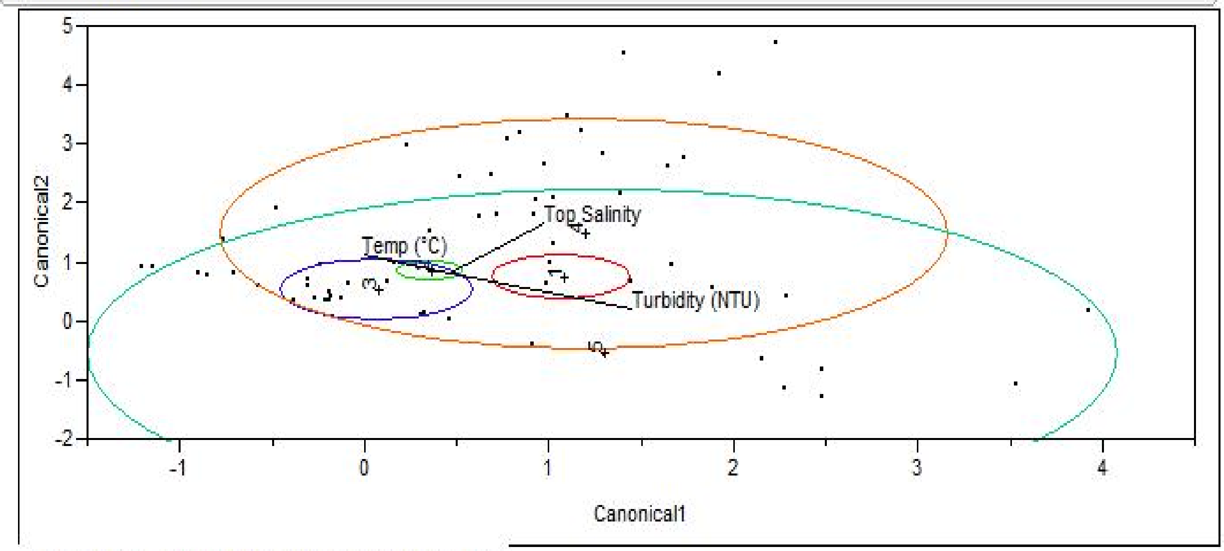


Figure C.3.13. Canonical analysis for Hepatosomatic Index by temperature, salinity and turbidity of delta smelt collected from August to December 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.09018067	83.0221	83.0221	0.28761232
0.01690141	15.5598	98.5819	0.12892052
0.00154041	1.4181	100.0000	0.03921781

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.9006462	2.8207	12	838.99	0.0008
Pillai's Trace	0.1008794	2.7750	12	957	0.0010
Hotelling-Lawley	0.1086225	2.8574	12	947	0.0007
Roy's Max Root	0.0901807	7.1919	4	319	<.0001

Within Matrix
Between Matrix
Eigenvectors
Scoring Coefs

Figure C.3.14. Gonadosomatic index (GSI) over salinity (psu) of delta smelt collected from August to December 2011.

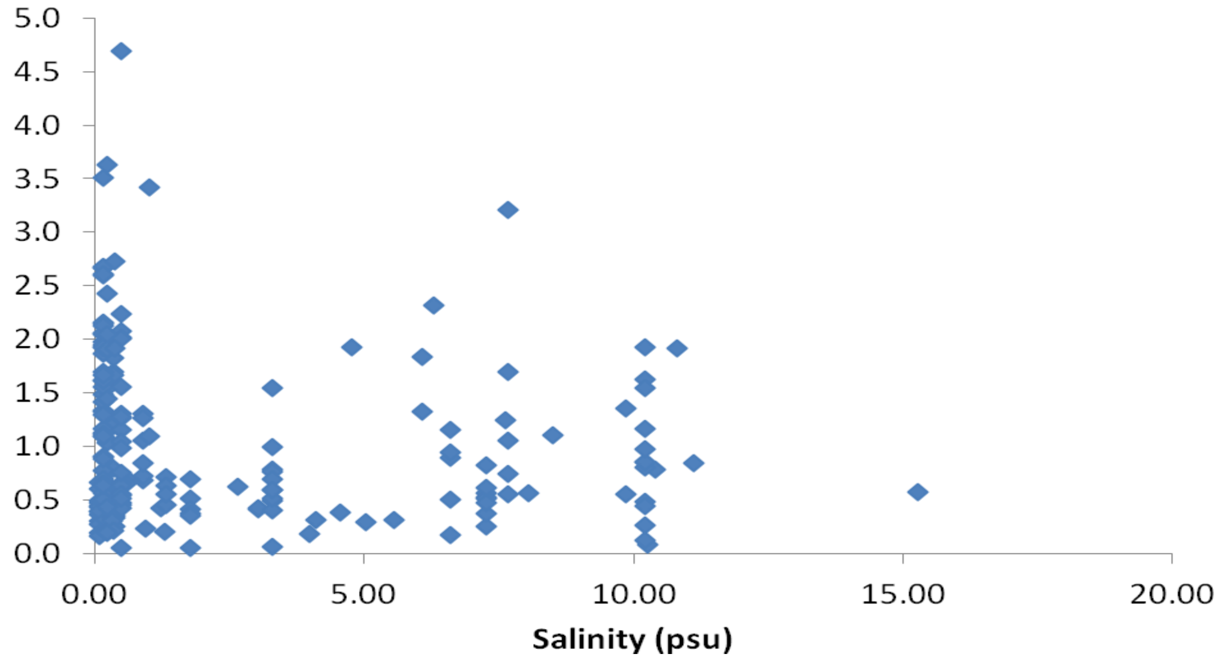


Figure C.3.15. Gonadosomatc index (GSI) of delta smelt collected from August to December 2011.

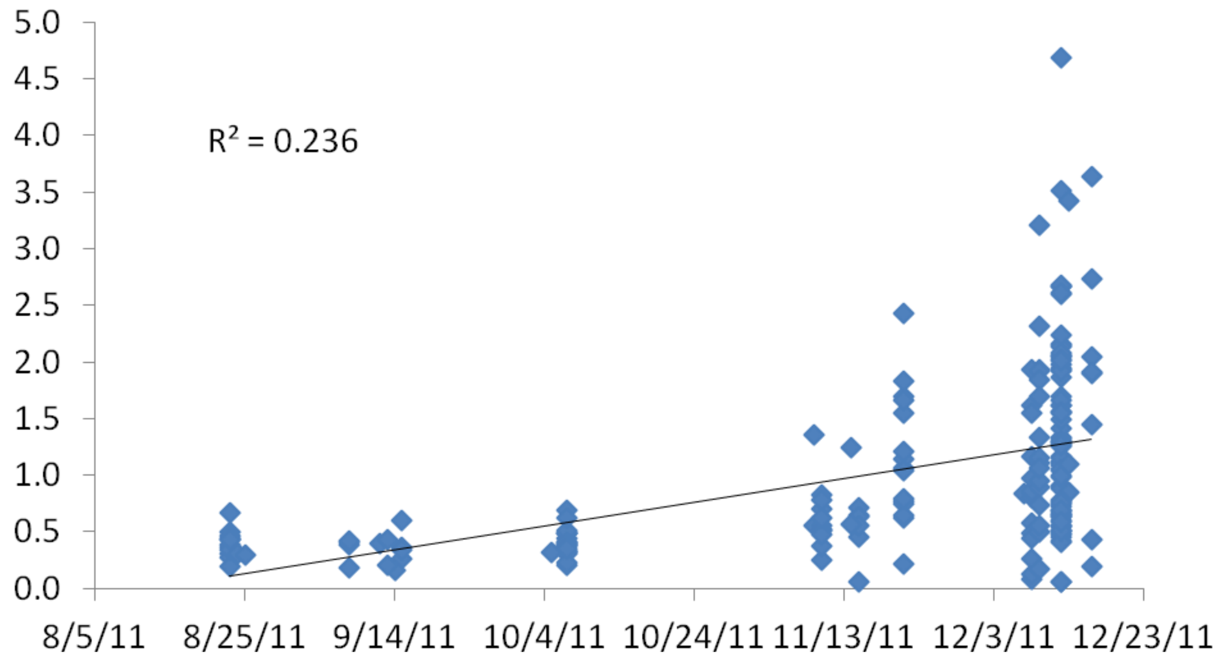


Figure C.3.16. Average gonadosomatic index (GSI) for Cache/SDWSC (C/S), <1 psu, 1-6 psu, and >6 psu for delta smelt from August to December.

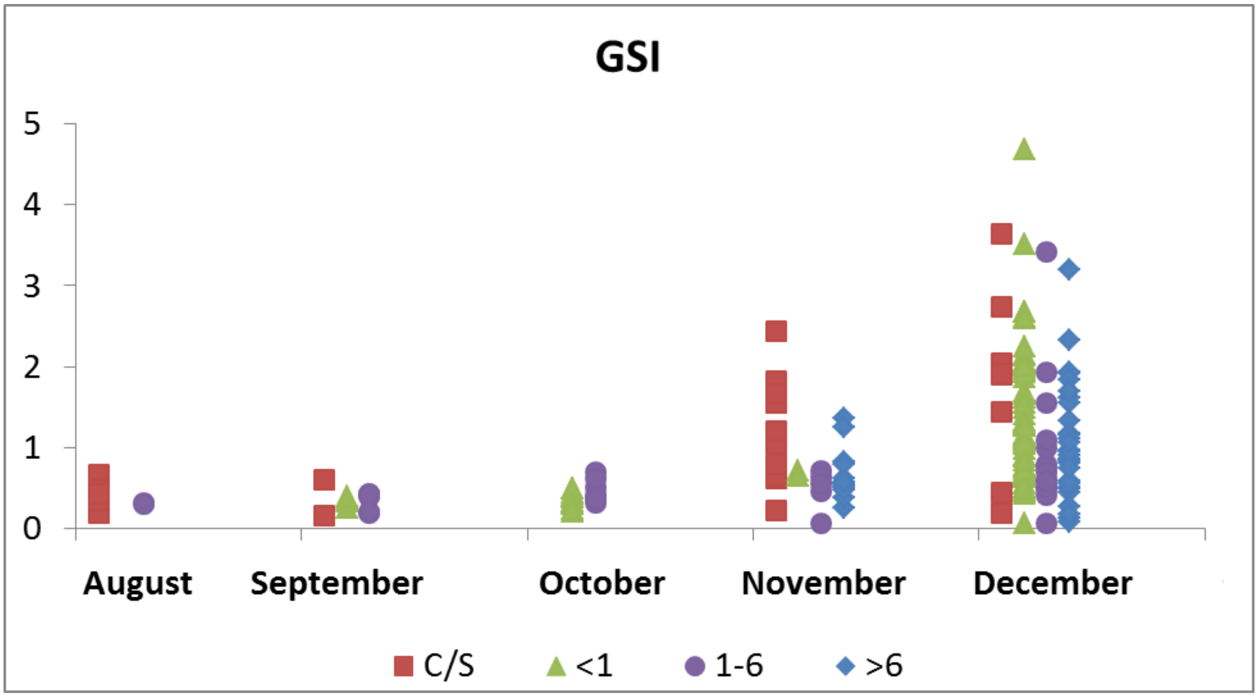


Figure C.6.17. Gonadosomatc index (GSI) by region of delta smelt collected from August to December 2011. Each bar represents the Mean ± Standard Deviation.

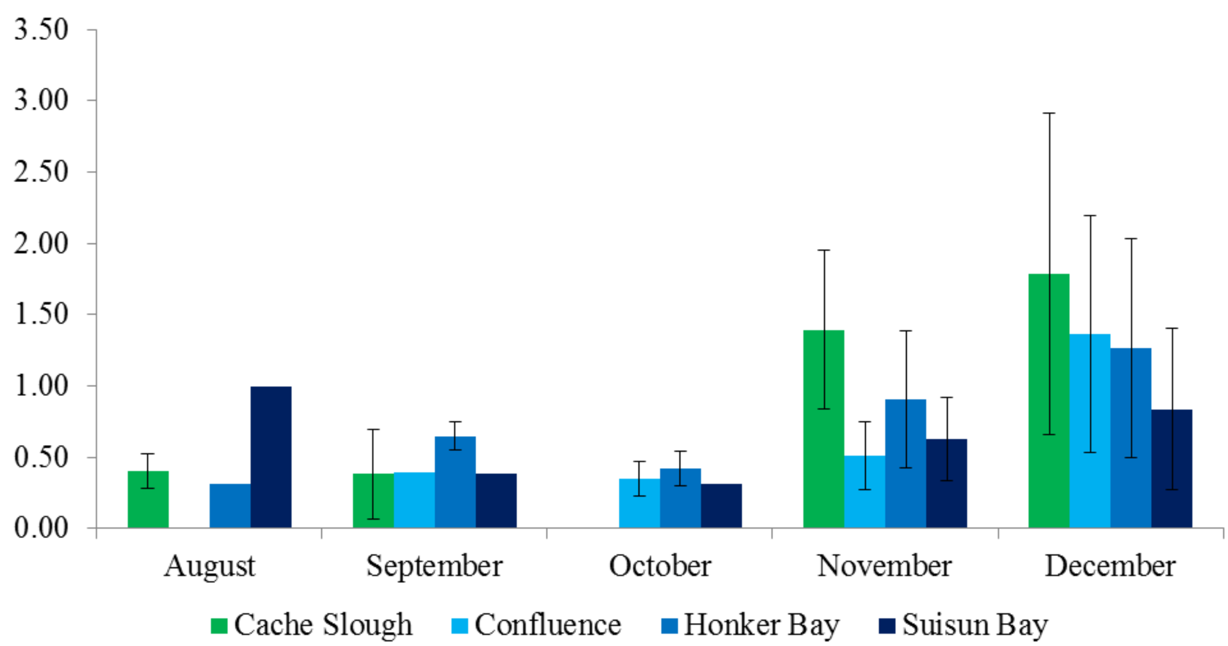
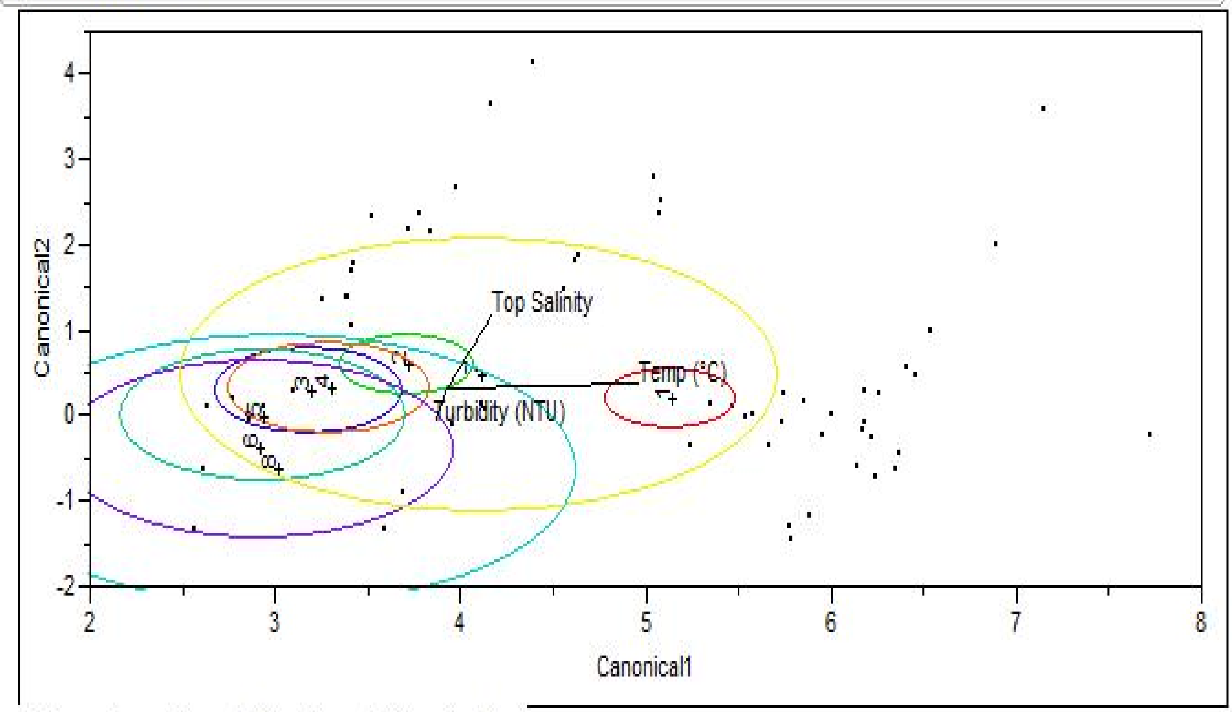


Figure C.6.18. Canonical analysis for Gonadosomatic Index by temperature, salinity and turbidity of delta smelt collected from August to December 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.72882589	86.9638	86.9638	0.64928634
0.06816588	8.1336	95.0974	0.25261793
0.04108738	4.9026	100.0000	0.19866008

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.5201431	6.7178	21	551.87	<.0001
Pillai's Trace	0.5248544	5.8768	21	582	<.0001
Hotelling-Lawley	0.8380791	7.6092	21	572	<.0001
Roy's Max Root	0.7288259	20.1989	7	194	<.0001

Within Matrix

Between Matrix

Eigenvectors

Scoring Coefs

C-4. Nutritional Analysis

The nutritional status of the delta smelt was characterized by measuring the concentrations of triglycerides (TAG) and determining the RNA/DNA ratio in the muscle. TAG is stored energy that is critical for growth, maturation and overwintering (Adams, 1999) while the RNA/DNA ratio indicates the capacity for short term growth and nutritional status of fish (Buckley et al., 1999). TAG concentration in muscle was measured as described by Lenz et al. (2011) utilizing the enzymatic Adipogensis Assay Kit (BioVision, CA). Protein concentration was measured by the Lowry Method (Lowry et al., 1951) and the TAG concentration was reported in millimoles of triglyceride per mg of protein in the muscle. For RNA/DNA ratio, muscle nucleic acids were measured by an ethidium bromide fluorometric technique (Caldarone et al., 2001). Sample protein was dissociated from nucleic acid and the fluorophore ethidium bromide (DNA staining dye) was used to measure total nucleic acids. RNAse was added to digest RNA to differentiate RNA concentration and DNA concentration. All TAG and RNA/DNA were reported in Tables C.4.1-5. For RNA/DNA, some sites were not reported (Table C.4.1-2) due to insufficient sample and reported as N/A.

