The Role of Contaminants, within the Context of Multiple Stressors, in the Collapse of the Striped Bass Population in the San Francisco Estuary and its Watershed

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EXECUTIVE SUMMARY

DESCRIPTION OF THE PROBLEM

Abundances of several fish species in the Delta/Suisun Bay have declined recently. Although these recent declines are, in part, consistent with natural long-term decadal-scale oscillations, certain features suggest additional anthropogenic-induced mechanisms. These unusual features include a possible step-like decline near the turn of the century and a failure of abundance measures to favorably respond to recent years of high precipitation and Delta inflows. Possible anthropogenic-induced mechanisms, as currently outlined in the POD conceptual model, include: (1) food web interactions, (2) toxicity, and (3) water project operations.

PROJECT OBJECTIVES AND APPROACHES

The factors and associated interactions contributing to the decline of pelagic fish in the Bay-Delta are admittedly complicated and multifaceted. Understanding issues of these types generally require a breadth of analytical techniques and a highly interdisciplinary team of investigators. For the study proposed herein, we have assembled a group of individuals encompassing backgrounds in histopathology, analytical chemistry, geochemistry, toxicology, protein profiling, ecological modeling, spatial mapping, microbiology and parasitology.

The underlying hypothesis of our research is that interactions of multiple stressors in the Bay Delta region have substantially contributed to the decline of the striped bass population. Specific hypotheses include:

1. Sublethal exposures of early life stages of striped bass to environmentally relevant concentrations of contaminants induce pathological and physiological changes that adversely affect subsequent survival and ultimately population numbers (Okihiro 1990; Bailey, Ostrach et al. 1991; Bailey et al. 1993; Bennett et al. 1995; Ostrach 2006; Ostrach, et al., 2008)
2. Evaluating contaminant interactions in regions with the broad range and history of uses such as the Bay Delta is more productively accomplished by addressing the contaminants as a unit, rather than a list. This uncovers indirect as well as direct toxic effects such as depletion of effective immune response mechanisms and reproductive efficacy while addressing the interactions of multiple stressors (Springman 2005)
3. Health effects associated with the interaction of multiple stressors in the Bay Delta have substantially contributed to the decline of the striped bass population. These include: (i) sublethal exposure to contaminants, (ii) food limitation, and (iii) disease.

The overall goal of our research program is to assess the significance of contaminants relative to other factors in the POD conceptual model on the observed decline of the striped bass population. The primary objective of the study outlined herein is to assess the health status of larval, juvenile, and adult female striped bass collected from selected locations in the Bay Delta using morphometric, histopathological, otolith (aging, growth and microgeochemical analyses) and biochemical metrics. The primary objective of this study is a component of our overall research goal, and findings from this study will help shape and guide research in outgoing years. Specific study objectives, associated tasks, progress and findings to date are outlined below.

SUMMARY OF FINDINGS

1) 2006 Egg contaminant data has been completed and reported in the Summary and Findings section of this report. However, due to a lack of funds POD managers were unable to analyze egg samples from the 2007 developmental studies or those provided by the Chesapeake Biological Laboratory from 3 locations on the East Coast of the United States for comparative analysis. Results indicate that maternal transfer of xenobiotics is continuing to occur as confirmed by the 2006 egg data. PCBs, PBDEs and pesticide levels in the 2006 egg samples from all Sacramento River collected female striped bass, one San Joaquin female and several females collected from the Mokelumne River are similar to or greater than those seen in the earlier studies conducted

in 1999 & 2001. Egg contaminant data verifies developmental abnormalities seen in the 2006 larvae from Sacramento River collected female striped bass and is consistent with results from the earlier studies in 1999 and 2001 (Ostrach et al., 2008). In 2007 the developmental abnormalities were virtually identical to those of the 2006 study inferring the continual maternal transfer of xenobiotics and adverse affects on larval striped bass. However, without chemical analysis of the egg samples no definitive conclusion can be made regarding the 2007 developmental studies. The 2007 egg samples and those from the East Coast of the United States are archived and stored at -80^{o} C for analysis when/if funding becomes available.

2) Gross lesions seen in developing 2006 larvae (abdominal edema, finfold edema, brain edema and necrosis of epithelial tissues) from Sacramento River female striped bass were seen with similar frequency and severity in the 2007 developmental studies of Sacramento River female's developing larvae. The majority of these lesions were not seen in hatchery larvae and the incidence of any lesions in hatchery larvae was extremely small in comparison (Figures 4-7). These lesions have been confirmed by histopathology for both years studied. In addition, histopathology revealed lesions in the brains of the river larvae from both 2006 and 2007 consistent with xenobiotic exposure as reported in other studies (figure 7).

3) Acetylcholinesterase (AChE) in the 2007 larval study did not show differing levels of AChE between river, river-domestic cross and control domestic progenies. This result is in contrast to the previous 2006 larval study and the explanation is found in the corresponding section below. The 2007 findings did confirm the 3 fold increase in AChE reported during development between day 1 and day 5 post-hatching.

4) Histopathological results from 2007 were similar to the findings in juvenile striped bass from surveys conducted in 2005 and 2006. Specifically, the most common findings include external protozoan parasitic infections that primarily involved the branchial tissues and included *Trichodina* sp., coelomic granulomatous foci that were presumed to be due to a trematode infection and sessile ciliated protozoans. The level and severity of the infections seen in 2007 are considered abnormal for striped bass and juvenile fish in general. Viral isolation performed on selected fish from the 2007 survey did not result in the isolation of any viral agents. The level and severity of the inflammation in the fish from 2005 with trematodiasis, and the external protozoan infections in fish from 2006 and 2007 are considered a significant impact on the health status of the fish and subsequently the population that may result in morbidity and/or mortality in affected fish or may further compromise fish especially juvenile fish. Results may be attributed to immuno-suppression caused by sub lethal contaminant exposure.

5) Immunohistochemical (IHC) findings in 2007 indicate that the vast majority juvenile striped bass are under sub lethal contaminant exposure as measured by P450-1A1 expression. This is the 3rd year in a row where IHC results indicate that the vast majority of juvenile striped bass are under sub lethal contaminant exposure throughout their entire range and first 6 months of life. This type of sub lethal contaminant exposure causes physiological stress and likely immuno-suppression which may explain the abnormal findings of parasitism and disease in these juvenile striped bass.

6) EROD (quantitative measure of P450-1A1 induction/sub lethal contaminant exposure) results are completed and indicate that the majority of juvenile striped bass (~65%) collected for biochemical metrics from August 2007 through January 2008 were found to be under EROD induction. This corroborates the IHC findings using this biochemical assay. This is the 3rd year in a row where EROD results indicate that the vast majority of juvenile striped bass are under sub lethal contaminant exposure throughout their entire range and first 6 months of life

7) Vitellogenin and metallothionein results indicate a significant portion of juvenile striped bass are expressing these two biomarkers in addition to the P4501A1 biomarker at elevated levels. Vitellogenin expression indicates exposure to estrogenic compounds/estrogenic mimics. Vitellogenin expression was found in 22% of the juvenile striped bass evaluated for this biomarker. Metallothionein is induced by heavy metals including zinc, cadmium, and mercury as well as by other chemical factors and severe oxidative stress and was found in

34% of juvenile striped bass evaluated for this biomarker. These findings coupled with the IHC and EROD findings indicate that these juvenile striped bass are under sub lethal contaminant exposure of several types causing physiological stress and likely immuno-suppression.

8) In the 2007 juvenile striped bass evaluated with these 3 biomarkers of contaminant exposure 33% were found to be under multiple types of sub-lethal contaminant exposure. Interactions between the physiological systems involved when multiple types of contaminant exposure such as these is complicated and can lead to an underestimation of exposure. Therefore these findings of positive single and multiple biomarkers are a conservative if not underestimate of the percentage of the juvenile striped bass population that are under sub-lethal contaminant exposure in the San Francisco Estuary causing physiological stress and likely immuno-suppression. These findings combined likely result in immuno-incompetence, morbidity and further compromise the health status of this population of juvenile striped bass during all 3 years of investigations.

9) AChE results in juvenile striped bass from 2007 are consistent with 2005 and 2006 findings. None of the individuals sampled in the field indicate a significant inhibition of AChE. This is not an unexpected finding. AChE inhibition occurs when fish are being exposed to significant levels of pesticides or neurotoxic agents and levels required for inhibition are not typically seen where these fish were collected. Findings indicate that AChE levels in juvenile striped bass vary with age and temperature. In addition, fish sampled in the San Joaquin River had lower AChE activity than fish from the Sacramento River in August, in October and January fish collected in Suisun Bay had lower AChE activity than fish sampled in the Sacramento and San Joaquin rivers. The lower levels observed may be due to physiological differences at those sites but not resulting from these natural factors. Further investigation is required.

10) Juvenile striped bass age and growth through ~100 days of age was determined by otolith analysis in 2006 and 2007. We compared growth between 2006 a relatively wet climactic year and 2007 an extremely dry year. We had hoped to compare growth regionally but unfortunately sampling was not performed in a uniform manner during both years so the comparison was made across all regions sampled in the estuary. In 2007 the instantaneous growth rate was 0.250 d^{-1} with an r^2 value of 0.705 (Figure 26). In 2006 the instantaneous growth rate from all locations sampled was 0.235 d^{-1} with an r^2 value of 0.718 (Figure 25) indicating very similar growth in both years despite the difference in climate and that the samples were not from all of the same locations. To better determine age and growth differences in juvenile striped bass collected from various regions and climactic conditions in differing years a more consistent yearly sampling protocol must be put in place. It is likely that during dry versus wet years juvenile striped bass are found in different abundance and in different habitats with some being more suitable for better growth and survival. We can state that from the limited samples collected in 2006 and 2007 across all regions growth was very similar in these two very different climactic years

11) Otolith microgeochemical analysis of habitat use in adult striped bass indicates that the vast majority of adult female and male striped bass evaluated in this study (n=170) encompassing a very broad area of the San Francisco Bay Estuary system including fifteen fish collected in the Pacific Ocean are exploiting the freshwater and delta habitats while not spending any appreciable time in the Pacific Ocean environment. A few fish make periodic trips to the ocean presumably to feed. Although subgroups may exist within three of the locations evaluated thus far (Mokelumne River, Clifton Court & the outer bays) additional striped bass collected at each of these locations appear to be nonresident fish exploiting other areas of the estuary as well as the location of capture.