Univariate analysis indicated that there was no significant correlation between salinity or region with TAG (Fig.C.4.1-3) and RNA/DNA ratio (Fig. C.4.5-7). Although there was no relationship between TAG and temperature, there was a negative correlation between temperature and RNA/DNA ratio (Fig. C.4.6). As an indicator of short term growth and nutritional status this result suggests that fish were good nutritional status and had greater capacity for growth at low temperatures.

When using canonical analysis on nutritional data, the results show that a combination of temperature, salinity and turbidity had significant effect on the RNA/DNA ratios of delta smelt (Fig. C.4.4) but not TAG (Fig C.4.9). For RNA/DNA, the result further suggests that a combination of three factors and no single factor significantly drives the short term nutritional status of delta smelt. While for TAG, the environmental factors measured may not have a n effect since they may not reflect the entire life history of exposure. Further analysis is needed to determine the magnitude of the effect of these three factors and at what range is optimal for delta smelt. Threshold limits for TAG and RNA/DNA for delta smelt will be determined in the future with additional yearly sampling.

Table C.4.1. Nutritional data of delta smelt collected during the Summer Tow Net Survey in August 2011. Indices of RNA/DNA and triglyceride concentration were reported as Means ± Standard Deviation. N/A represents no data was available.

Site	n	Date	RNA/DNA	TAG
418	3	8/25/11	0.85	13.23 ± 7.57
602	20	8/25/11	1.29 ± 0.82	23.01 ± 3.17
716	38	8/23/11	0.85 ± 0.28	14.76 ± 13.78
797	4	8/23/11	N/A	7.80 ± 2.86

Table C.4.2. Nutritional data of delta smelt collected during the Summer Tow Net Survey in September 2011. Indices of RNA/DNA and triglyceride concentration were reported as Means ± Standard Deviation. N/A represents no data was available.

Site	n	Date	RNA/DNA			TAG		
405	5	9/8/11	N/A			16.43		
411	2	9/8/11	N/A			10.59		
413	3	9/8/11	N/A			10.83		
501	1	9/8/11	N/A			7.91		
502	4	9/8/11	N/A			19.61		
503	3	9/8/11	N/A			13.53	±	1.29
507	5	9/15/11	0.86	±	0.45	25.03	±	14.67
512	1	9/13/11	0.67			23.74		
515	2	9/15/11	1.40	±	0.72	15.26	±	3.61
516	1	9/13/11	0.19			7.41		
518	4	9/13/11	0.50	±	0.25	20.13	±	11.80
519	9	9/13/11	1.16	±	0.96	18.39	±	15.00
715	2	9/14/11	0.64	±	0.07	15.73	±	8.28
802	1	9/12/11	0.42			15.31		

Table C.4.3. Nutritional data of delta smelt collected during the Tow Net Survey in October 2011. Indices of RNA/DNA and triglyceride concentration were reported as Means ± Standard Deviation.

Site	n	Date	RNA/DNA			TAG		
505	1	10/5/11		1.30			14.14	
507	24	10/7/11	1.15	±	0.30	16.15	±	1.49
508	2	10/7/11	1.12	±	0.27	19.33	±	11.43
509	3	10/7/11	1.21	±	0.04	17.26	±	1.74
510	1	10/7/11		0.89			22.90	
511	1	10/7/11		0.88			16.82	
517	1	10/7/11		1.34			19.44	
518	7	10/7/11	1.07	±	0.38	17.64	±	4.12
519	2	10/7/11	1.33	±	0.01		16.92	
721	3	10/11/11	1.30	±	0.23	16.25	±	1.93

Table C.4.4. Nutritional data of delta smelt collected during the Tow Net Survey in November 2011. Indices of RNA/DNA and triglyceride concentration were reported as Means ± Standard Deviation.

Site	n	Date	RNA/DNA			TAG		
407	1	11/9/11		1.95			17.21	
412	2	11/9/11	1.38	±	0.10	20.52	±	2.99
517	2	11/14/11	1.42	±	0.54	12.72	±	4.10
518	1	11/14/11		1.51			22.53	
601	1	11/10/11		1.42			12.10	
606	10	11/10/11	1.62	±	0.23	23.66	±	5.24
609	1	11/10/11		1.91			20.95	
703	1	11/15/11		1.92			11.48	
704	4	11/15/11	1.46	±	0.51	17.98	±	1.96
705	1	11/15/11		1.29			3.66	
719	10	11/21/11	1.99	±	0.29	26.68	±	9.12
797	8	11/21/11	1.71	±	0.38	21.80	±	3.59

Table C.4.5. Nutritional data of delta smelt collected during the Tow Net Survey in December 2011. Indices of RNA/DNA and triglyceride concentration were reported as Means ± Standard Deviation.

Site	n	Date	RNA/DNA	TAG
417	1	12/8/11	0.86	6.23
502	1	12/7/11	1.75	5.78
507	2	12/9/11	2.01	8.29 ± 6.34
509	1	12/9/11	1.81	12.61
516	1	12/9/11	1.92	24.35
517	3	12/9/11	1.56 ± 0.60	10.46 ± 5.58
518	6	12/9/11	1.76 ± 0.15	15.05 ± 2.84
519	6	12/9/11	1.82 ± 0.13	13.89 ± 9.67
605	1	12/8/11	0.93	9.84
606	10	12/8/11	1.90 ± 0.10	23.19 ± 9.98
703	10	12/12/11	1.76 ± 0.18	4.97 ± 2.82
704	6	12/12/11	1.88 ± 0.10	7.30 ± 1.86
705	19	12/12/11	1.71 ± 0.52	10.55 ± 3.95
706	32	12/12/11	1.72 ± 0.20	11.55 ± 4.51
719	6	12/16/11	1.69 ± 0.37	13.68 ± 8.62
797	2	12/16/11	1.49 ± 0.11	21.34 ± 12.99
806	1	12/13/11	1.52	8.74
807	2	12/13/11	1.38 ± 0.31	6.23 ± 0.62

Figure C.4.1. Triglyceride (TAG) versus salinity (psu) of delta smelt collected from August to December 2011.

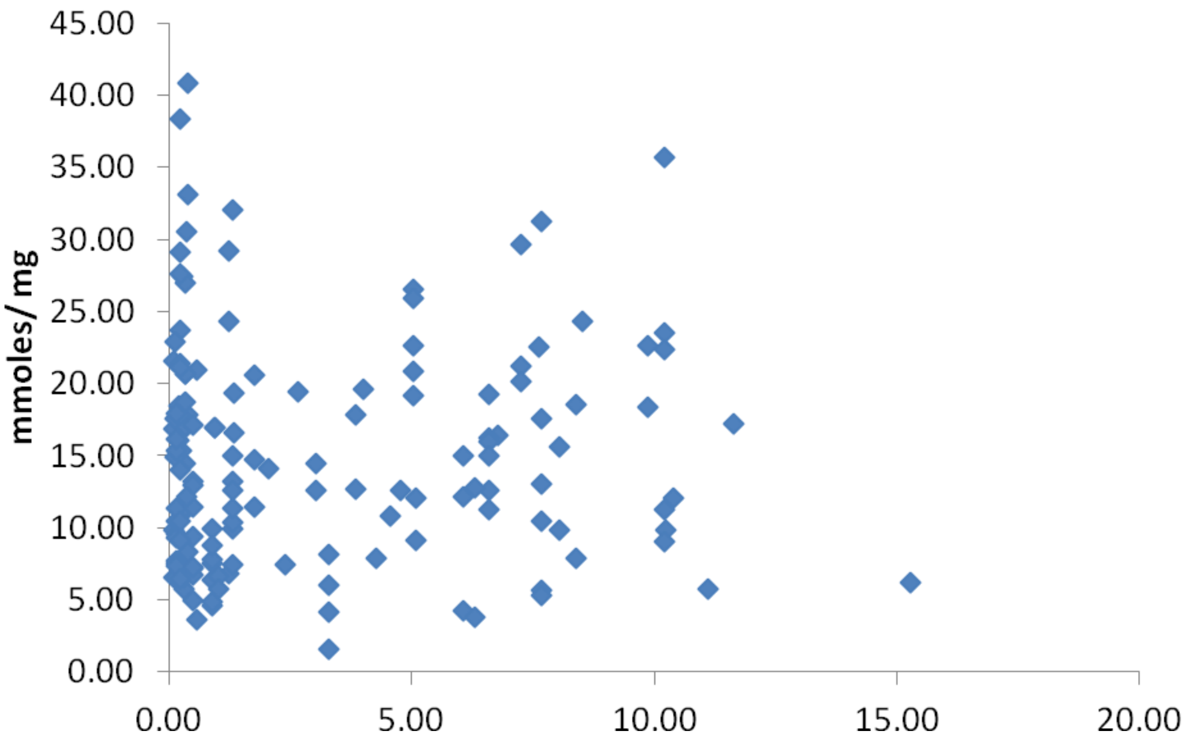


Figure C.4.2. Means ± Standard Deviation of triglyceride (TAG) in delta smelt collected from Cache/SDWSC (C/S), <1 psu, 1-6 psu, and >6 psu between August and December.

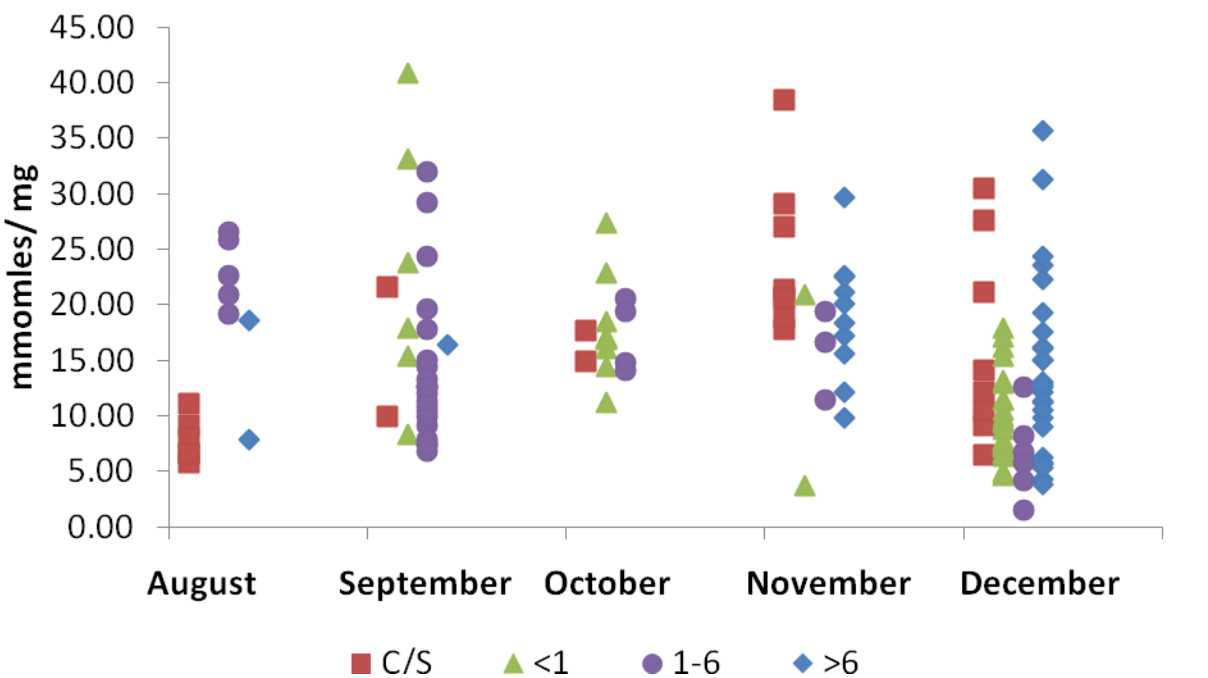


Figure C.4.3. Means ± Standard Deviation of Triglyceride (TAG) by region of delta smelt collected from August to December 2011.

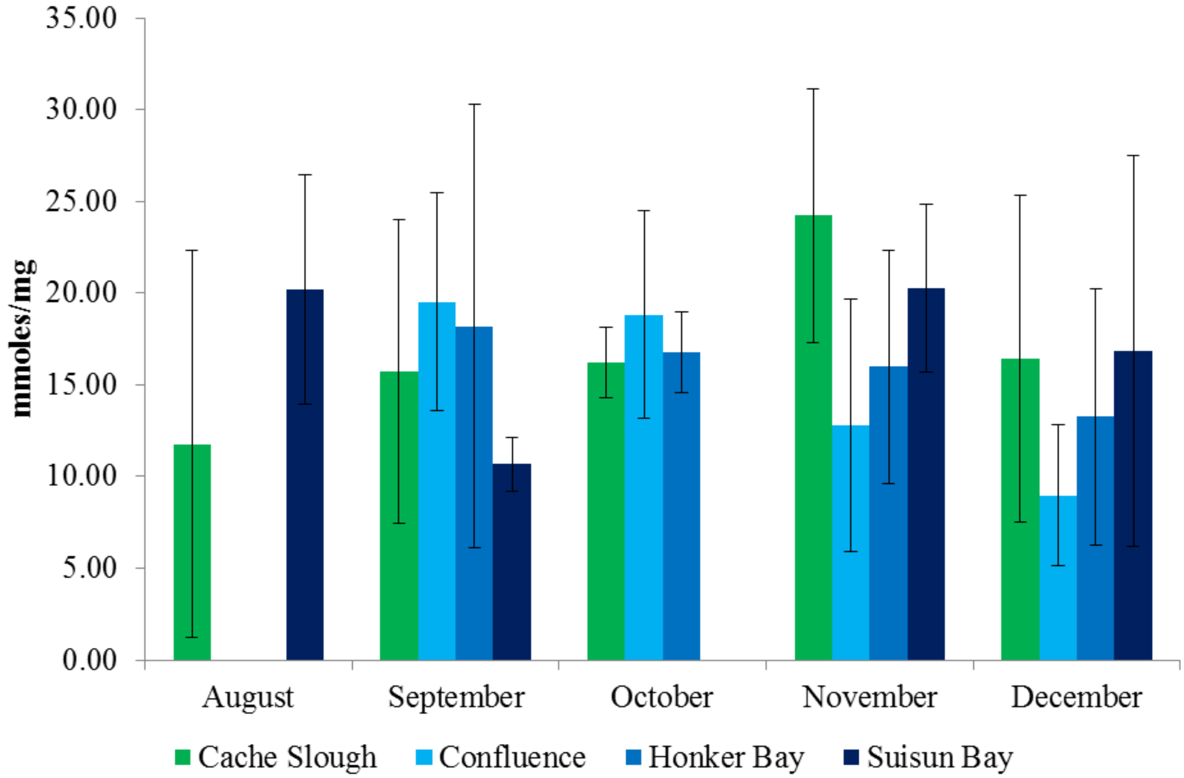
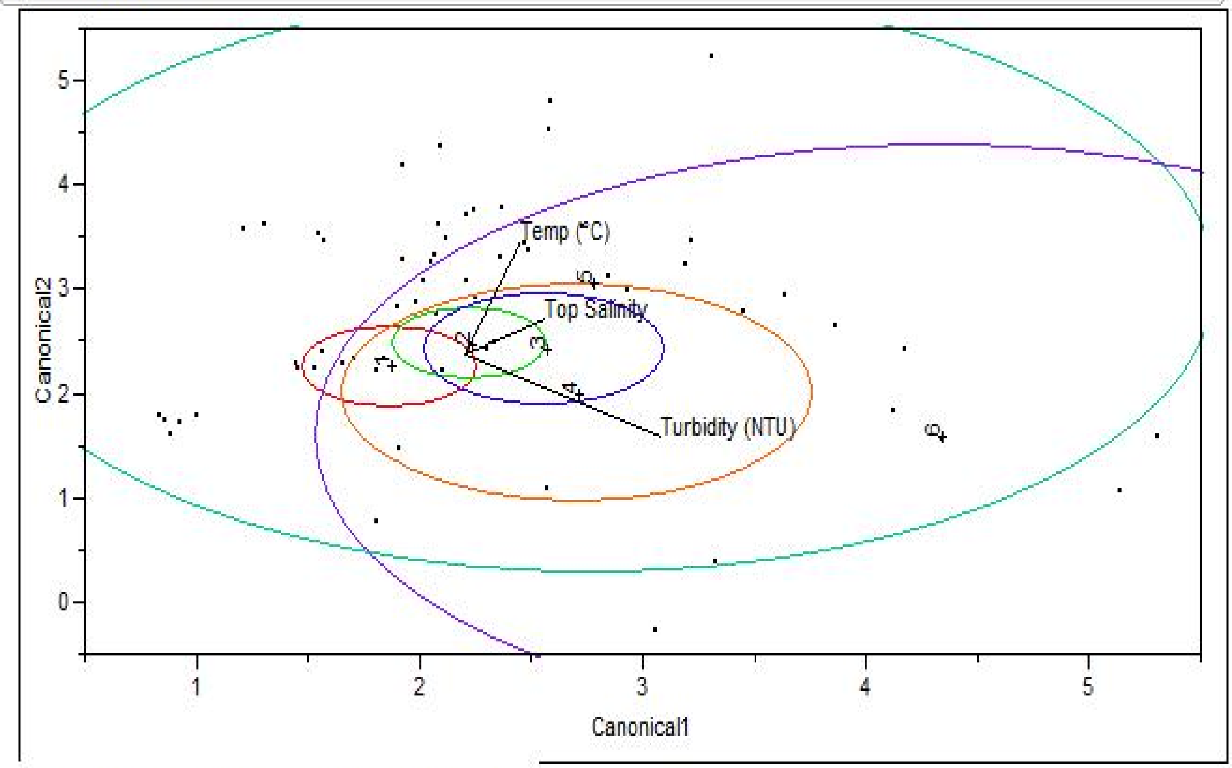


Figure C.4.4. Canonical analysis for triglyceride by temperature, salinity and turbidity of delta smelt collected from August to December 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.10906448	75.0072	75.0072	0.31359077
0.02437397	16.7628	91.7700	0.15425309
0.01196685	8.2300	100.0000	0.10874437

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.869798	1.3654	15	395.16	0.1609
Pillai's Trace	0.1339585	1.3555	15	435	0.1657
Hotelling-Lawley	0.1454053	1.3733	15	425	0.1564
Roy's Max Root	0.1090645	3.1629	5	145	0.0097

Within Matrix
Between Matrix
Eigenvectors
Scoring Coefs

Figure C.4.5. RNA/DNA versus salinity (psu) of delta smelt collected from August to December 2011.