12) PBDE & PCB levels of 31 female striped bass collected in 1999, 2001 and 2006 were examined and correlated with their habitat use. A slightly positive correlation was found between delta residence and PBDE levels. However with the addition the 2006 samples this correlation was less than previously reported. No correlation was found between PBDE accumulation and residence time of striped bass between Carquinez Strait and the Pacific Ocean. <u>However, findings indicate that PBDE bioaccumulation is positively correlated to fork length of female striped bass.</u> This is a new finding that may indicate that the bioaccumulation of PBDE's

and maternal transfer is different as compared to other lipophilic contaminants such as PCBs and pesticides. The correlation between PBDE levels and fork length suggests that female striped bass do not maternally transfer the entire PBDE load to their eggs but retain and continue bioaccumulate higher levels as the fish get larger/older. It may also suggest that the metabolism of PBDE in striped bass and maternal transfer to the eggs is different in this relatively new class of compounds than in other lipophilic contaminants. These results indicate that further investigation of PBDE bioaccumulation and physiology is necessary to fully understand these results. No correlation was seen between PCB levels and delta residence as previously reported. A slightly positive correlation was found between PCB bioaccumulation and residence time of striped bass between Carquinez Strait and the Pacific Ocean. These results are consistent with sediment sampling that indicates higher PCB levels in the outer bays and higher PBDE levels in the Delta. No correlation was found between PCB accumulation and fork length of female striped bass in this study.

SUMMARY CONCLUSIONS

The overall goal of this research program was to assess the significance of contaminants relative to other factors in the POD conceptual model on the observed decline of the striped bass population. The primary objective of the study focused on assessing the health status of larval, juvenile, and adult female striped bass collected from selected locations in the Bay Delta using morphometric, histopathological, otolith (aging, growth and microgeochemical analyses) and biochemical metrics. In 2005 investigations began on a limited basis followed in 2006 and 2007 by more comprehensive investigations. Significant progress has been made during these past three years and has identified contaminants as a significant stressor on early life stage striped bass throughout the first 6-8 months of life. Maternal transfer of xenobiotics and severe adverse effects on larval development and subsequent survival has been documented over an extended period of time (1999-2006) and is likely causing population level effects. It has been determined that the vast majority of juvenile striped bass are suffering from sub-lethal contaminant exposure of several types during all three years studied causing severe physiological stress, morbidity and likely compromising the immune systems of these fish. Findings of abnormal disease and parasitism were found in juvenile striped bass in all three years studied and are considered to have a significant impact on the health status of the fish and subsequently the population. In addition, data suggests that adult striped bass are also likely adversely affected by the bioaccumulation of contaminants such as PBDEs. As such contaminant effects need to be considered a significant stressor that is affecting the decline of striped bass and are likely causing population level effects in the early life stages. It must be noted that contaminant effects are one of many stressors affecting this and the other pelagic fish species in the San Francisco Estuary. The results from this three year investigation clearly indicate contaminants are one of several significant stressors adversely affecting the pelagic fish and the ecosystem in this estuary.

Objective 1: Assess health status of gravid females and mature males.

Task 1.1. Collect gravid female and male striped bass twice during the spawning season.

Task completed.

May 15, 2007: five gravid female striped bass ranging in size from 6 lbs.- 25 lbs. and twelve adult male striped bass were collected upstream of Knights Landing on the Sacramento River using standard electro-fishing techniques.

May 30, 2007: five gravid female striped bass ranging in size from 8 lbs.-45 lbs. and eight adult male striped bass were collected upstream of Knights landing on the Sacramento River. This was a poor year spawning run (short season, very hot weather and low flows) and it was difficult to find gravid females on the river. Three males and one female were collected at the fyke traps with DFG personnel during this second sampling trip so we could have enough fish to attempt to spawn.

Task 1.2. Assess the health status of gravid female and male striped bass at the time of capture as evident by gross observations and parasitology.

Task completed.

Gross observations were recorded at the time of capture noted in task 1.1 above. Fish captured appeared healthy, few gross lesions & /internal/external parasites detected. More detailed observations were recorded during necropsy.

Task 1.3. Analyze the type and concentration of selected compounds in the eggs collected during spawning. Compounds will include but are not limited to, trace elements, pesticides, PCBs, PBDEs, PAHs, pharmaceuticals, total lipids & lipid type and protein. Chemical analyses will be performed by DFG Fish and Wildlife Water Pollution Control Laboratory.

Task 75% complete.

Eggs were collected just prior to spawning from four domestic/control females and eleven Sacramento River collected striped bass. Egg samples are archived at -80 C.

In addition, 25 eggs samples were obtained from the Chesapeake Bay and Albemarle Sound (near the Roanoke River) on the East Coast of the United States from 2007 spawning runs for comparative analysis. Egg samples are archived at -80 C.

POD managers indicated that they did not have sufficient funds to analyze these egg samples as per the terms of this contract (egg samples were to be analyzed by DFG's Fish and Wildlife Pollution Control Laboratory using POD chemical analysis funds). The egg samples from the SFE and East Coast of the US will be archived until/if funding can be obtained to perform the required analysis. As a result no definitive conclusions can be made from the 2007 study regarding the developmental comparisons of progeny from River collected female striped bass and Hatchery reared female striped bass.

Task 1.4. Histopathological and immunohistochemical analysis of visceral organs (of adult female & male striped bass collected for spawning)**.**

Task completed.

Female and male striped bass from the Sacramento River were euthanized and necropsied post-spawning. Histopathological results were unremarkable. There were no significant findings to report.

Task 1.5. Age and microgeochemical analyses of otoliths to assess possible migration patterns and habitat use.

Task completed.