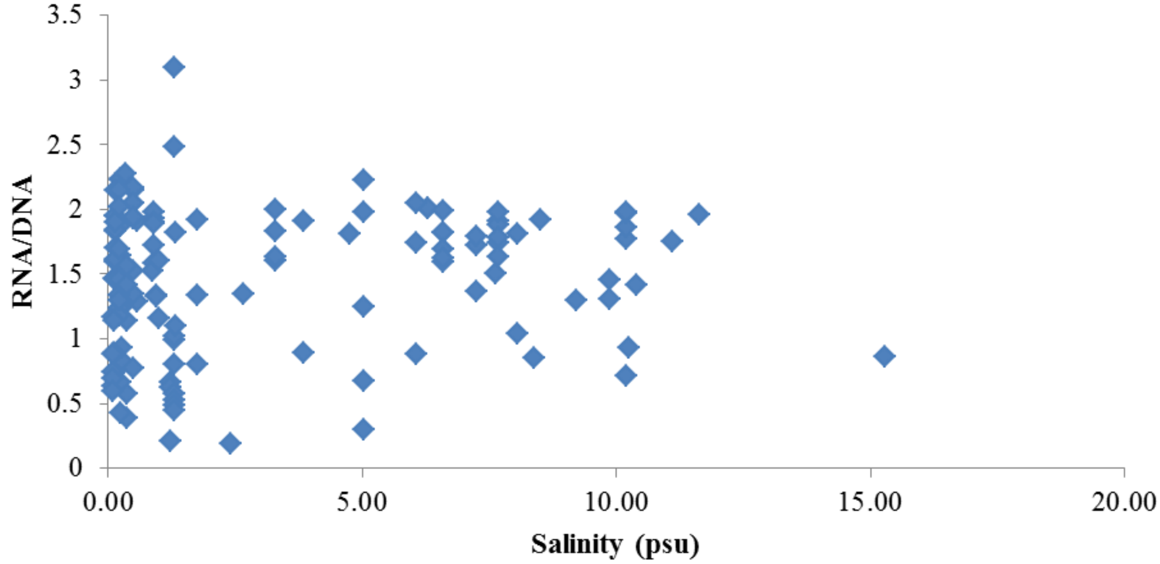


Figure C.4.6. RNA/DNA versus temperature (°C) of delta smelt collected from August to December 2011.

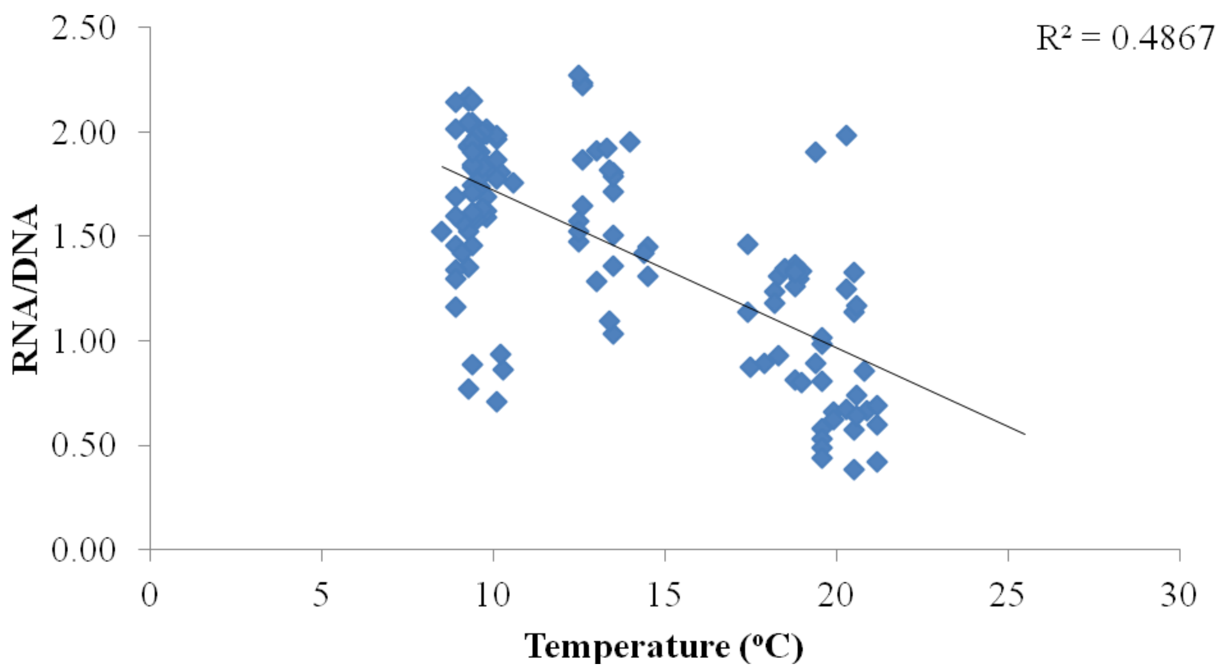


Figure C.4.7. Mean ± Standard Deviation RNA/DNA for Cache/SDWSC (C/S), <1 psu, 1-6 psu, and >6 psu for delta smelt from August to December.

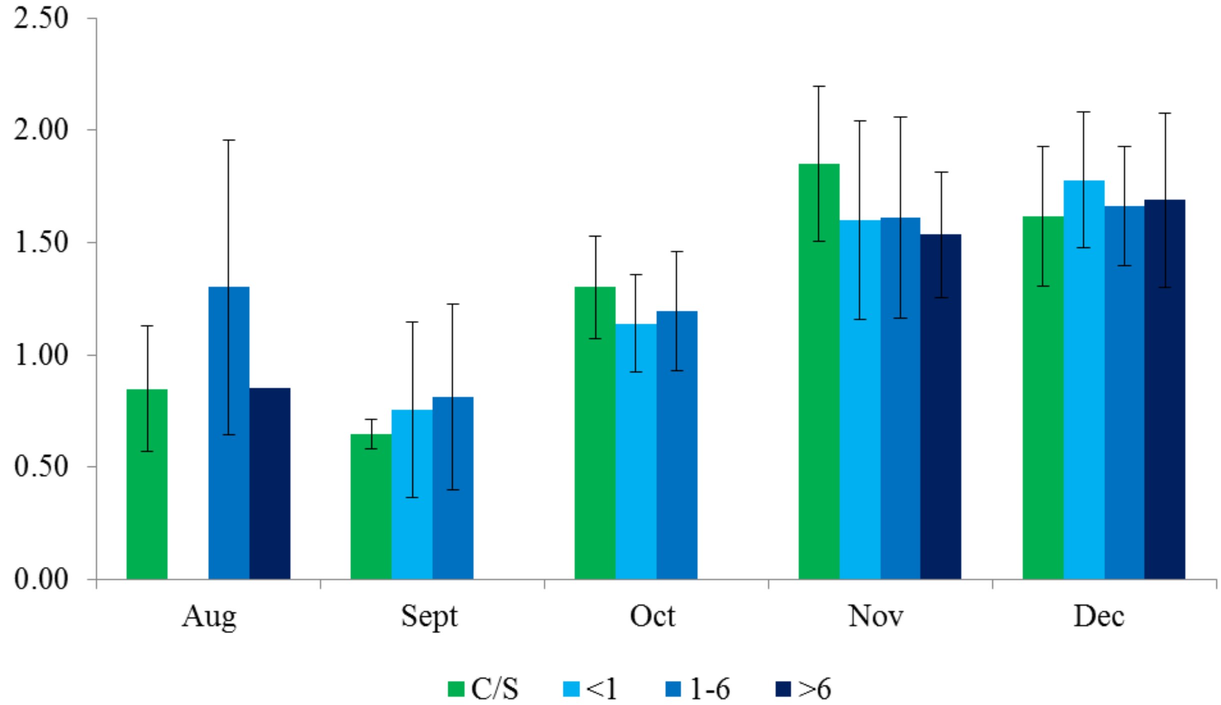


Figure C.4.8. RNA/DNA by region of delta smelt collected from August to December 2011. Each bar represents the mean with error bars of standard deviation.

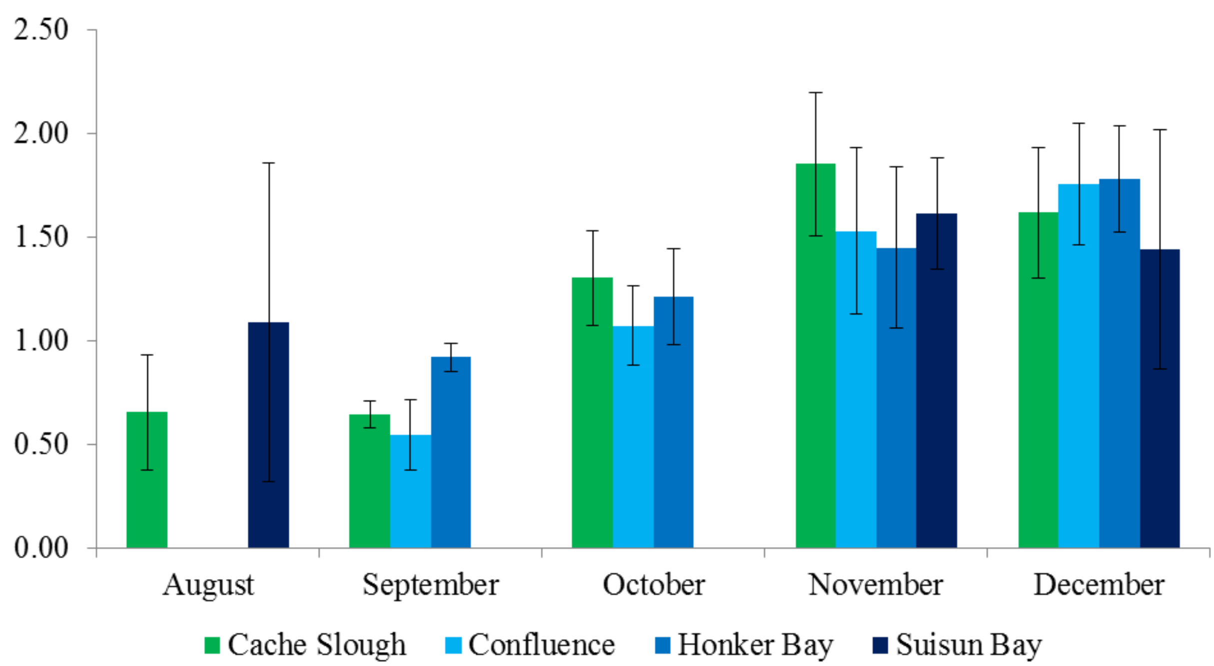
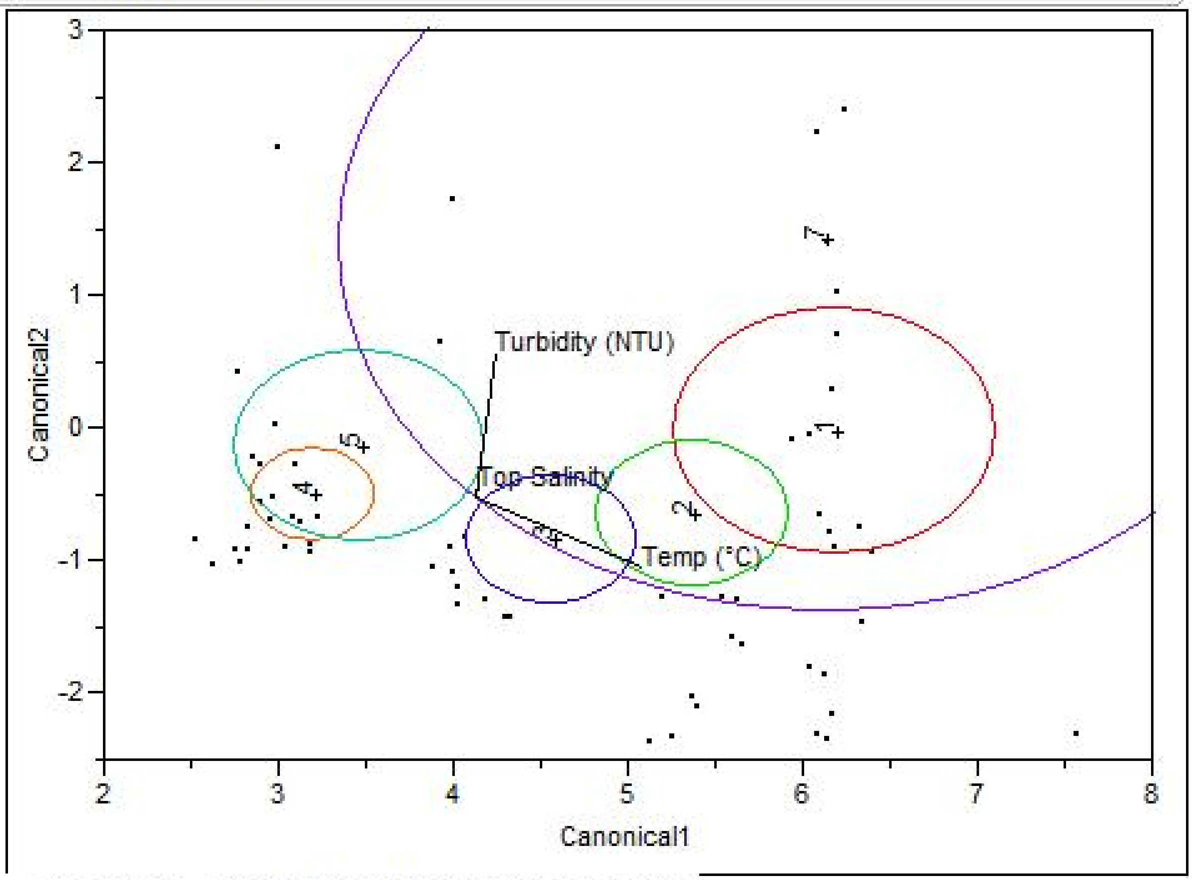


Figure C.4.9. Canonical analysis for RNA/DNA by temperature, salinity and turbidity of delta smelt collected from August to December 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
1.06532973	89.9193	89.9193	0.71820318
0.08674741	7.3219	97.2412	0.2825296
0.03268532	2.7588	100.0000	0.17790672

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.4314336	8.7882	15	370.32	<.0001
Pillai's Trace	0.6272896	7.1910	15	408	<.0001
Hotelling-Lawley	1.1847625	10.4786	15	398	<.0001
Roy's Max Root	1.0653297	28.9770	5	136	<.0001

Within Matrix

Between Matrix

Eigenvectors

Scoring Coefs

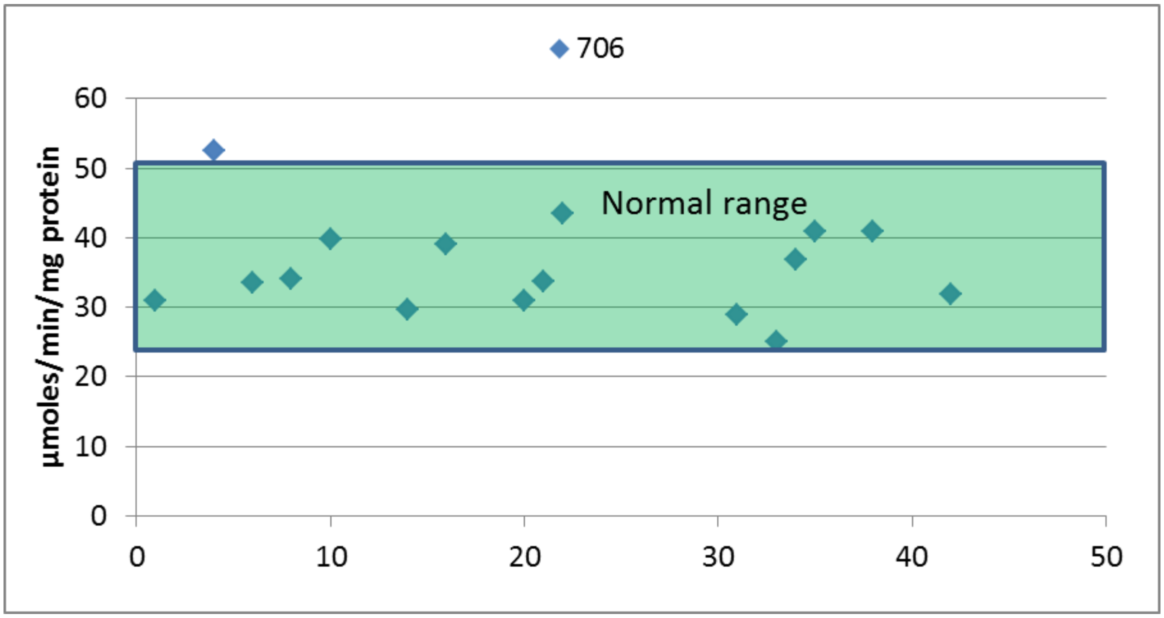
C-5. Enzymatic Biomarkers

C5-1 Acetylcholinesterase (AChE) is an enzyme found mainly at the neuromuscular junction and cholinergic synapses in the central nervous system of animals, and its activity serves to terminate synaptic transmission (Greig-Smith 1991; Zinkl et al. 1991). Exposure to OP pesticides, carbamates and metal contamination can be determined using this biomarker. The exposure of the animal to OP pesticides causes depression in AChE activity. Fulton and Key (2001) stated that, given the variability of individual AChE levels, a depression of 13% is often necessary to be indicative of effect of OPs. Recovery of delta smelt is possible after a period of time depending on the level of depression.

Due to time constraints, only121 brains were analyzed for AChE activity. The methodology of Ellman et al. (1961) was followed with modification to optimize the assay specific to delta smelt at the start of the project. The enzyme activity was normalized to protein concentration, where protein was determined according to Lowry method (Lowry et al., 1951). From our preliminary results, the threshold limit of AChE activity for delta smelt was estimated to be between 24-52 µmoles/min/mg protein, which was similar to the AChE activity in the wild strain zebrafish (Yang et al., 2011). There is no other previously available AChE data for Delta smelt. In this study, we found an abnormal decrease and increase in AChE activity, indicating potential exposure due to OP pesticides, carbamates or metal exposure. Sant'Anna et al., (2011) demonstrated an increase in AChE activity of zebrafish exposed to iron.

The AChE activity for each location is summarized in Table C.5.1. Delta smelts collected from sites 705, 706, and 716 had normal AChE activities while fish collected from 412,418,507 had low activity. This is shown in Fig C.5.1 by comparing the site 706 (fish collected in December) with site 507 (fish collected in Sep, October). This indicate that the fish has been exposed to contaminants such as OP, metals either from 412, 418,507 or exposed to contaminants at some other location and moved in recently to that location. With the available data for AChE activity for the fish collected in different locations, 705,706,716 may be the potential site for delta smelt to recuperate from contaminants or potential sites for spawning. Based on the threshold concentration range of 24-52 µmoles/min/mg protein estimated for delta smelt, 59% of fish were considered normal with AChE activity regardless of salinity concentration in 2011(Fig C.5.2).Univariate analysis indicates that there were no significant correlations between salinity, turbidity, secchi depth or site on AChE activity. When subjected to canonical analysis (Fig. C.5.3), it is shown that a combination of temperature, salinity and turbidity had significant effect on AChE activity Thus the results suggest that the three factors are important to show the significance of enzyme activity in delta smelt and also contribute to the effect of contaminants. Furthermore, these results can be strengthened by conducting further contamination studies using other enzymatic biomarkers, such as detoxification cytochrome P450 or ethoxyresorufin-O-deethylase (EROD) enzymes.

Figure C.5.1. Comparison of AChE activity for the delta smelt collected in 706 (n=16) and 507 (n=20).



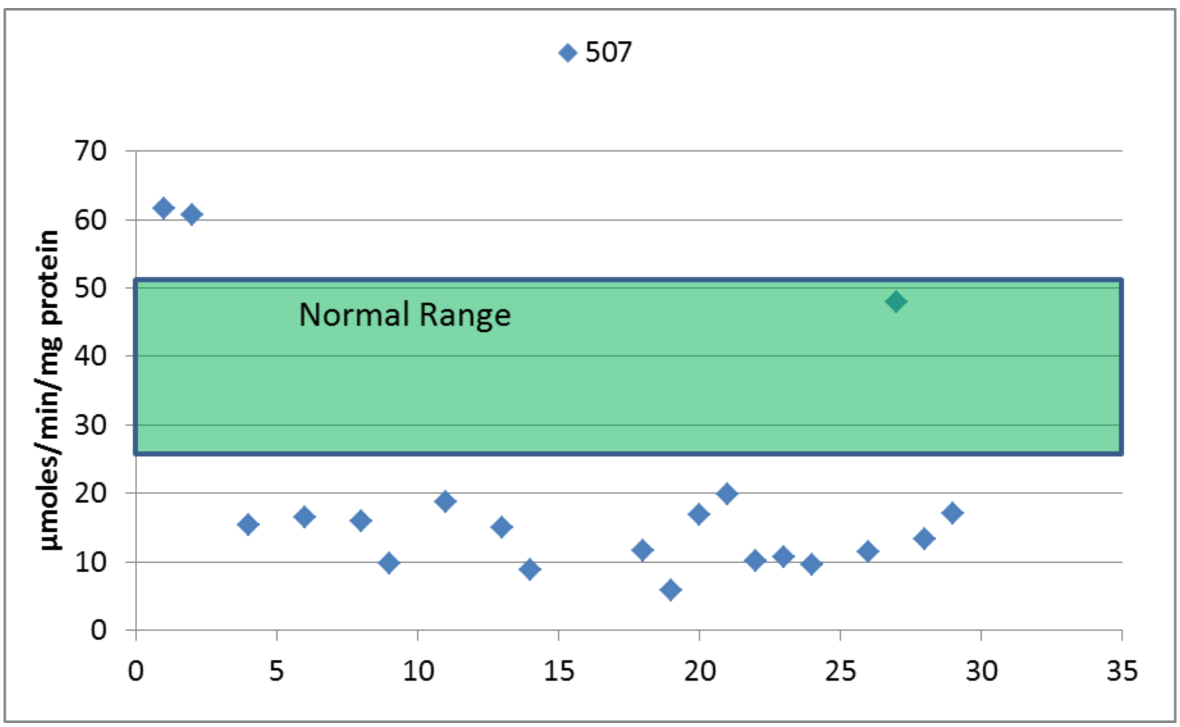


Table. C.5.1. AChE activity of delta smelt collected from August to December 2011 were categorized: Good (Normal AChE activity), Bad (inhibition and abnormal increase in AChE activity) and reported as Means ± Standard Deviation. Normal range: 24-52 µmoles/min/mg protein.

Site	Month	AChE activity (µmoles/min/mg protein)					
		Normal		High		Depressed	
		n	Activity	n	Activity	n	Activity
412	November	-	-	-	-	2	17.6 ± 1.5
418	August	-	-	-	-	2	17.7 ± 5.49
507	September	-	-	3	62.14 ± 1.50	1	15.27
	October	1	48	-	-	15	12.89 ± 3.90
518	September	1	28.12	-	-	2	12.6
	October	1	48	-	-	4	15.44 ± 4.47
	December	2	35.65 ± 3.47	-	-	-	-
519	September	4	34.37 ± 2.1	-	-	1	18.03
	October	1	43	-	-	1	17.08
	December	-	-	-	-	2	16.68 ± 2.72
606	November	-	-	-	-	5	20.15 ± 5.1
	December	4	36.40 ± 8.4	-	-	-	-
703	November	-	-	-	-	1	18.56
	December	5	42.8 ± 4.9	-	-	2	10.54 ± 0.42
705	December	10	42.1 ± 8	-	-	2	17.07 ± 5.95
706	December	15	35.48 ± 6.77	-	-	-	-
716	August	4	42.09 ± 6.1	-	-	-	-
719	November	4	31.4 ± 5.4	-	-	4	21.96 ± 5.2
797	November	-	-	-	-	3	19.50 ± 2.58
508	October	1	33.3	-	-	-	-
509	October	-	-	-	-	2	-
515	September	1	29	-	-	-	-
516	December	1	29	-	-	-	-
517	December	4	32.1 ± 12.2	-	-	-	-
721	October	-	-	-	-	1	19.2
602	August	-	-	-	-	3	22
601	November	1	29	-	-	-	-
609	November	1	27.3	-	-	-	-

Figure C.5.2. AChE activity versus different salinity range from August to December 2011.

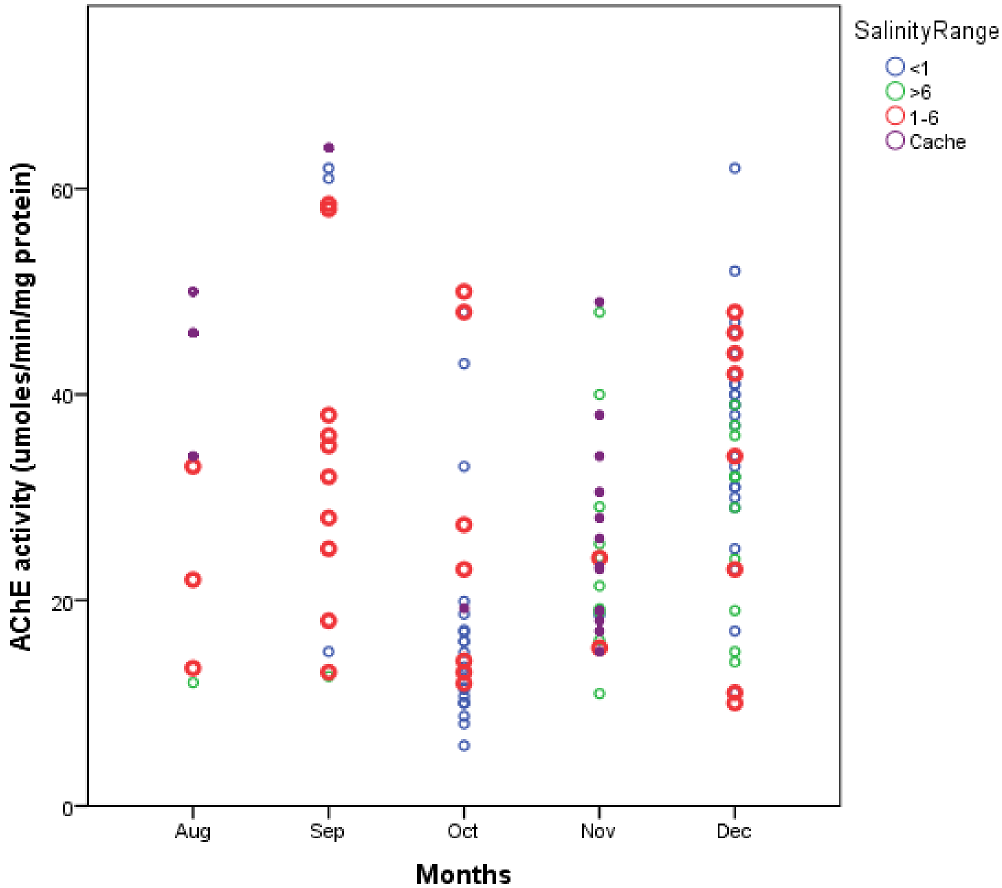
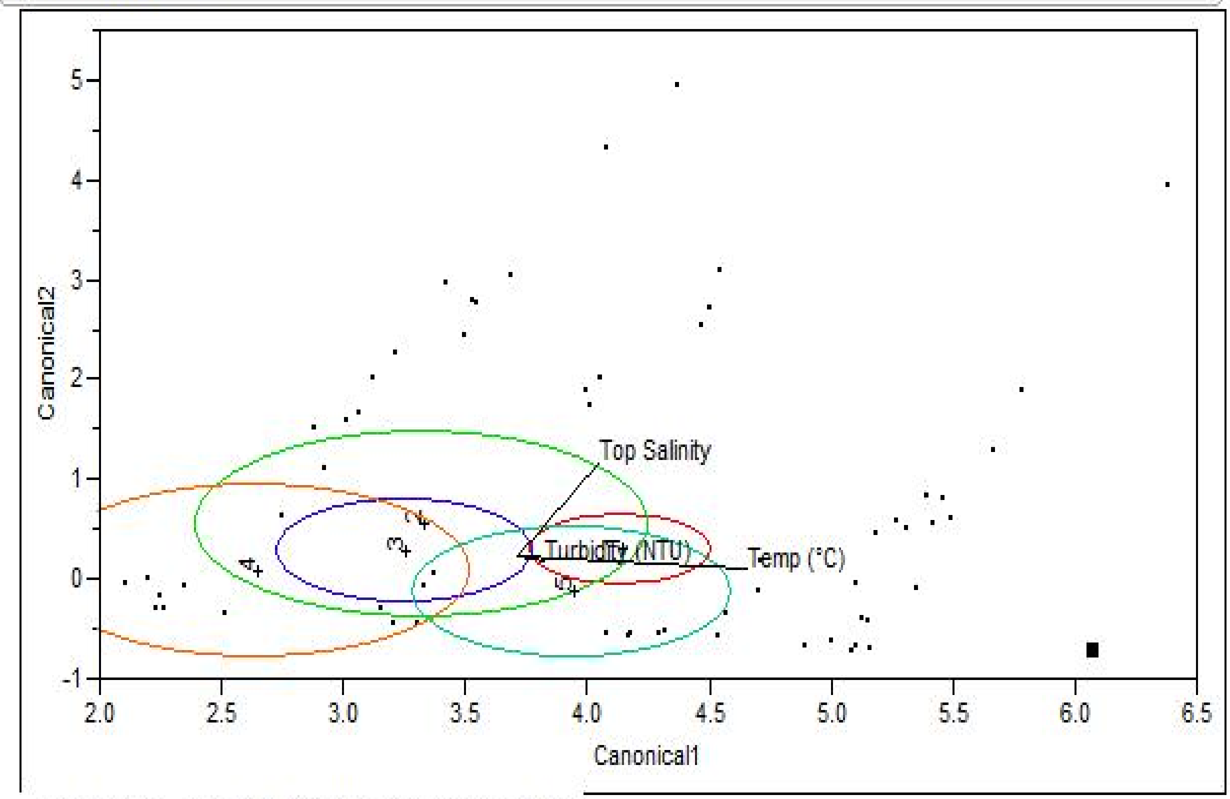


Figure C.5.3. Canonical analysis for AChE by temperature, salinity and turbidity of delta smelt collected from August to December 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.2587676	85.1589	85.1589	0.45340068
0.03251858	10.7017	95.8606	0.1774667
0.0125781	4.1394	100.0000	0.11145336

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.7598503	2.7519	12	301.91	0.0015
Pillai's Trace	0.2494885	2.6305	12	348	0.0022
Hotelling-Lawley	0.3038643	2.8529	12	338	0.0009
Roy's Max Root	0.2587676	7.5043	4	116	<.0001

Within Matrix

Between Matrix

Eigenvectors

Scoring Coefs

C5-2. Sodium-Potassium-Adenosine Triphosphate (Na^+/K^+-ATPase) enzyme mainly helps to maintain resting potential, avail transport and regulate cell volume. The major role of the Na^+/K^+-ATPase is to maintain ion electrochemical gradients across the plasma membrane (Lingwood et al. 2005). Na^+/K^+-ATPase is responsible for cells energy expenditure. The elevated or depressed Na^+/K^+-ATPase activity indicates that the fish is under stress. Factors such as salinity, temperature and turbidity and sudden exposure to certain metal contamination (Ay et al. 1999, Hwang and Tsai 1993, Yoshikawa et al. 1993) can elevate the Na^+/K^+-ATPase activity while contaminants and diseases typically depresses NaK ATPase activity. Na^+/K^+-ATPase activity in gill was evaluated by the method of Holliday (1985) with modification to optimize the assay specific to delta smelt at the start of the project. The enzyme activity was normalized to protein concentration, where protein was determined according to BCA protein assay. In this study, the mean ATPase activity of smelt was estimated to range from 7-24 µmoles/Pi/hr/mg protein, which is similar to the activity expressed by Quabius et al. (1997) for tilapia and by Foott and Bigelow (2010) for delta smelt collected during Jan-May. Na^+/K^+-ATPase activity (n=121) was determined for the fish collected from the sites 418, 507, 518, 519, 606, 703, 705, 706, 719, and 797 (Table C.5.2). The Na^+/K^+-ATPase was lower than the normal range in some of the fish collected from sites 507, 518, and 519 and these suggest the potential effect of contaminants. Some fish in sites 606 and 719 expressed higher activity than the normal range and these are likely due to environmental factors such as temperature, turbidity, salinity or may also be the result of using more energy to get acclimatized to the environmental conditions when they migrate from one location to another. Univariate analysis indicates that there were no significant correlations between salinity, turbidity, secchi depth or site on Na^+/K^+-ATPase activity. Based on the estimated threshold concentration of Na^+/K^+-ATPase approximately 62% of the fish were considered normal Na^+/K^+-ATPase activity regardless of salinity concentration (Fig. C.5.4).When subjected to canonical analysis (Fig. C.5.5) a lack of significant relationship between Na/K ATPase and the predicted three water parameters have been noted. Thus the confounding factors such as migration and exposure to contaminants such as ammonia, metals, and pesticides may have affected the outcome of this result.

Table. C.5.2. Means ± Standard Deviation of Na/K-ATPase of delta smelt collected from August to December 2011were categorized as Good =Normal Na/K ATPase activity and Bad = inhibition in Na/K ATPase activity reported as. Normal range: 7-24 µmoles/pi/hr/mg protein.

		Na,K ATPase activity(µmoles/pi/hr/mg protein)					
		Normal		High		Depressed	
Site	Month	n	Activity	n	Activity	n	Activity
412	August	2	8.55 ± 1.25	-	-	-	-
507	September	-	-	-	-	5	2.85 ± 1.27
	October	14	14.45 ± 4.96	-	24.45 ± 0.15	3	4.33 ± 0.75
518	September	2	10.8 ± 1.8	-	-	1	1.6
	October	1	10.85 ± 3.85	-	-	1	3.8
	December	3	8.63 ± 0.38	-	-	2	3.95 ± 2.15
519	September	-	-	-	-	6	4.46 ± 2.14
	October	8	14.34 ± 5.1	-	-	3	0.83 ± 0.28
606	December	10	12.98 ± 3.74	1	24.9	-	-
517	December	4	18.15 ± 5.66	-	-	1	1.1
703	December	4	13.9 ± 3.6	-	-	1	1.9
704	December	2	17.1 ± 1.9	-	-	-	-
705	December	3	14 ± 2.88	-	-	-	-
706	December	3	14.9 ± 2.87	4	25.15 ± 0.54	-	-
716	August	3	9.1 ± 1.23	-	-	1	2.6
719	November	3	12.2 ± 2.3	3	24.75 ± 1.58	-	-
797	November	3	19.2 ± 4.8	-	-	-	-
510	October	-	-	1	25	1	0.8
511	October	-	-	-	-	-	-
515	September	1	7.7	-	-	-	-
601	November	-	-	-	-	1	6.3
602	November	-	-	-	-	2	6.3
721	October	1	11.1	-	-	-	-

Figure C.5.4. Na/K-ATPase versus different salinity range from August to December 2011