Otoliths from nine female Sacramento River collected striped bass ranging in weight from 8 – 45 pounds and nine adult male striped bass ranging in weight from 5 – 13 pounds were successfully removed, coded, cleaned and measured (Table 10a). Otoliths were prepared for aging and microgeochemical analysis. Analysis is complete, habitat use plots were generated (see Appendix A, Figures 12a-28a) and data included in histograms of habitat use by region (Figures 32, 33).

Objective 2: Assess egg and larval development.

Task 2.1. Spawn and fertilize eggs obtained from hatchery controls and striped bass collected in the field. Rear embryos/larvae through five days post hatch.

Task completed.

Three domestic broodstock female striped bass were spawned successfully. One female was spawned on May 2, 2007 and two females on May 9, 2007. Eggs and larval samples were collected from 12 hrs post fertilization through seven days post hatch for developmental and biochemical studies.

Six female striped bass were captured using electro-fishing on the Sacramento River on May 15, 2007 and were successfully spawned between May 16 & May 17, 2007. Developmental samples were collected from three females (the other three crashed during development).

Four female striped bass were captured using electro-fishing on the Sacramento River on May 30, 2007 and two females were collected from DFG's fyke traps located just upstream of Knights Landing. Three of the females collected on the river were successfully spawned between May 31 & June 1, 2007.

Attempts were made to spawn the two females collected from the fyke traps. One fish was not spawned as it was determined that the eggs were breaking down and in the process of being reabsorbed likely due to the stress of fyke trapping and transport. The other female was spawned but the eggs died during development with none surviving to hatching.

Task 2.2. At days 1, 3, & 5 post hatch, analyze developing embryos/larvae for morphology and biochemical metrics.

Task completed.

Morphological studies of larvae at 1, 3 & 5 days post hatch were successfully conducted *in vivo* for the domestic larvae from three female striped bass and the larvae from five river collected striped bass. In addition we successfully fertilized a portion of two of the river collected females eggs with sperm from domestic striped bass for comparative analysis. Three larvae from each female at each of the developmental periods were anaesthetized using MS-222 and photographed in various orientations using an inverted compound Olympus microscope. A total of 205 micrographs (microscopic photographs) were obtained from the domestic larval study and 630 micrographs were obtained from the larvae of river collected female striped bass.

Larvae from three female domestic striped bass and larvae from six river collected striped bass were collected and preserved in 10 % formalin at the above listed developmental stages for morphological and histological studies. In most cases ≥40 larvae/female/developmental stage were preserved for these analyses.

During these sampling events larvae were also sampled in triplicates, frozen and stored at -80° C for biochemical and DNA fragmentation analyses. AChE analysis is completed (Figure 11). The EROD assay was not sensitive enough to detect CYP1A1 activity in the 2006 larval sample. Development of a more sensitive competitive ELISA has been completed. The ELISA has been optimized and 2006 and 2007 samples were evaluated for CYP1A1 activity/induction.

Morphometric measurements obtained from archived photographs of the larvae from the maternal transfer study (Ostrach 2006; Ostrach et al., 2008) were be used to compare larvae from the 2007 spawning of domestic striped bass, river collected striped bass and river/domestic crosses to this previous study and to morphometry collected from 2006 larvae.

Objective 3: Assess health status of 2007 field collected striped bass larvae and juveniles.

Task 3.1. Analyze for diseases, parasites, viral load and biochemical markers in fish collected during 'special sampling events' (CDFG arranged sampling for POD researchers ~ bimonthly).

Task completed.

Special sampling events were conducted on the following dates: May 8 & 23, 2007; July 2 & 17, 2007; August 16, 17, 28 & 29, 2007; September 21, 24 & 25, 2007; October 30, 2007; November 19, 20, 2007; 27 & 28; December 19 & 20 2007; January 29 & 30 2008 (Table 4a-7a).

A total of 154 striped bass were collected for histopathological evaluation, 194 for biochemical analysis and 32 for viral assessment during these sampling events. Histopathological analysis is complete for all these fish (see summary in Summary and Findings section below).

EROD and AChE, vitellogenin and metallothionein biochemical assays have been completed (n=177- 192). Results are complete and shown below in the Summary & Findings section.

Samples collected for viral assessment for the months of June through December 2007 have been analyzed. No cyto-pathological effects (CPE) were observed indicating an absence of virus in these samples.

Task 3.2. Histopathological and immunohistochemical analyses of fish collected by CDFG surveys and special sampling events.

Task completed.

DFG staff delivered Townet Survey (n=121), Summer Kodiak Trawl (n=10) and Fall Midwater Trawl (n=62) to our laboratory (Table 4a-7a). Samples totaled 193 fish bodies preserved in formalin and the heads preserved in ETOH for otolith ageing and growth studies. From POD special sample surveys 154 juvenile striped bass were collected with bodies and one half heads preserved in formalin and the other half head preserved in ETOH for otolith analysis (Table 8a). All fish have been prepared and analyzed for histopathology with a subset subjected to immunohistochemistry analyses.

A summary of findings is found below in the Summary and Findings section. The final full pathology reports are found in the Appendix.

Task 3.3. Age and growth analyses of a subset of otoliths collected by DFG surveys and special sampling events.

Task completed.

DFG staff delivered 193 juvenile striped bass heads preserved in ETOH (Table 4a-7a) and during our POD special survey our staff collected 140 half heads for otolith analysis (Table 8a) the other ½ head was preserved in formalin for histopathological analysis). Otoliths have been extracted and prepared for analysis. Otolith priorities were given to fish from the early summer when fish are most likely subjected to high rates of predation. Daily aging of otoliths from striped bass beyond ~120 days of age is difficult and accuracy limited (Secor personal communication and past experience) so samples believed to be over 120 days old are the lowest priority. Final results are found below in the Summary and Findings section.