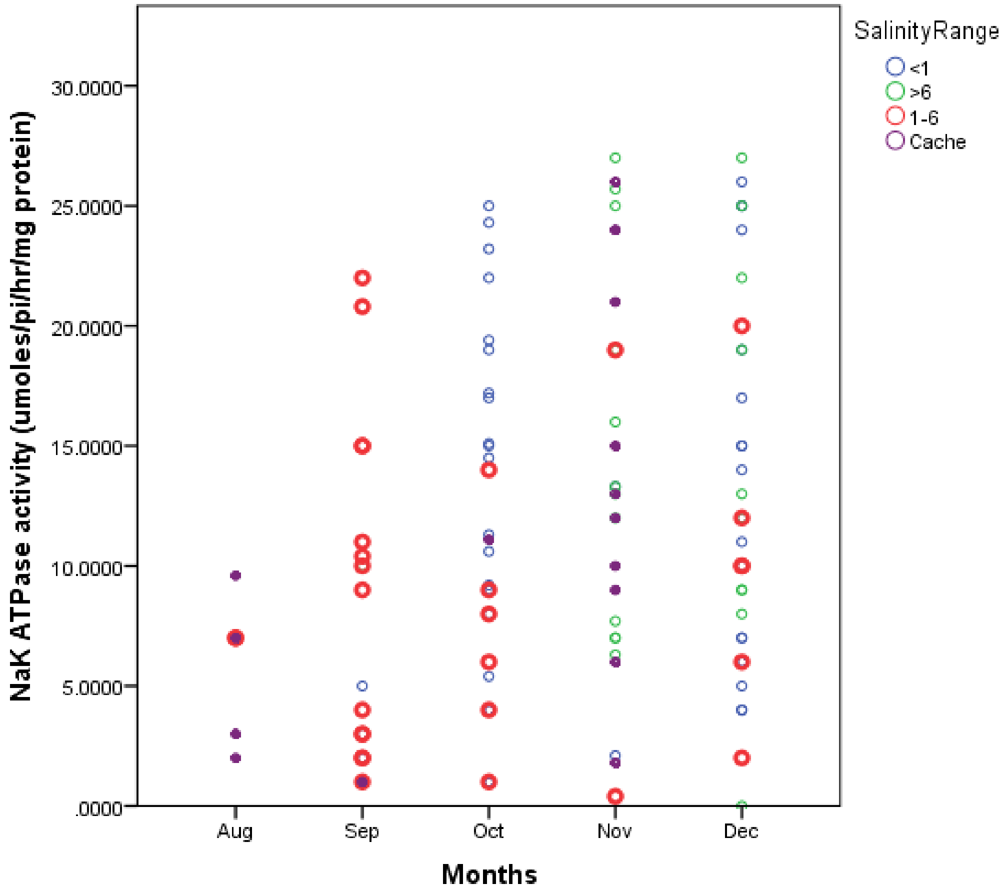
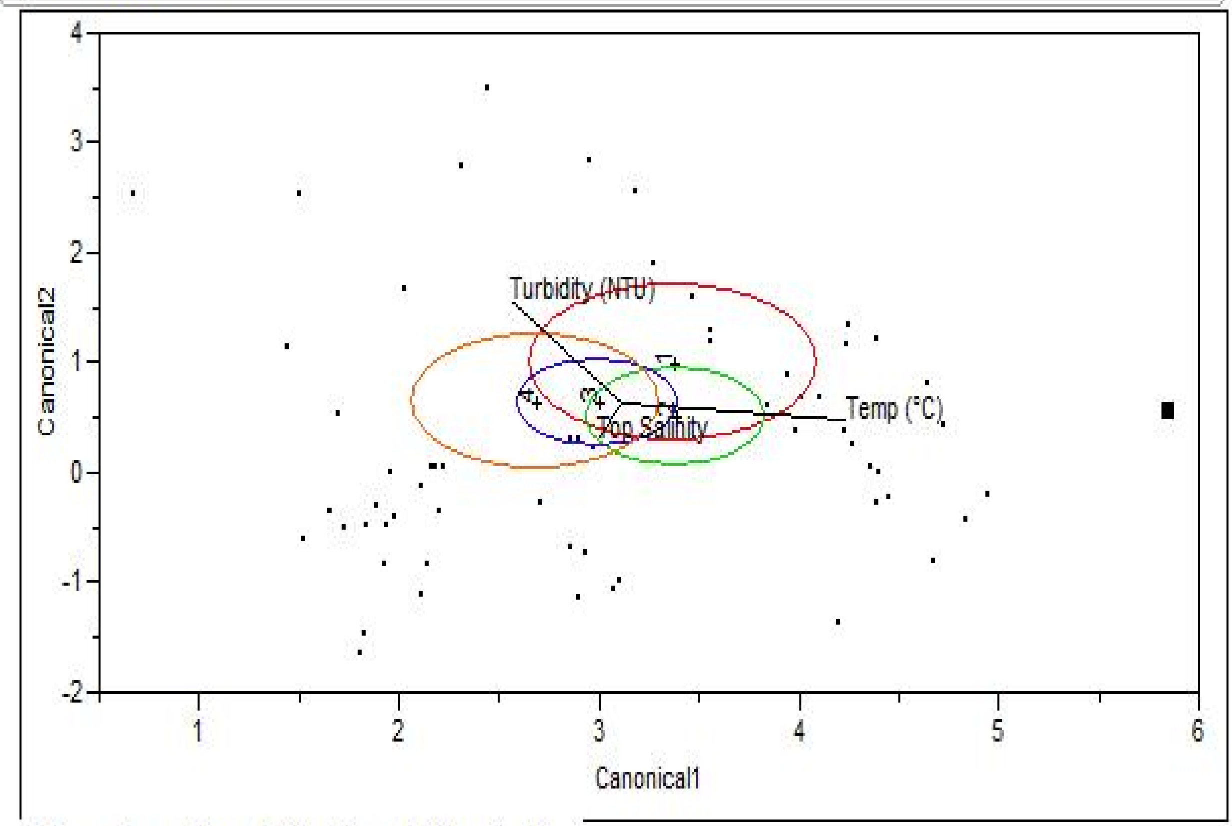


Figure C.5.5. Canonical analysis for Na/K ATPase by temperature, salinity and turbidity of delta smelt collected from August to December 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.06964592	74.8379	74.8379	0.25516894
0.02278952	24.4884	99.3263	0.14927067
0.00062692	0.6737	100.0000	0.02503053

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.9134852	1.1786	9	280.03	0.3085
Pillai's Trace	0.0880194	1.1788	9	351	0.3074
Hotelling-Lawley	0.0930624	1.1753	9	341	0.3098
Roy's Max Root	0.0696459	2.7162	3	117	0.0479

Within Matrix

Between Matrix

Eigenvectors

Scoring Coefs

C-6. Pathogens and Disease

A total of 329 delta smelt from the FLaSH have been processed for bacterial and viral isolation. The kidney and spleen of each fish were collected and held on ice for 18h in Minimal Essential medium (MEM) with no antibiotics until the samples were used for bacterial isolation. Tissues were disrupted by homogenizing for 30 sec–1 min and then used for bacterial and viral isolation (AFS-Fish Health Section, 2007) as described below.

C6-1. Bacteriology – sub-samples (ca. 20 μl) of the homogenized kidney and spleen tissues from each fish were inoculated onto Blood agar for general isolation of pathogens and onto Tryptone Yeast Extract Medium (TYES) to enhance the isolation of fastidious organisms such as flexibacteria (*Flexibacter, Cytophaga*). The plates were incubated at 15°C and examined for bacterial growth for 7 – 14 days.

Identification of bacterial isolates

Isolates on Blood agar and TYES were examined on phenotypic (morphology and biochemical reactions) characteristics (American Fisheries Society – Fish Health Section, 2007). The identity of dominant isolates were confirmed using molecular methods by sequencing the 16S ribosomal RNA gene commonly found in all bacteria. The universal bacterial primers EUBA/EUBB were used for initial PCR to amplify the 16S rDNA regions from which the amplified products were submitted to Davis Sequencing to determine the DNA sequences of representative dominant isolates.

Growth of bacterial flora was observed on Blood agar and TYES plates. The number of colonies ranged from low to moderate, with high colony numbers in a few high cases. Pure isolates of bacteria were isolated from few fish samples, but generally mixed in most of the fish examined. Bacterial isolates were mostly Gram-negative that were identified following analysis of basic phenotypic characteristics (Table C.6.1) and confirmed by sequencing of the 16S rDNA gene region. The dominant bacteria that have been isolated and identified to date from the different fish species are: *Flavobacterium*, *Aeromonas*, *Pseudomonas*, *Microbacterium*, *Sphingopyxis*, *Zooglea*, and *Bacillus* (Table C.6.1). Although these organisms are commonly found in the aquatic environment, certain species belonging to these genera are known as serious fish pathogens such as *Flavobacterium*.

The presence of bacterial assemblages was scored from 0 – 4 according to the number of bacterial colonies present on the plates: 0=absence of bacterial growth, 1=minimal number of bacterial colonies (N=1–10), 2= moderate number of colonies (N=11–20), 3=high number of colonies (>20–200), and 4=too numerous to be counted. The pathogen scores are recorded in the data sets corresponding to the results of each individual fish examined for the various health indices.

Multivariate analysis using canonical method was conducted on 329 delta smelt with corresponding pathogen scores. The results showed that none of the environmental factors alone significantly correlated to bacterial presence. Interestingly, a combination of specific factors including turbidity, salinity, and water temperature significantly interact to cause the presence of bacterial organisms among the delta smelt that were tested (Fig. C.6.1).

Mycobacterium qPCR analysis of delta smelt

In addition to other bacteria, delta smelt (N=88, kidney and spleen tissues) that were collected during the 2011 FLaSH Fish Survey were randomly chosen and analyzed for the presence of *Mycobacterium* using the proprietary *Mycobacterium tuberculosis* complex (MTC) PCR assay developed at the UC Davis Real-time PCR Research and Diagnostic Core Facility. The PCR assay detects DNA of closely related *Mycobacterium* species as causative agents of human and animal tuberculosis. Among the 88 delta smelt tested, the spleen and kidney tissues of 37 fish were positive for *Mycobacterium* DNA (42% incidence).

We have modified the MTC PCR assay to quantify the abundance of *Mycobacterium* present in the delta smelt tissues. Initial results demonstrate that delta smelt collected from various sites in the San Francisco Estuary in 2011 had minimal to moderate abundance of *Mycobacterium* spp. from kidney and spleen tissues that approximately correspond to ranges of $1 \times 10^2 – 1 \times 10^4$ gene copies of *Mycobacterium* spp. Refinement of the qPCR assay to verify the accuracy of the standard curve (known concentrations of *Mycobacterium*) and corresponding abundances of *Mycobacterium* in unknown samples is in progress. For this reason, Ct values were used for preliminary correlation analysis (Table C.6.2)**.** Ct refers to cycle threshold, the number of cycles required for the fluorescent signal to cross the threshold (exceed background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample such that the Ct values were grouped into different categories:
Cts < 29: strong positive reactions indicative of abundant target nucleic acid in sample
Cts of 30-37: positive reactions indicative of moderate amounts of target nucleic acid
Cts of 38-40: weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or environmental contamination.

Using the Ct values, initial multivariate analysis of qPCR data using canonical method showed that location significantly interact with surface conductivity and temperature to cause *Mycobacterium* DNA presence in delta smelt in the Fall of 2011 (Fig. C.6.2). The canonical analysis suggests that the locations in the San Francisco Estuary from which *Mycobacterium*-infected DS are more likely to occur are in sites 340, 418, 413, and 515 with corresponding ranges of temperature (19 – 20˚C), top conductivity (6220 – 22430 microsiemens per centimeter, μScm^{-3}), and salinity (3.84 – 15.06 ppt) (Table C.6.2)**.**

C6-2. Virology – following bacterial inoculation, the remaining homogenized tissues were centrifuged at 3,000 rpm, the supernatant diluted 1:1 with 2x antibiotic antimycotic solution, and incubated overnight at 10°C. Duplicate wells of EPC (Epithelioma papulosum cyprinid carp), CHSE-214 (Chinook salmon embryo), BF2 (Bluegill fry), and threadfin shad gill (TFSG) cells were inoculated with 200 µl of each sample. The TFSG cell line has been used for virus isolation from fish species in the San Francisco estuary in addition to the traditional cell lines stated above. Cell cultures were incubated at 15°C for a minimum of 21 days and subcultured for 14 days. No replicating viral agents have been isolated from any of the species tested by cell culture following incubation at 15°C for a minimum of 21 days using the different cell lines and a subculture of 14 days.

Table C.6.1. Basic phenotypic characteristics and significance of bacteria isolated from the different species from the Fall X2 Fish Health Survey.

Bacteria	Basic phenotypic characteristics	Significance in fish
Flavobacterium	Rod-shaped Non motile (gliding) Yellow pigments Gram-negative	Widely distributed in soil and water Normal flora of salmon/trout skin Pathogenic under unfavorable conditions (e.g. *F. psychrophilum*) Associated with bacterial gill disease of trout Non-salmonid infections occur at 8-12°C
Aeromonas	Facultative, motile Rod-shaped Gram-negative	Ubiquitous in fresh water, sewage, and eutrophic waters Some species are pathogenic to fish and humans (e.g. *A. hydrophila*)
Pseudomonas	Rod-shaped, motile Gram-negative	Widely distributed in nature Opportunistic, some species are pathogenic to fish and humans (e.g. *P. aeruginosa*)
Zooglea	Gram-negative, rod Proteobacteria form yellow gelatinous flocs	Common in wastewater treatment plants
Sphingopyxis	Gram-negative rod	Dominant in oligotrophic (low nutrient flux) environments Present in activated sludge
Microbacterium	Gram-positive rod	Found in dairy products, sewage, and insects
Bacillus	Gram-positive rod	Present in a wide range of habitats Some species are pathogenic to animals and humans (e.g. *B.cereus*, *B. thuringiensis*, which cause GI infections)

Table C.6.2. Collection sites from the San Francisco estuary from which delta smelt harboring *Mycobacterium* DNA are most likely to occur as determined by qPCR. Water temperature and top conductivity ranges for each site are indicated.

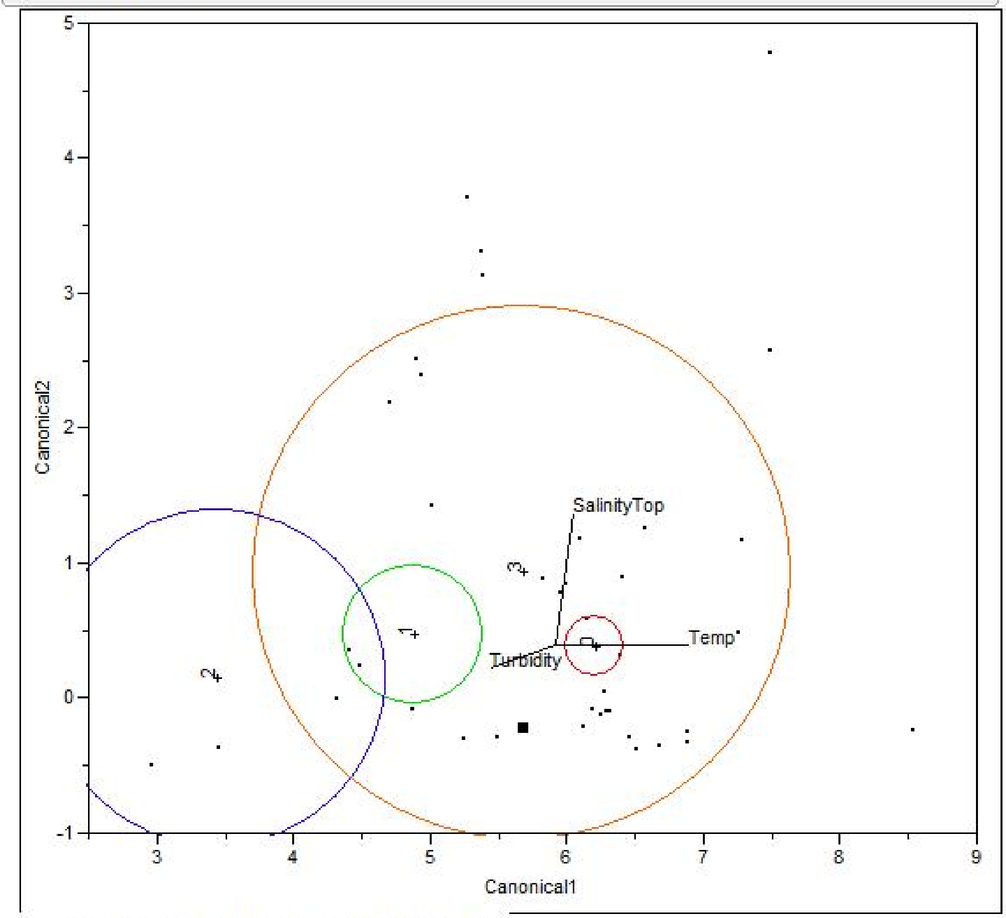
Site	Water temp	Top Conductivity ($\mu S/cm^3$)	Top Salinity (psu)	*Mycobacterium* (qPCR Ct value)*
340	20.2	22430	15.06	33.68
418	20.8	13250	8.38	33.4
413	20	7380	4.54	33.59
515	19.4	6220	3.84	32.61

*Ct = cycle threshold, the number of cycles required for the fluorescent signal to cross the threshold (exceed background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. Cts of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid.

Figure C.6.1. Multivariate analyses using canonical method showing the significant interaction of turbidity, salinity, and water temperature to cause the presence of bacterial organisms in Delta smelt from the San Francisco Estuary from Aug-Dec 2011.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.39875535	98.2785	98.2785	0.53392761
0.00570981	1.4073	99.6858	0.0753485
0.00127486	0.3142	100.0000	0.03568247

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.7099573	7.6452	9	455.26	<.0001
Pillai's Trace	0.2920293	6.7940	9	567	<.0001
Hotelling-Lawley	0.40574	8.3703	9	557	<.0001
Roy's Max Root	0.3987553	25.1216	3	189	<.0001

Within Matrix

Between Matrix

Eigenvectors

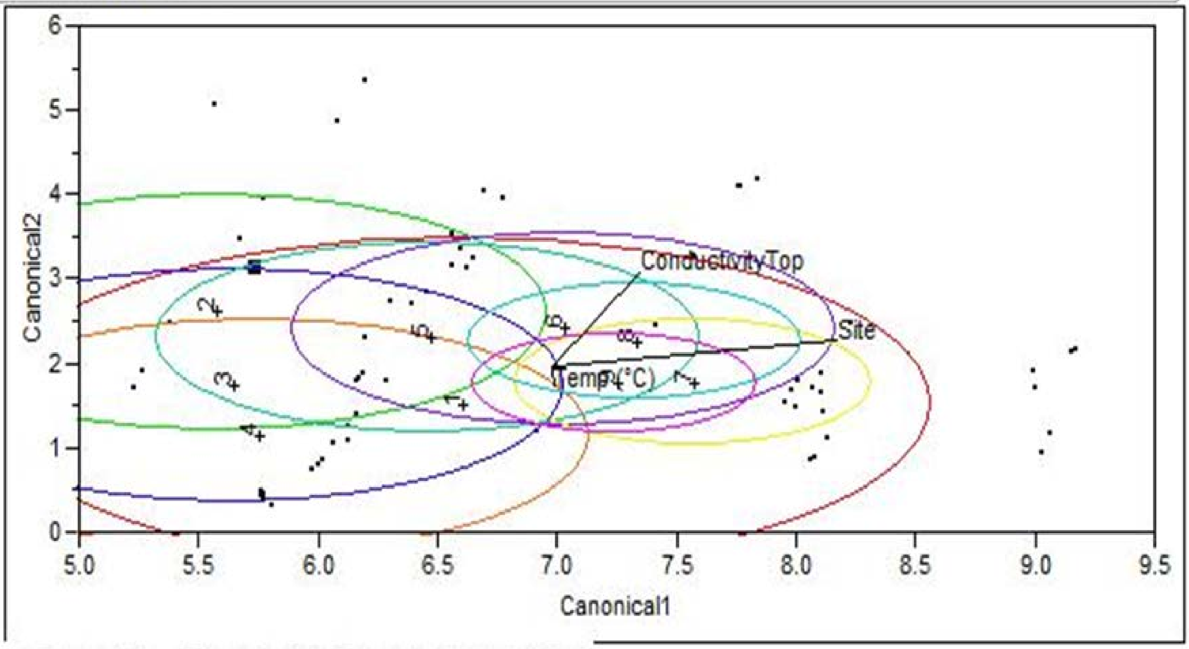
	Temp	Turbidity	SalinityTop
Canon1	0.3641654	-0.021313	0.0399678
Canon2	-0.001954	-0.00746	0.3326357
Canon3	0.1294272	0.0390366	0.0803477

Scoring Coefs

Figure C.6.2. Multivariate analyses using canonical method showing the significant interaction of location, top conductivity, and water temperature factors to cause the presence of *Mycobacterium* DNA in Delta smelt from the San Francisco Estuary.

Discriminant Analysis

Canonical Plot



Eigenvalue	Percent	Cum Percent	Canonical Corr
0.4563059	69.4061	69.4061	0.55975984
0.13732595	20.8879	90.2940	0.34748322
0.06381164	9.7060	100.0000	0.24491624

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.5675416	1.7517	24	194.92	0.0206
Pillai's Trace	0.4940596	1.7005	24	207	0.0261
Hotelling-Lawley	0.6574435	1.7988	24	197	0.0160
Roy's Max Root	0.4563059	3.9356	8	69	0.0007

Within Matrix

Between Matrix

Eigenvectors

Scoring Coefs

C7. Histopathology Biomarker

Gill, liver, and gonads of individual delta smelt were assigned a random alpha-numeric identification code to perform a blind study. Processed tissues were embedded in paraffin and sectioned at 3-5 microns thickness. Tissue sections were mounted on glass slides and stained with hematoxylin and eosin (H&E). Severity scores were semiquantitative and based on a scale of 0 to 3 (0 = not present, 1 = mild, 2 = moderate, and 3 = severe).

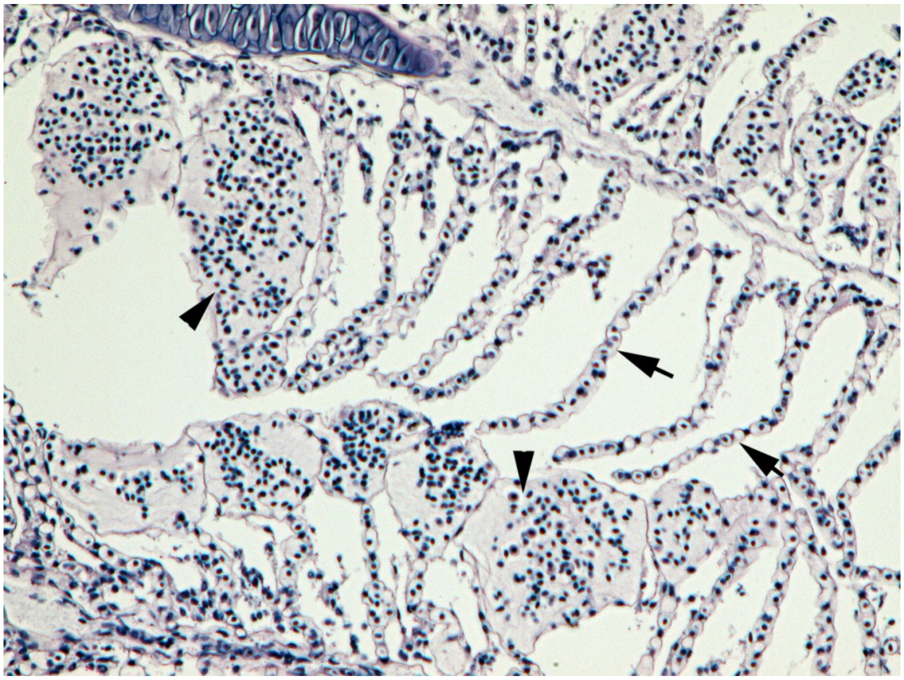
115 delta smelt (August n=46; September n=13, October n=33; November N= 7; and December N= 16) were randomly selected for histopathological analysis. Fish from various locations were grouped based on salinity of <1, 1-6, and >6 for each month (Table C.7.1). The prevalence of significant liver lesions (LGD, LIP, and LCN , Fig. C.7.3) were higher in fish sampled between August and October than between November and December and the significant gill lesions were higher in September, October and December compared to other months (Table C.7.1). It should also be noted that Gill secondary lamellar edema (GLE, Fig.C.7.1), aneurysm (GAN), and chloride cell hyperplasia (GCH, Fig. C.7.2) were higher in fish collected at <1 psu in September and October while GCH and GLE were higher in fish collected at 1-6 and >6 psu in December of 2012. In addition, two males collected in November had mild to severe testicular necrosis (Table C.7.1 and Figure C.7.4).

Table C.7.1 Prevalence of Liver and Gill Lesions

Month	N	LGD*	LIP	LCN	LMA	LINF	GAN	GCH	GLE
Aug (<1 psu)	20	19	9	3	2	6	1	0	0
Aug (1-6 psu)	22	21	3	1	0	3	0	0	0
Aug (>6 psu)	4	4	2	0	0	1	2	0	0
Sep (<1psu)	8	6	6	2	3	1	6[a]	2[a]	1
Sep (1-6 psu)	5	2	0	0	0	0	0	0	0
Oct (<1 psu)	32	25	12	1	1	4	15	5	1
Oct (1-6 psu)	1	1	0	0	0	0	1	0	0
Nov (<1psu)	5[b]	2	2	0	0	0	0	5	2
Nov (>6psu)	2	1	0	0	0	1	1	0	0
Dec (<1psu)	5	4	1	0	0	0	1	2	0
Dec (1-6psu)	5	4	1	1	0	0	0	4[c]	1
Dec (>6psu)	6	5	2	0	1	0	0	3	0

LGD= liver glycogen depletion; LIP = liver lipidosis; LCN = liver cell necrosis; LMA= liver macrophage aggregate; LINF = focal inflammation and granuloma in liver; GAN = gill aneurysm in secondary lamellae; GCH= gill chloride cell hyperplasia; GLE = gill secondary lamella edema. *For liver glycogen depletion, only moderate and severe scores were considered significant. [a] Severe GAN and GCH in one fish. [b] Mild to severe primordial germ cell in testis of two males. [c] Severe gill epithelial cell necrosis in one fish and gill chloride cell atrophy in another fish.

Figure C.7.1. Gill of delta smelt collected from site 507 in September 13, 2011 showing severe secondary lamellar aneurysm (arrowheads). Arrows point to normal secondary gill lamellae.



FigureC.7.2. Gill of delta smelt collected from site 509 in December 9, 2011 showing severe chloride cell proliferation (arrows) and fusion of secondary lamellae (*) resulting in obliteration of the interlamellar space. Arrowheads point to increase mucus secretion between the gill filaments.

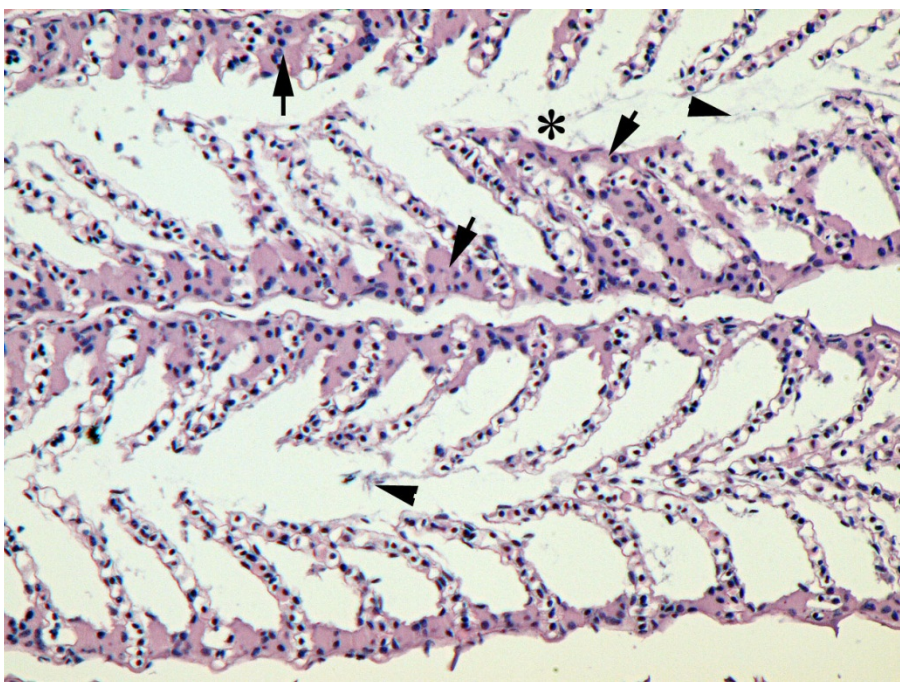


Figure C.7.3. Liver of delta smelt collected from site 797 in November 21, 2011 showing moderate lipidosis (arrowheads) and single cell necrosis (Arrows).

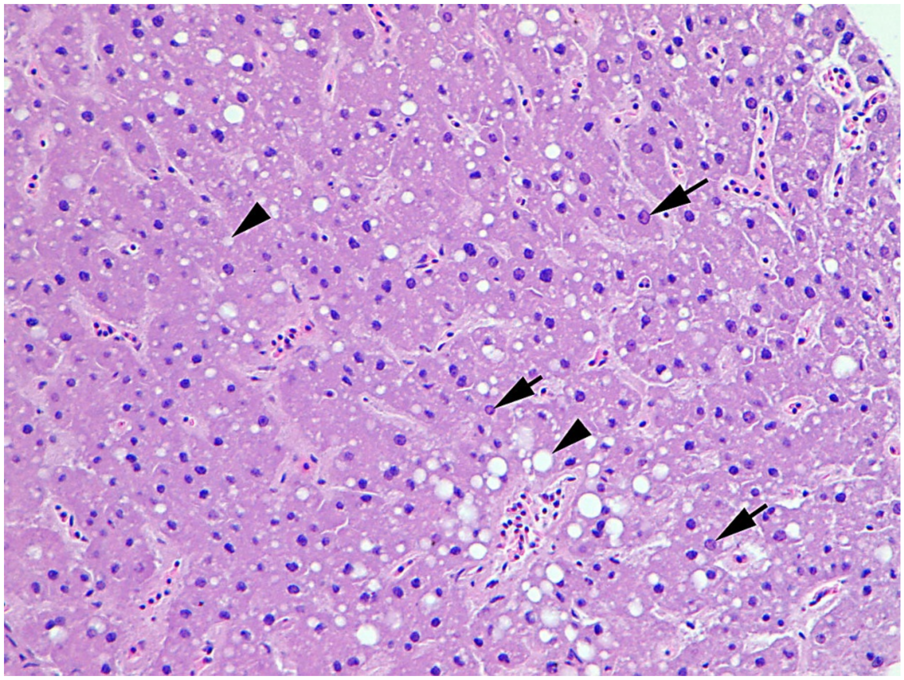
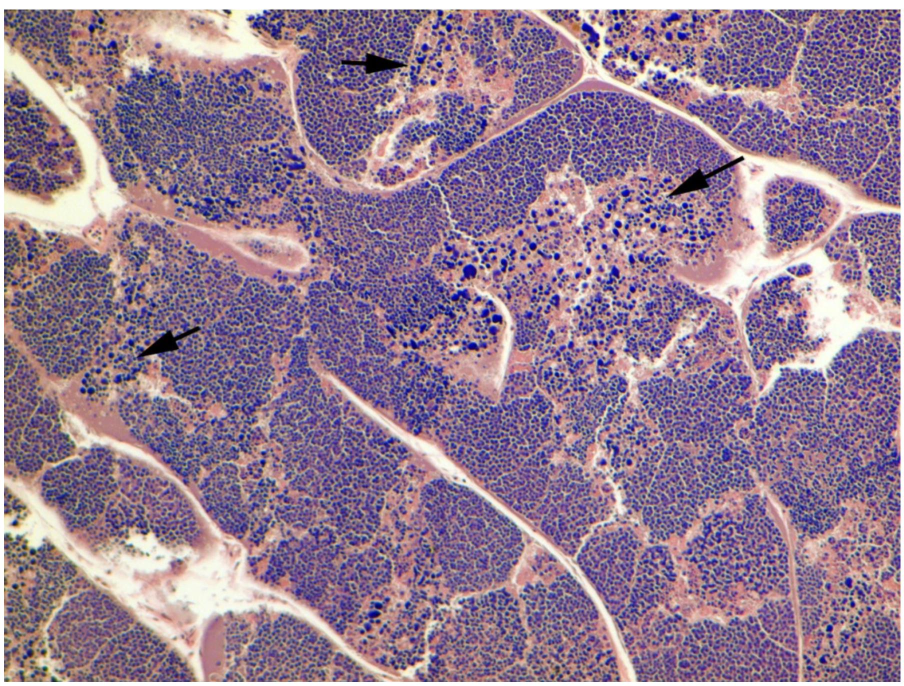


Figure C.7.4. Testis of delta smelt collected from site 797 in November 21, 2011 showing severe primordial germ cell necrosis (arrows).



C-8. Statistics

During this project, univariate statistics was utilized and investigated the potential for developing a multivariate statistical method for delta smelt health modeling in the San Francisco Estuary. In order to fully characterize any causal relationship between delta smelt health indices and water quality factors a comprehensive statistical approach was performed. All health indices such as FL, BW, CF, RNA/DNA, TAG, Na/K ATPase, and Acetylcholinesterase (AChE) were compared to physical factors in the environment (salinity, temperature and turbidity). In addition, two region classifications were used to compare the health indices. The first classification was determined by DFG as the Cache/SDWSC, <1 psu, 1-6 psu and >6 psu. The second classification was based on the geographical region of the SFE; Suisun Bay, Confluence, Honker Bay and Cache Slough Complex. For this study, 400 and 600 sites are grouped as Suisun Bay, 508-512, 700-706 and all the 800's are grouped as Confluence, and 507 and 517-519 are grouped as Honker bay. The Cache Slough Complex includes all the sites within the Cache Slough region and the Sacramento Deep Water Ship Channel.

For the canonical analysis, all health indices are ranked and converted to categorical endpoint based on frequency distribution or observed natural threshold (Table C.8.1). Then all possible predictor variables such as Temperature, Secchi, Electrical Conductivity, Turbidity and Salinity were examined to discover possible causative relationship between a group of predictor variables and a categorical outcome by using Canonical method. In statistics, canonical analysis belongs to the multivariate methods for data analysis. Canonical analysis is used to model the value of a dependent categorical variable based on its relationship to one or more predictors. It catches a relationship between a set of predictor variables and single categorical variable by the multiple correlations. All statistical analyses in this project were performed using customized Excel spreadsheets (Office Excel 2010, Microsoft Inc., USA), SPSS Ver. 20, IBM Inc,. USA and JMP Ver. 5.01, SAS Institute Inc., USA.

Table C.8.1. Ranges for each category among measured health indices during study are assigned based on frequency distribution or observed natural thresholds.

Biomarkers	Unit	Canonical Categories							
		1	2	3	4	5	6	7	8
AChE	µmoles/min/mg	<25	25-30	30-40	40-45	45<			
ATPase	µmoles/pi/hr/mg	<10	10-20	20<					
TAG	mmoles/mg	<10	10-20	20-30	30-40	40-50	50<		
RNA/DNA		<0.5	0.5-1	1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5<
GSI	%	<0.5	0.5-1	1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5<
HSI	%	<0.5	0.5-1	1-1.5	1.5-2	2<			
CF	mg/mm^3	<0.4	0.4-0.6	0.6-0.8	0.8-1	1<			
FL	mm	<45	45-53	53-58	58<				

D. Discussions:

D-1. Otolith growth rate and microchemistry

Total growth rates for delta smelt in fall of 2011 did not differ between defined habitats. However, due to low sample numbers during some survey months and habitats, it was difficult to conduct proper statistical analyses accounting for spatial and temporal variability. Temporal variability in total growth rates was apparent for the November and December surveys. Reduced growth during the November-December time period could be the result of reduced water temperatures during these months or an ontogenetic change in the growth rates of individuals beginning the reproductive maturation process. It is likely that both factors interact to result in reduced growth rate. Survival to the December Fall Midwater Trawl Survey did appear to depend on growth rates during the months of July and August, however Month Specific Growth Rates trends for the November and December survey months alternative for the July and August months, being low in July, but high in August, thus the selection on growth rate is not a clear process. To examine this process further, we will use a repeated measures MANOVA approach to distinguish the effects of size and the hatch-date at each Month Specific Growth period, as both can be autocorrelated with growth rate. Otolith chemistry revealed that two distinct life history types exist for the delta smelt, a resident freshwater form and the migratory form. We did observe a large proportion of the resident form in the Cache-SDWSC, where most of these individuals were collected in August when migration to the low salinity zone could still occur. Furthermore, we did observe several individuals in the Cache-SDWSC region during each survey month. A few resident individuals were found in the low to higher salinities, suggesting either the fish had recently migrated to these habitats or the otolith chemistry may not always accurately reflect the migration history. Considering those individuals that occurred in the higher salinity habitats did so in August and the low salinity later in the season, it is likely these fish had just migrated to these habitats within a short period of time prior to being captured. In the future we will be quantifying the residence time for each individual in the four habitats types with the otolith microchemistry. In addition to resident time in each habitat we will estimate individual growth rates in each habitat to determine if habitat specific differences exist in growth rates and how residence time in each habitat may be associated with the growth and health metrics.