These results were compared to results from 2006 to determine if growth rates differ significantly during different climactic years (wet vs. dry year) and results are found in the Summary & Findings section.

Objective 4: Characterize habitat use of striped bass.

Task 4.1. Obtain female striped bass heads and intact otoliths collected by CDFG from the Sacramento River, San Joaquin River, American River, Feather River and Pacific Ocean.

Task completed.

Ten Sacramento River female striped bass and nine male striped bass were collected during two electro-fishing events under task 1.1 (one female died and was not coded so the otoliths were not analyzed).

Eleven adult striped bass heads were collected by DFG's Creel Census and provided to us for aging/habitat use analysis. DFG/IEP staff was unable to obtain any additional fish heads/otoliths from other locations. However, otoliths from other locations were obtained in collaboration with SFEI as follows.

In collaboration with SFEI 90 adult male and female striped bass adult striped bass otoliths were provided from the RMP & FMP surveys in support of our research from various areas in the estuary system including: San Pablo Bay (n=23); Berkeley/Central Bay(1); South Bay (n=1); Rio Vista Fish Derby (n=2); Cache Slough (n=1); Cosumnes River (n=1); Miner Slough (n=1); Clifton Court Forebay (n=20) collected outside the radial gates); Liberty Island (n=21); Toe Drain (n=3); and Knights Landing (n=16). We reported results of twelve of these in our last report but have included all 90 here.

Otoliths were successfully removed, coded, cleaned and measured (Table 10a). Otoliths have been prepared for aging and microgeochemical analysis.

Task 4.2. Age and microgeochemical analyses of otoliths to assess possible migration patterns and habitat use.

Task completed.

We were able to schedule 6 weeks of time at the LA-MC-ICPMS facility since our September 2007 report. All otoliths collected to date from sample collections listed in task 4.1 above and otoliths collected from Mokelumne River (n=11) and one from the San Joaquin River collected during the spring of 2007 that could not be analyzed due to time constraints on the LA-MC-ICPMS have been completed.

Analyses of the data collected from the LA-MC-ICPMS are completed. Results are presented in the Summary and Findings section following. Habitat use/life history plots and graphs of habitat use by region and during the last two years of life by region are found in Appendix A, Figures 1a-129a and Figure 32, 33.

Objective 5: Rear larval and juvenile striped bass.

Task 5.1. Attempt to rear hatchery and river collected striped bass larvae at UCD facilities through juvenile stages to support Dr. Inge Werner's & Dr. Lindsay Sullivan's POD studies.

Task completed.

Larvae and juvenile striped bass were successfully raised during the spawning events listed in Task 2.1 above and offered to Dr. Inge Werner and Dr. Lindsay Sullivan for their research purposes.

Dr. Werner's laboratory was involved in delta smelt assays and decided not to use any striped bass in her 2007 spring studies.

Early life stage striped bass (9-19 days post-hatching) were provided to Lindsay Sullivan for her POD/CALFED funded feeding investigations on May 30, 2007, June 1, 2007 and June 19, 2007. Dr. Sullivan was also provided 30-33 day-post hatch larvae on July 3rd and July 6th.

Larval striped bass were moved to ponds for rearing. Rearing was moderately successful. This was mainly due to predation by backswimmer beetles (*Notonecta undulate*). Spring 2007 was very hot and dry and it was difficult to control or contain *Notonecta undulate* infestations. However, juveniles were produced in sufficient numbers for our and other researcher's requirements.

Juvenile striped bass are being maintained at UC Davis at the Putah Creek facilities and are being used for method development, verification studies for our biochemical metrics and for our USF&W SPMD/POCIS POD related physiological effects of water borne contaminants studies.

Juvenile striped bass from this effort are being provided on an ongoing basis to Dr. Juan Korenbrot of UCSF for use in his NSF funded studies on eye and retinal development. Dr. Korenbrot used to obtain striped bass for his research through a fish farm in Chico/CA that was forced to close. Our efforts have helped him to continue his important NSF research as there is no other source of control juvenile striped bass available.

Supplemental objective 6 (not in this contract): Additional research performed in support of this POD project.

6.3. Studies pending in support of POD research

As mentioned in section 6.2. above there is currently an ongoing collaboration between fellow POD researcher Dr. Inge Werner's laboratory in the development and use of new molecular biomarkers to assess sublethal stress, immune responses and xenobiotic impacts on striped bass. We have continued to collaborate with Dr. Werner in our ongoing POD field and experimental investigations throughout 2007 and 2008.

Our laboratory is collaborating with Dr. Lindsay Sullivan a postdoctoral fellow in Dr. Wim Kimmerer's laboratory on her CalFed funded project investigating feeding behavior of larval and juvenile striped bass. We provided fish, technical support, experimental support and facilities at UC Davis to Dr. Sullivan in support of her CalFed project during the 2007 & 2008spawning seasons.