D-2. Morphometic indices

Morphometric indices of FL, BW, CF, HSI, and GSI were highly variable. Correlations were detected between FL and BW with time suggesting delta smelt grew during the fall months as temperature decreased with time. No differences were detected over time and temperature for CF and HSI suggesting that the delta smelt were relatively similar health throughout the sampling period. Therefore comparing all the fish together was feasible when examining biomarkers of health such as enzyme activity and histopathological lesions and biomarkers of nutrition such as RNA/DNA, TAG and histopathological lesions. In contrast GSI changed over time with maturation appearing to occur in November with temperatures dipping below 15^{o}C. As a one year spawner, maturation was expected to begin by late fall. In December, GSI continued to increase with temperatures around 10^{o}C. Visual identification of sex was possible in December facilitating future comparative analyses between sexes for all biomarkers for the month of December. In addition, with further analyses of GSI for winter 2012, correlations with development and reproductive biomarkers can be examined.

Univariate statistics was unable to determine a significant causative relationship between water quality and the morphometric indices. In addition, comparing salinity regions and geographical regions with morphometric indices was variable. Some significant differences were detected but they were few and not consistent throughout the sampling period of August through September. Suggesting that other factors occurred that may have confounded the relationships such as sampling techniques, temporal and/or geographical variances, tidal fluctuations and fish behavior. Sampling issues occurred multiple times through the sampling period. Some geographic and salinity regions had either few fish or none were collected at all. Perhaps continued sampling in a region in order to insure fish were collected may be warranted. Obtaining consistent samples throughout the region, salinity range and/or sampling period would increase the robustness of the univariate analyses. As a tidal marsh, fluctuations in water quality can happen throughout the day. The daily variations combined with movement behavior of delta smelt suggest that site fidelity is weakly correlated. The recorded water quality only reflects a small period of time for what the delta smelt experience, therefore relating water quality and regional variations to the morphometric biomarker recorded will also be weakly correlated.

Due to the complex relationships of water quality, geographic factors, monthly effects and fish swimming behavior a multivariate approach was warranted. Initial multivariate approach utilized canonical analyses of all the water quality factors recorded by the Department of Fish and Game, conductivity, salinity, temperature, turbidity and secchi depth. Initial results detected significant causative correlative effects on all morphometric indices by temperature, turbidity and salinity suggesting that these factors are important in characterizing the changes in the morphometric indices. Although the three water quality parameters were detected to have an effect the results do not suggest which factor was the most significant portion of the effect, whether the parameter has a positive or negative relationship or whether there is an optimal range. More advanced multivariate analyses of a Generalized Linear Model or Multivariate Logistic Model will be attempted in the future to determine the magnitude and direction of the correlative effects from temperature, turbidity and salinity.

D-3 Nutritional analyses

Nutritional indices of RNA/DNA and TAG were highly variable. A negative correlation was detected between RNA/DNA with temperature. RNA/DNA is an indicator of relative nutritional status (Buckley et al., 1999). Temperature decreased from August to December therefore the delta smelt nutritional status was correlated with time suggesting that nutritional stress decreases with time or fish with relatively poor nutritional status do not persist later in the sampling period. The median RNA/DNA value was 1.58 with values of greater than 1 representative of good nutritional status therefore most (>80%) of the fish were considered to be at good nutritional status. This verifies the characterization of 2011 being a good year for delta smelt. For TAG no differences were detected over time and temperature suggesting that the delta smelt from August to December had relatively similar long term nutritional status. To date there are no TAG analyses performed on delta smelt and only a small handful on osmeridaes. The three osmeridae analyzed were rainbow (*Osmerus mordax*), surf smelt (*Hypomesus pretiosus*), and sweet smelt (*Plecoglossus altivelis*). Rainbow and surf smelt produce glycerol to tolerate the cold temperatures of their environment (Raymond 1993, Raymond et al., 1996). The glycerol would artificially elevate the TAG analysis as the assay detects TAG by using a probe that is specific to the glycerol backbone of TAG. Sweet smelt values were calculated using whole body weight therefore it is difficult to compare the results of this study in mmoles of TAG/mg of protein from muscle. In addition, sweet smelt live in colder water (Chyung 1991; Jeong et al., 2000). Therefore the values recorded for 2011 cannot be compared with other species or with other studies to determine whether the concentrations of TAG in the muscle are sufficient to promote overwintering and increased productivity. Comparisons from multiply years will be helpful to determine what the normal range for RNA/DNA and TAG for delta smelt.

Univariate statistics was unable to determine a significant causative relationship between water quality and the nutritional indices. In addition, comparing salinity regions and geographical regions with morphometric indices was variable. Some significant differences were detected but they were few and not consistent throughout the sampling period of August through

September. Suggesting that other factors occurred that may have confounded the relationships such as sampling techniques, temporal and/or geographical variances, tidal fluctuations and fish behavior. Sampling issues occurred multiple times through the sampling period. Some geographic and salinity regions had either few fish or none were collected at all. Perhaps continued sampling in a region in order to insure fish were collected may be warranted. Obtaining consistent samples throughout the region, salinity range and/or sampling period would increase the robustness of the univariate analyses. As a tidal marsh, fluctuations in water quality can happen throughout the day. The daily variations combined with movement behavior of delta smelt suggest that site fidelity is weakly correlated. The recorded water quality only reflects a small period of time for what the delta smelt experience, therefore relating water quality and regional variations to the morphometric biomarker recorded will also be weakly correlated.

Due to the complex relationships of water quality, geographic factors, monthly effects and fish swimming behavior a multivariate approach was warranted. Initial multivariate approach utilized canonical analyses of all the water quality factors recorded by the Department of Fish and Game, conductivity, salinity, temperature, turbidity and secchi depth. Results of the canonical analysis detected significant causative correlative effects on RNA/DNA and TAG biomarkers by temperature, turbidity and salinity. Although the three water quality parameters were detected to have an effect the results do not suggest which factor was the most significant portion of the effect, whether the parameter has a positive or negative relationship or whether there is an optimal range. More advanced multivariate analyses of a Generalized Linear Model or Multivariate Logistic Model will be attempted in the future to determine the severity and direction of the correlative effects from temperature, turbidity and salinity.

<u>D.4 Enzymatic biomarkers</u>

Enzyme activities for AChE and Na^+/K^+-ATPase were variable at different locations indicating that other environmental stressors such as contaminants also affect the health of Delta smelt. Most of the fish collected from Sacramento River and Cache Slough had normal enzyme activity compared to the fish collected from Suisun bay. Thus the fish with low AChE and ATPase activity indicate that fish has likely been exposed and survive contaminant effects and the chance for recovery occurs if the fish migrates to a clean site. Pesticide analysis at certain locations and further studies on biomarkers such as EROD, Metallothionein can help to identify the cause for specific stressors in certain locations.

The preliminary results based on AChE and Na^+/K^+-ATPase indicate 59% of the fish had normal AChE and 62% had normal Na^+/K^+-ATPase activities according to the estimated threshold range suggested for 2011. These threshold ranges can vary for delta smelt, as it is species dependent. Comparisons of these enzyme activities among wet and dry season for multiple years will be helpful to determine exact threshold range of AChE and Na^+/K^+-ATPase for delta smelt.

D.5 Histopathologic biomarkers

Delta smelt caught in August, September and October had lower health condition than those caught in December suggest multiple stressors effects including 1) exposure to contaminants as evidenced by higher number of fish with lipidosis, cell necrosis and macrophage aggregates in liver, 2) exposure to changing in water conditions such as salinity as fish migrated from high salinity zone to low salinity zone as evidenced by the loss of gill osmoregulation resulting in gill secondary lamellar aneurysm and chloride cell hyperplasia, and/or 3) the combination of contaminants and water quality changes as they migrate upstream. Similarly, when compared fish caught based on salinity zone within month, gill secondary lamellar aneurysms were mostly found in fish at <1 PSU and not at 1-6 or >6psu further suggested fish is migrating to or have migrated to the sites and trying to adapted to freshwater (Table C.7.1).

D.6 Pathogens and Disease

The presence of pathogens/disease was examined mainly from delta smelt and striped bass among the different species collected from the Fall X2 Fish Health Survey. The bacteria that were isolated from the different fish species are mostly Gram negative and belong to genera that commonly occur in freshwater and marine environments. Some species of these genera are known pathogens of fish. For example, *Flavobacterium psychrophilum* is the agent of cold water disease in salmonids or systemic infections in rainbow trout. Multivariate analysis (canonical method) of delta smelt (N=329) collected in 2011 indicates that the presence of bacterial pathogens is not influenced by a single environmental factor. However, significant interactions were observed among three environmental factors: turbidity, salinity, and water temperature to cause the occurrence of bacteria in wild delta smelt.

While a high incidence of delta smelt carrying *Mycobacterium* DNA (42% incidence) was observed by qPCR among randomly selected wild delta smelt in 2011, *Mycobacterium* was not isolated by traditional culture method and mycobacteriosis was not observed. The presence of *Mycobacterium* DNA in wild delta smelt indicates exposure to *Mycobacterium* present in the habitat. Canonical analysis demonstrates that wild delta smelt harboring *Mycobacterium* DNA (carriers) is influenced by the significant interaction of conductivity and water temperature at certain locations. In addition to water temperature and conductivity, other unmeasured factors present in the location such as contaminants may influence the incidence of latent infections with *Mycobacterium* among wild delta smelt. To illustrate this hypothesis, canonical analysis suggested that sites 340, 418, 413, and 515 from which delta smelt harboring *Mycobacterium* are most likely to occur are adjacent to naval shipyards and other anthropogenic inputs from local industries.

It is important to note that delta smelt in captivity may develop infections that progress from latent to clinical disease due to various stress factors under intensive culture conditions. For example, delta smelt (most are sexually mature) from the Fish Conservation and Culture Lab

(FCCL) that was studied for temperature effects in our laboratory (8/25-11/7/2011 at 15°C) suffered mortalities due to severe infections with *Mycobacterium* (N=8 positive for *Mycobacterium* by culture and disease from 20 delta smelt that died); 16SrDNA sequencing confirmed the bacterium as *M. salmoniphilum*. Infections may progress from latent to clinical disease due to various stress factors in captivity such as frequent handling, crowding, and spawning stress under intensive culture conditions such as those currently observed among refugial populations of delta smelt at FCCL, broodstock delta smelt at Livingstone National Fish Hatchery, and at various applied research programs at UC Davis using delta smelt originating from FCCL.

Viral agents have not been isolated from among the different species of fish examined to date. Although these results imply the absence of virus infections among the fish tested, there is also a possibility that samples that were caught and examined represented healthier life stages precluding the analysis of unfit fish that may have already died in the wild. In this context, the role of pathogens/disease and other risk factors affecting fish fitness in the estuary can be easily dismissed. Integrating disease/pathogens as a key component of the Fall X2 Fish Health Survey will determine their potential correlation with the different health indices that may be relevant to growth, reproduction, and general health of the different fish species collected from the 3 contrasting regions. A multi-year monitoring of pathogens/disease among the fish species will provide critical baseline on the relevance of pathogens that can be used for evaluating future changes on fish health status due to the combined effects of multiple stressors and water infrastructures. Correlation analyses between pathogens/disease presence and environmental stressors among the other species (i.e. striped bass, threadfin shad, and American shad) will be examined to determine which factors are more likely to affect general fish health in the contrasting habitats in the FLaSH.

D.7 Multivariate and Clustering Analyses

Because of the presence of multiple confounding factors in San Francisco ecosystem, results of univariate statistical analyses in Tasks C2 to C6 did not show any clear trends or causative effects among measured health indices or recorded water quality parameters. Therefore, correlation analyses and multivariate methods to interpret patterns and potential causative factors affecting Delta smelt health between August and December of 2011 were investigated in this study. These statistical approaches were based on epidemiologic methods for the study of populations by first identifying strongly correlated health parameters followed by determining environmental factors that are most representative of the correlated health parameters. Once determined, the environmental factors were then assigned as predictive variables for the multivariate analyses.

D7-1 Correlation analysis

Correlation analyses among measured health indices are summarized in Table D.7.1. There are strong correlation between Body weight and length to Gonadosomatic index. Using a similar approach, strong correlations were found among water quality parameters such as salinity, turbidity and temperature. Additional correlation studies indicate significant correlations among predicted water quality parameters and health indices (Table D.7.2). Temperature was significantly negatively correlated with RNA/DNA, GSI and Pathogen score ($p<0.05$), and weak positively correlated with TAG (Table D.7.2).

Table D.7.1. Correlation analyses of morphmetrics, nutritional, enzymatic and disease indices. Highlighted values indicated positive (blue) and negative (red) correlations.

Correlations

	FL	Weight	CF	HSI	GSI	RNA/DNA	TAG	ATPase	AChE	Pathogen Score
FL	1.0000	0.9070	0.1849	-0.0940	0.6805	0.2095	0.0487	0.1724	0.2246	0.2747
Weight	0.9070	1.0000	0.4280	-0.0400	0.6802	0.2506	0.1146	0.1555	0.1576	0.2842
CF	0.1849	0.4280	1.0000	0.0495	0.2238	0.2544	0.1504	0.0467	0.0125	0.1793
HSI	-0.0940	-0.0400	0.0495	1.0000	0.0717	-0.0908	0.2264	0.0853	-0.1492	0.0277
GSI	0.6805	0.6802	0.2238	0.0717	1.0000	0.3315	0.0501	0.2397	0.1624	0.2195
RNA/DNA	0.2095	0.2506	0.2544	-0.0908	0.3315	1.0000	-0.1145	0.2376	-0.1274	0.1800
TAG	0.0487	0.1146	0.1504	0.2264	0.0501	-0.1145	1.0000	-0.0987	0.0856	0.0024
ATPase	0.1724	0.1555	0.0467	0.0853	0.2397	0.2376	-0.0987	1.0000	-0.1035	0.0789
AChE	0.2246	0.1576	0.0125	-0.1492	0.1624	-0.1274	0.0856	-0.1035	1.0000	0.0328
Pathogen Score	0.2747	0.2842	0.1793	0.0277	0.2195	0.1800	0.0024	0.0789	0.0328	1.0000

Table D.7.2. Correlation analyses among predicted environmental factors and health parameters. Highlighted values indicated positive (blue) and negative (red) correlations.

Correlations

	Weight	CF	HSI	GSI	RNA/DNA	TAG	ATPase	AChE	Pathogen Score	Temp (°C)	Turbidity (NTU)	Top Salinity
Weight	1.0000	0.4276	-0.0410	0.6163	0.2126	0.1585	0.1420	0.0512	0.2842	-0.5560	-0.1204	-0.0579
CF	0.4276	1.0000	0.0495	0.1738	0.2381	0.1453	0.0353	-0.0706	0.1794	-0.2083	0.1809	-0.0021
HSI	-0.0410	0.0495	1.0000	0.1072	-0.0421	0.1872	0.0928	-0.1172	0.0306	0.1294	0.0296	-0.1012
GSI	0.6163	0.1738	0.1072	1.0000	0.2383	0.0763	0.2121	0.0480	0.1422	-0.4279	-0.0923	-0.1406
RNA/DNA	0.2126	0.2381	-0.0421	0.2383	1.0000	-0.1140	0.2629	-0.2240	0.1840	-0.6125	-0.3157	0.0975
TAG	0.1585	0.1453	0.1872	0.0763	-0.1140	1.0000	-0.0487	0.0746	0.0003	0.2384	0.2771	0.0100
ATPase	0.1420	0.0353	0.0928	0.2121	0.2629	-0.0487	1.0000	-0.1409	0.0535	-0.2926	-0.1155	0.1234
AChE	0.0512	-0.0706	-0.1172	0.0480	-0.2240	0.0746	-0.1409	1.0000	-0.0399	-0.1838	-0.0139	-0.1268
Pathogen Score	0.2842	0.1794	0.0306	0.1422	0.1840	0.0003	0.0535	-0.0399	1.0000	-0.3672	-0.0911	0.0463
Temp (°C)	-0.5560	-0.2083	0.1294	-0.4279	-0.6125	0.2384	-0.2926	-0.1838	-0.3672	1.0000	0.4894	-0.1318
Turbidity (NTU)	-0.1204	0.1809	0.0296	-0.0923	-0.3157	0.2771	-0.1155	-0.0139	-0.0911	0.4894	1.0000	-0.0363
Top Salinity	-0.0579	-0.0021	-0.1012	-0.1406	0.0975	0.0100	0.1234	-0.1268	0.0463	-0.1318	-0.0363	1.0000

D7-2 Canonical and Clustering analyses

Canonical analysis widely applied in modeling categorical outcomes based on interactions of one or more predictors was selected as the multivariate approach to verify the relationships between predictor variables and measured outcomes. Canonical analyses among predicted environmental factors and health parameters are summarized in Table D.7.3 And Figures D.7.1A and B. Results indicated combinations of predicted water parameters significantly affect the outcome of all health parameters except TAG and ATPase. The lacks of significant relationship between TAG or ATPase and the predicted three water parameters have been noted in TAG and ATPase under the discussion section (D3 and D4). In the nutritional section, there are TAG no differences detected over time and temperature suggesting that the delta smelt from August to December had relatively similar long term nutritional status in the relative good 2011. For ATPase, the confounding factors such as migration and exposure to contaminants such as ammonia, metals, and pesticides may have affected the outcome of this result.

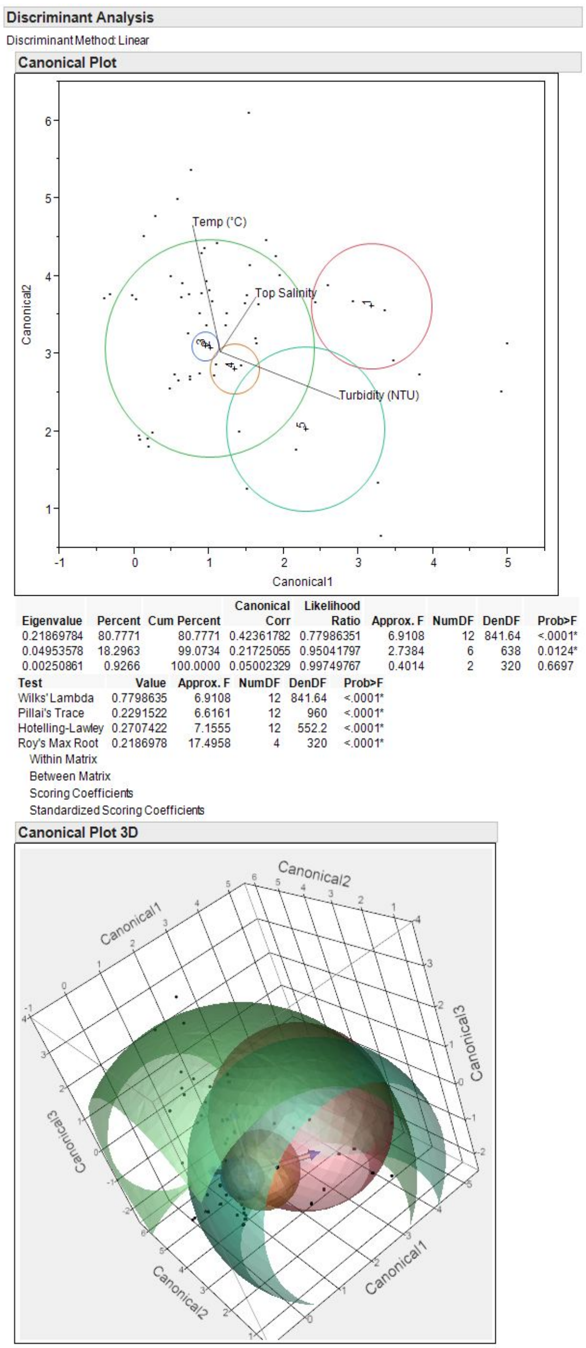
Once the causative relationship among health and water quality parameters were verified, data were then clustered based on salinity level to control for variations on effects across salinity zones. Clustering analyses revealed clear causative pattern between predictor factors (salinity, turbidity and temperature) and condition factor (Figures D.7.2.A-E). Results suggested that condition factor will be improving by reducing temperature from 16 °c to 14°c among fish in 0-2psu salinity and by increasing turbidity from 37 NTU to 60 NTU. A similar pattern of action was also observed among fish in >8psu salinity (Figure D.7.2.E). Despite small sample size and presence of outlier data, pattern of actions are almost similar among different groups.