We are partners in a U.S. Fish and Wildlife service funded contract led by Cathy Johnson with Dr. William Brumbaugh of the CERC USGS and the Department of Fish and Game's Wildlife Water Pollution Control Laboratory (Dave Crane) titled: **"**CA - Contaminants Profile for the San Francisco Bay – Sacramento San Joaquin Delta and Effects of Contaminants on Resident Fish Species." In these funded studies SPMD's and Polar Organic Chemical Integrated Samplers (POCIS) have been deployed and retrieved monthly at five sites in the estuary (Little Honker Bay, Goodyear Slough, Boynton Slough, Sherman Lake & North Cache Slough) year round to determine and quantify water borne contaminants found within the Bay Delta system. Our laboratory is performing experiments on juvenile striped bass to determine the effects of these waterborne complex contaminant mixtures from the various sites. We have offered as partners to dedicate significant personnel and resources to this project. We received funding in late September 2008 and work is scheduled to be completed by June 2009. The first 10 monthly samples from the five locations provided by USF&W extracts were injected into control juvenile striped bass for histopathological, biochemical and molecular biomarker evaluation. Preliminary results are completed and indicate several sites are positive for one or more of the following biomarkers of sub-lethal contaminate exposure/physiological stress: EROD, CYP1A, vitellogenin and metallothionein. Results indicate that complex contaminant mixtures found in the water from these sites is causing sub-lethal contaminant exposure/physiological stress to fish living at those sites during those time periods. This is an important new finding in that it indicates water quality at several delta sites alone can cause significant biomarker expression and physiological stress to fish potentially affecting growth, behavior, reproduction and survival.

Our laboratory has also entered into collaboration with SFEI and Moss Landing Marine Laboratory to further investigate striped bass migration histories as they relate to the bioaccumulation of contaminants using otolith microgeochemical techniques. The initial project involves working with Jon Walsh a master's student at Moss Landing and assisting him with the scientific and technical aspects of the project as well as providing support services here at UC Davis. This project has provided additional otoliths, and will provide additional contaminant data in support of our currently funded striped bass POD investigations. SFEI has provided us with 90 adult striped bass otoliths for microgeochemical analysis. All 90 have been analyzed and data from various sites is shown in (Figures 32, 33, 40a-129a). SFEI will also be providing tissue levels of contaminants found in these fish including mercury, organics and trace elements.

In collaboration with Dr. Stuart Meyers we have preserved sperm samples in 2007 for various assays (Annexin, TUNEL, motility, activation, lipid etc.) to investigate paternal effects caused by the bioaccumulation of xenobiotics.

We have also worked with Joan Lindberg, Brad Bridges and Theresa Rettinghouse in collaboration with Dr. Stuart Meyers to develop and refine methods to cryopreserve Delta Smelt sperm to maintain the genetic integrity of the wild population. We have made excellent progress in our first pilot studies in this area. Progress on this project will be reported by Theresa Rettinghouse, Joan Lindberg and Brad Bridges.

SUMMARY AND FINDINGS

Analyze the type and concentration of selected compounds in the eggs collected during 2006 spawning: Data unavailable during last reporting period.

Egg analysis was not completed when the final year 1 report was submitted in September 2007. The analysis is complete and the results shown below in Figures 1, 2, 3b, 3c and Table 1a-3a.

Developmental abnormalities, gross and histological lesions were observed in the progeny of all larvae from the river collected females and were consistent with maternal transfer of xenobiotics as documented in the earlier studies conducted in 1999 & 2001 (Ostrach et al., 2008). However, confirmation of the effects seen could not be verified until chemical analyses of the eggs were completed. The chemical analysis of the 2006 eggs was found to be similar to the findings in the earlier studies. The hatchery eggs contained far fewer contaminants and at lower levels than in the earlier study. This is likely due to increased regulation and better formulation of commercial fish food. A portion of commercially manufactured fish food is comprised of ground up fish which are the source of the low levels of contaminants seen in the eggs of hatchery fish. The eggs from Sacramento River collected female striped bass contained similar levels of PCB as in the earlier studies, slightly higher levels of PBDE and similar levels of pesticides (Figures 1, 2, 3b, 3c and Table 1a-3a). Although DDT levels were lower than in earlier studies levels of DDT degradation products were slightly higher. The chemical analysis of the eggs confirms developmental abnormalities/lesions seen in the 2006 larvae from Sacramento River collected females are the result of maternal transfer of xenobiotics.

In addition to eggs collected from Sacramento River striped bass egg samples were collected from females captured on the Mokelumne River and one captured on the San Joaquin River in 2006. Contaminant levels in 4 of 8 females collected on the Mokelumne and the female collected on the San Joaquin were similar to those collected on the Sacramento River (Table 1a-3a). Two eggs samples collected from females on the Mokelumne had contaminant levels about ½ of those seen in the Sacramento River samples (Table 1a-3a). However, contaminant levels in 2 of 6 females collected on the Mokelumne River were found to contain contaminant levels comparable to or lower than the hatchery controls (Table 1a-3a). Habitat use patterns of these fish were evaluated it was determined that the fish with very low levels of contaminants were resident fish that lived their entire life in the Mokelumne River (Figures 2a & 7a). The striped bass captured on the Mokelumne River whose eggs showed xenobiotics levels similar to those of Sacramento River females' habitat use patterns indicated that two were non-resident fish utilizing delta habitats in a manner similar to the Sacramento River collected females and two were resident fish (Figures 1a, 3a-5a).

The analysis of the 2006 egg samples coupled with the habitat use profiles of the females provides significant new findings that indicate fecundity estimates of striped bass must be reconsidered. All of the female striped bass from the Sacramento River, San Joaquin River and some Mokelumne River had high levels of contaminants in their eggs that would affect larval development and survival. Therefore traditional measures of fecundity (egg number/female = egg/larval production) are no longer a true estimate of larval production. The variability of contaminant levels in the resident Mokelumne fish is an interesting finding that requires further investigation. The strontium isotope signature of the resident fish is clearly a Mokelumne River signature. The difference seen in the resident fish eggs may be due to differing diets of the individual fish. In addition, findings from the Mokelumne River resident fish indicate larvae from these females would have a much higher probability of developing normally and higher survival than larvae produced from females' exploiting the delta habitat. Although the Mokelumne River is not the preferred spawning habitat for striped bass these results indicate that sub-groups of resident fish living in relatively uncontaminated sites within the delta system may be contributing more to the striped bass population than originally considered.

In summary the egg contaminant data from 2006 confirms that significant maternal transfer of xenobiotics continues to occur in female striped bass. Findings indicated that the river larvae grew more slowly, growth ceased between day 3 and day 5 and these larvae had significant lesions not seen in the domestic larvae. Growth and developmental trends parallel that of the earlier maternal transfer study and indicate that serious problems with progeny from river collected striped bass continued to occur in 2006. The combination of poor growth, abnormal development and lesions encountered lead to the conclusion that very few of the river larvae would make the transition from the larval to juvenile stage and potentially affecting recruitment and the subsequent adult population levels.

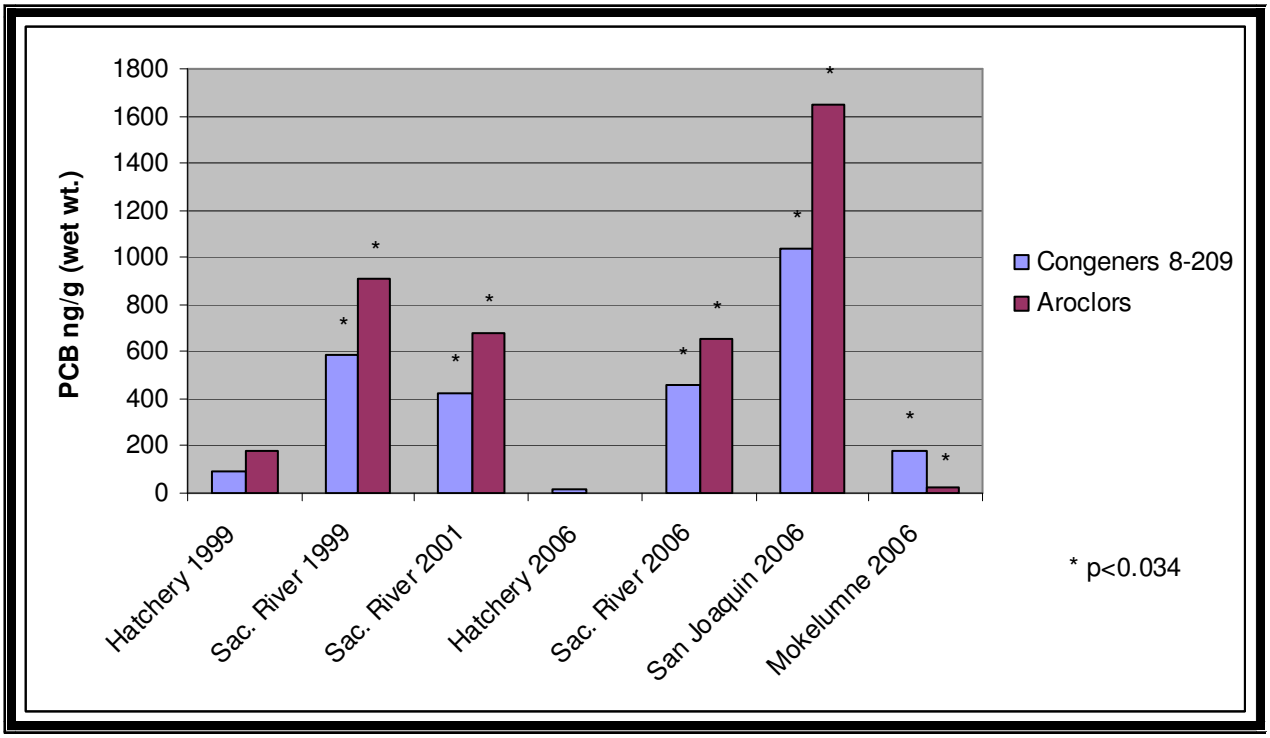


Figure 1. PCB contamination in striped bass eggs from river collected females compared with hatchery eggs in 2006 and from the earlier maternal transfer studies conducted in 1999 & 2001.