Table D.7.3. Canonical analyses among predicted environmental factors and health parameters.

	CF	HSI	GSI	RNA/ DNA	TAG	ATPase	AChE	Pathogen Score
Salinity	Sig.	Sig.	Sig.	Sig.	---	---	Sig.	Sig.
Turbidity	Sig.	Sig.	Sig.	Sig.	---	---	Sig.	Sig.
Temperature	Sig.	Sig.	Sig.	Sig.	---	---	Sig.	Sig.
P-Value	<0.0001	0.0008	<0.0001	<0.0001	0.1609	0.3085	0.0015	<0.0001

Figure D.7.1. Multivariate analysis using a 3D image of pertinent causative relationships of conditional factor and ATPase outcomes and selected water quality parameters. A) shows significant relationship between condition factor and water parameters (p<0.0001) and B) shows no significant relationship among ATPase and predictor variables (p=0.3085).

A.

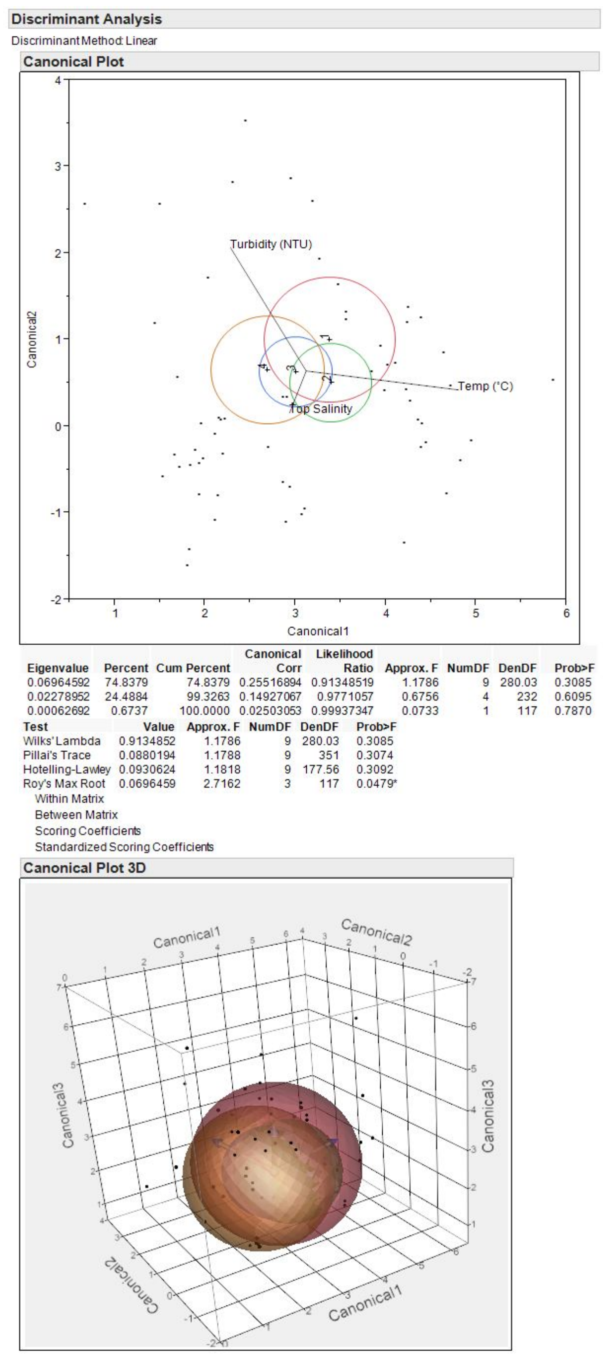


Eigenvalue	Percent	Cum Percent	Canonical Corr	Likelihood Ratio	Approx. F	NumDF	DenDF	Prob>F
0.21869784	80.7771	80.7771	0.42361782	0.77986351	6.9108	12	841.64	<.0001*
0.04953578	18.2963	99.0734	0.21725055	0.95041797	2.7384	6	638	0.0124*
0.00250861	0.9266	100.0000	0.05002329	0.99749767	0.4014	2	320	0.6697

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.7798635	6.9108	12	841.64	<.0001*
Pillai's Trace	0.2291522	6.6161	12	960	<.0001*
Hotelling-Lawley	0.2707422	7.1555	12	552.2	<.0001*
Roy's Max Root	0.2186978	17.4958	4	320	<.0001*

Within Matrix
Between Matrix
Scoring Coefficients
Standardized Scoring Coefficients

B.



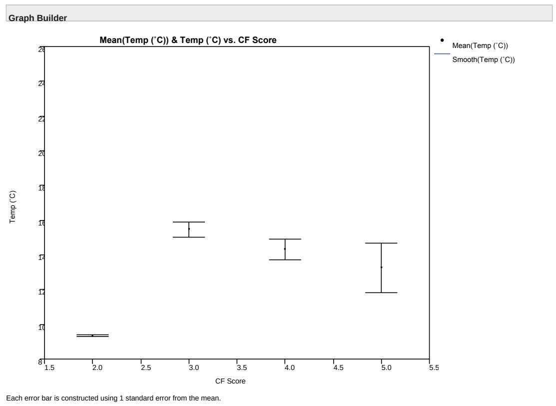
Eigenvalue	Percent	Cum Percent	Canonical Corr	Likelihood Ratio	Approx. F	NumDF	DenDF	Prob>F
0.06964592	74.8379	74.8379	0.25516894	0.91348519	1.1786	9	280.03	0.3085
0.02278952	24.4884	99.3263	0.14927067	0.9771057	0.6756	4	232	0.6095
0.00062692	0.6737	100.0000	0.02503053	0.99937347	0.0733	1	117	0.7870

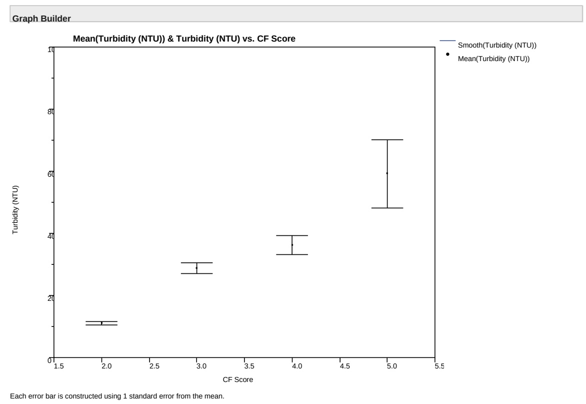
Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.9134852	1.1786	9	280.03	0.3085
Pillai's Trace	0.0880194	1.1788	9	351	0.3074
Hotelling-Lawley	0.0930624	1.1818	9	177.56	0.3092
Roy's Max Root	0.0696459	2.7162	3	117	0.0479*

Within Matrix
Between Matrix
Scoring Coefficients
Standardized Scoring Coefficients

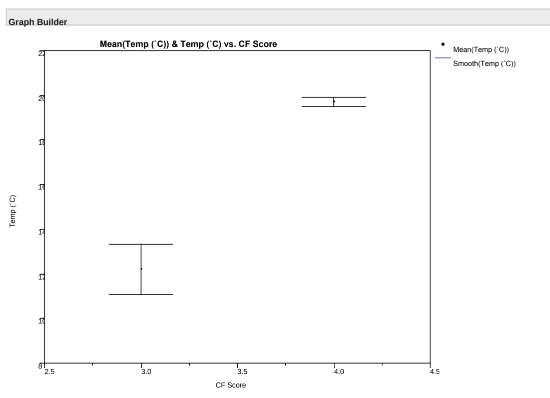
Figures D.7.2.A-E. Clustering correlations between condition factor (CF) versus temperature and turbidity in fish at different salinity levels.

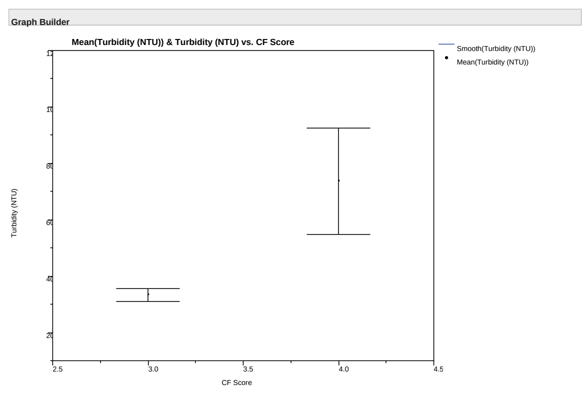
A. Salinity Level 1 (0-2 psu)



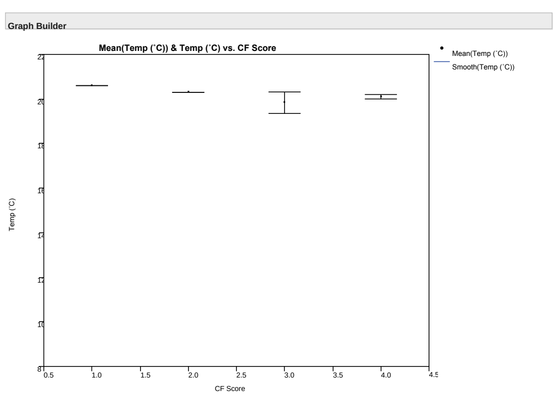


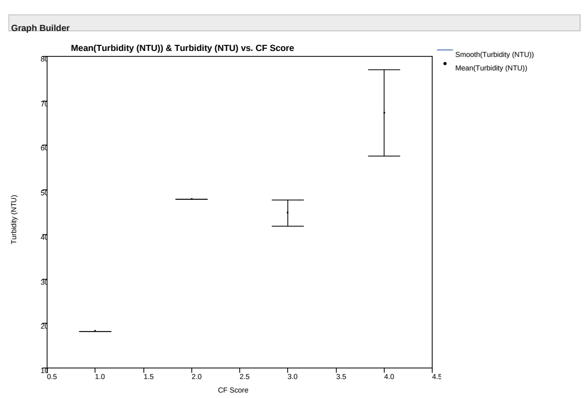
B. Salinity Level 2 (2-4 psu)



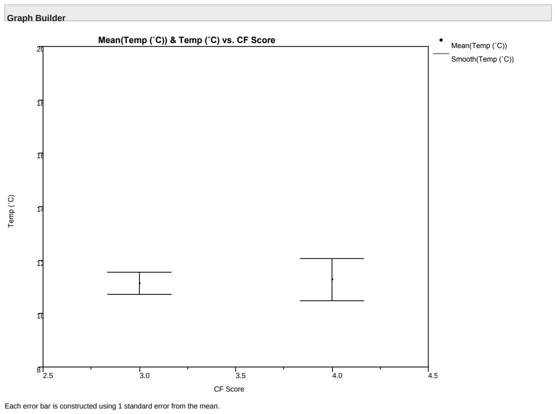


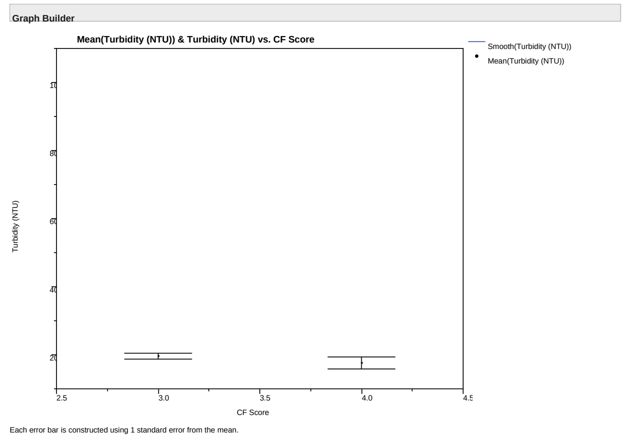
C. Salinity Level 3 (4-6 psu)



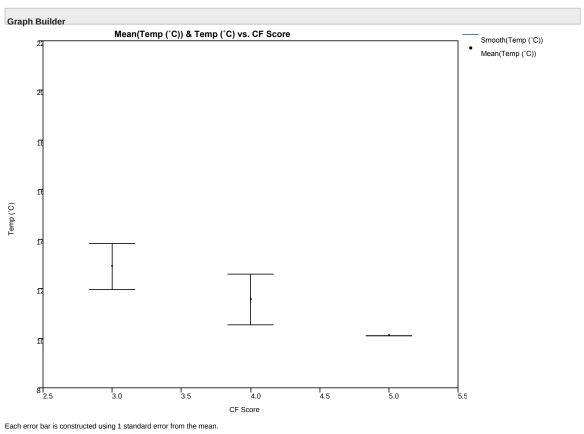


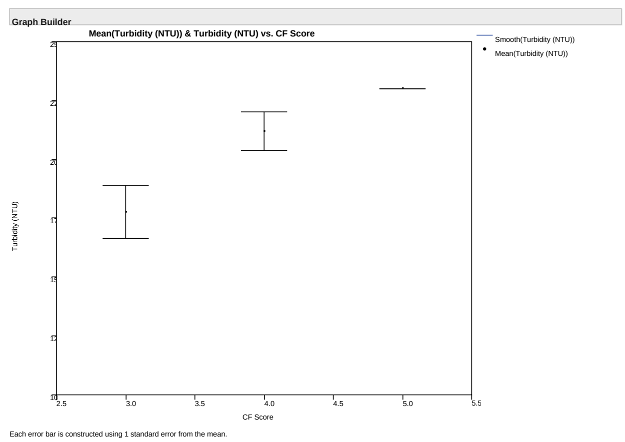
D. Salinity Level 4 (6-8 psu)





E. Salinity Level 5 (>8 psu)





E. Summary and implications of preliminary findings in 2011 pre-spawning delta smelt:

1. For the fall survey greater than 50% of all delta smelt were collected from the fresh to 2 psu habitats. Moreover catch distributions varied significantly from month to month among the different habitat and region scales, therefore conducting statistical analysis was difficult.

2. Otolith growth rates revealed fish during the fall of 2011 were growing at a high rate, highest since 2000, however we did not observe a difference in growth among different habitats. A majority of the fish collected during the FMWT survey were of the migratory life history type with only a few individuals being resident freshwater fish, however for the August TNS survey a large number of resident fish were observed in the Cache Slough Complex-Sacramento River Deep Water Ship Channel.

3. Morphometric and nutritional biomarkers may reflect relatively ‘good’ environmental conditions for 2011. Indices of CF and HSI were relatively similar from August to December suggesting that a majority of the fish were in relatively similar health throughout the fall. GSI increased with time and decreasing temperatures reflecting increased maturation going into the winter.

4. Nutritional biomarker, TAG, also exhibited similar indices from August to December suggesting nutritional status was similar throughout the fall. RNA/DNA increased with time and subsequently decreasing temperatures suggesting a relationship of improving RNA/DNA going into the winter.

5. Enzymatic biomarkers AChE and Na/K ATPase among the delta smelt sub-sampled from August-December 2011 indicate that approximately 60% were within normal ‘good’ range. However, some fish showed lower enzyme activity indicating that contaminants may also play a role in the health of Delta smelt.

6. Pathogens are present in most water bodies and only become problematic for fish when exposed to stressful environmental conditions. The current study showed that the presence of bacterial organisms among delta smelt is influenced by the synergistic interactions of three environmental stressors: turbidity, salinity, and water temperature. Delta smelt carrying *Mycobacterium* DNA are more pronounced in locations with significant interaction of conductivity and water temperature.

7. Histopathological analysis indicates fish surviving to December month in the <1 psu salinity are healthier than fish sampled in September –November months.

8. Correlation, canonical and clustering analysis indicated that combinations of salinity, turbidity, and temperature significantly affect the outcome of most health parameters except TAG and Na/K ATPase

9. The optimal mean turbidity and temperature for over 50% of the total fish sampled at 0-2 psu salinity with threshold condition factor of 0.6-0.8 were 59.15 NTU and 13.25 ^{o}C .

In this **preliminary report**, a concerted effort was initiated to develop a baseline fish health data. Fish health in the San Francisco Estuary is poorly understood because of the lack of baseline information on the health status of many threatened and indicator fish species. Baseline fish health is critically relevant due the prevalence of stressors in the SFE that have disrupted and will continue to render debilitating effects to the estuary and its fishery resources. Preliminary results of this current study provide a valuable reference to evaluate improvements or degradations of fish health. Due to time constraints, complete integrative analyses of multiple endpoints (otolith growth, migratory versus resident, histopathology, stomach contents, and abundances) to current data are in progress. Furthermore, growth condition and reproductive performance of adult smelt collected between Jan 2012 and May 2012 have not been studied.

Because an ecosystem perspective on fish health is critical to understanding the types of management approaches that need to be implemented in the SFE, fish health must be examined from multiple wet and dry years to provide robust data for future management protocols to address the degradation of fish health. By understanding the incremental and cumulative effects of multiple stressors, baseline fish health information will provide critical insights into system-wide patterns of normal prevalence and distribution (spatial and temporal) of different life stages and their ability to sustain growth and reproduction following exposures to environmental and anthropogenic stressors.

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