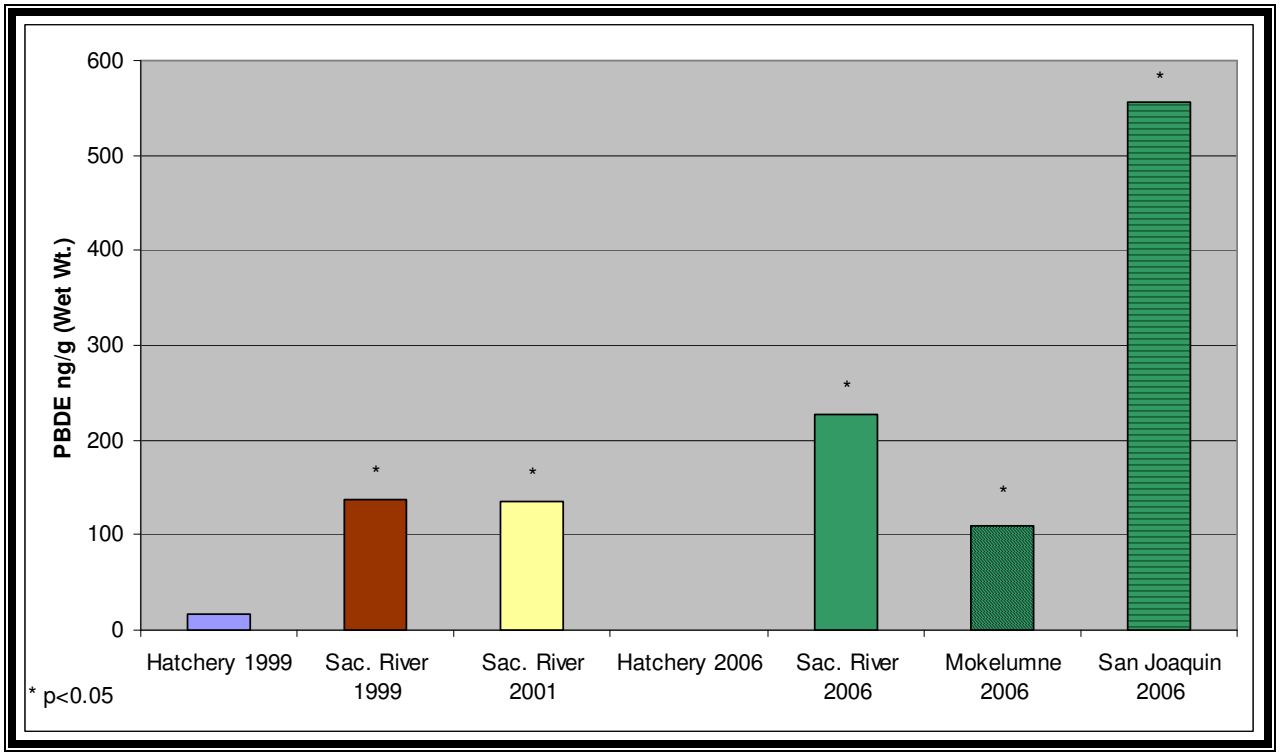


Figure 2. PBDE contamination in striped bass eggs from river collected females compared with hatchery eggs in 2006 and from the earlier maternal transfer studies conducted in 1999 & 2001.

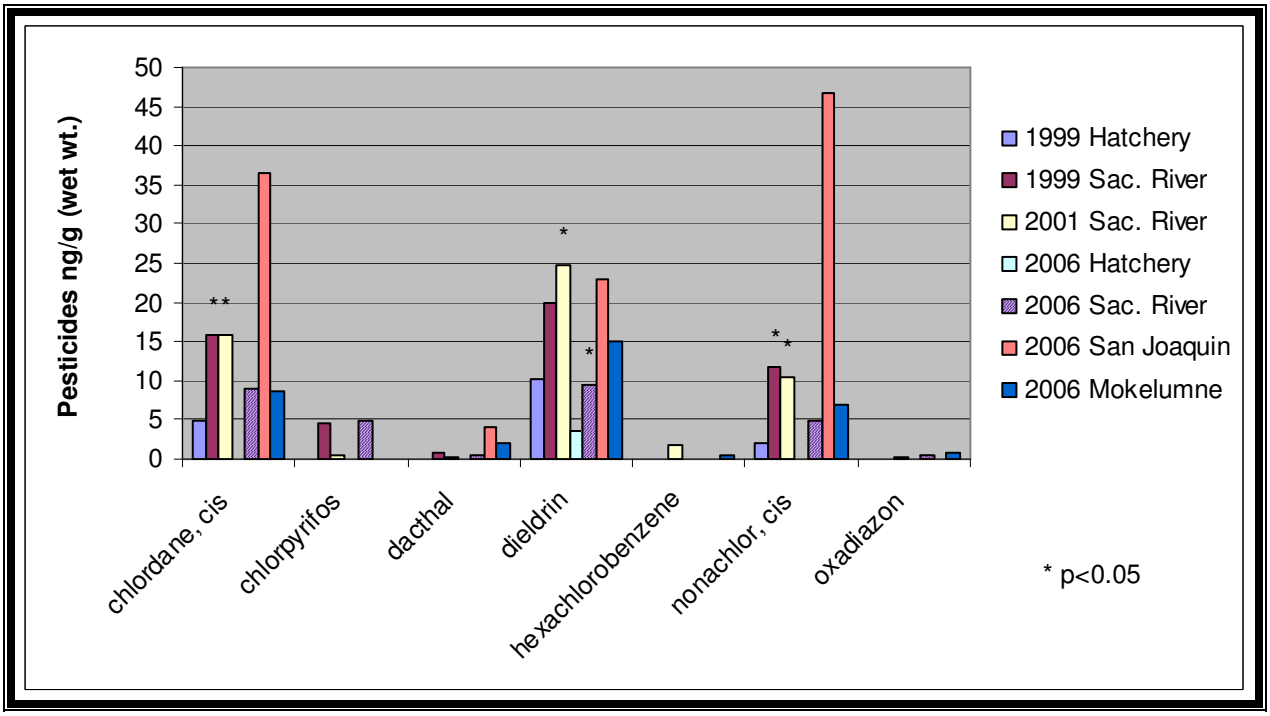


Figure 3b. Pesticide contamination in striped bass eggs from river collected females compared with hatchery eggs in 2006 and from the earlier maternal transfer studies conducted in 1999 & 2001.

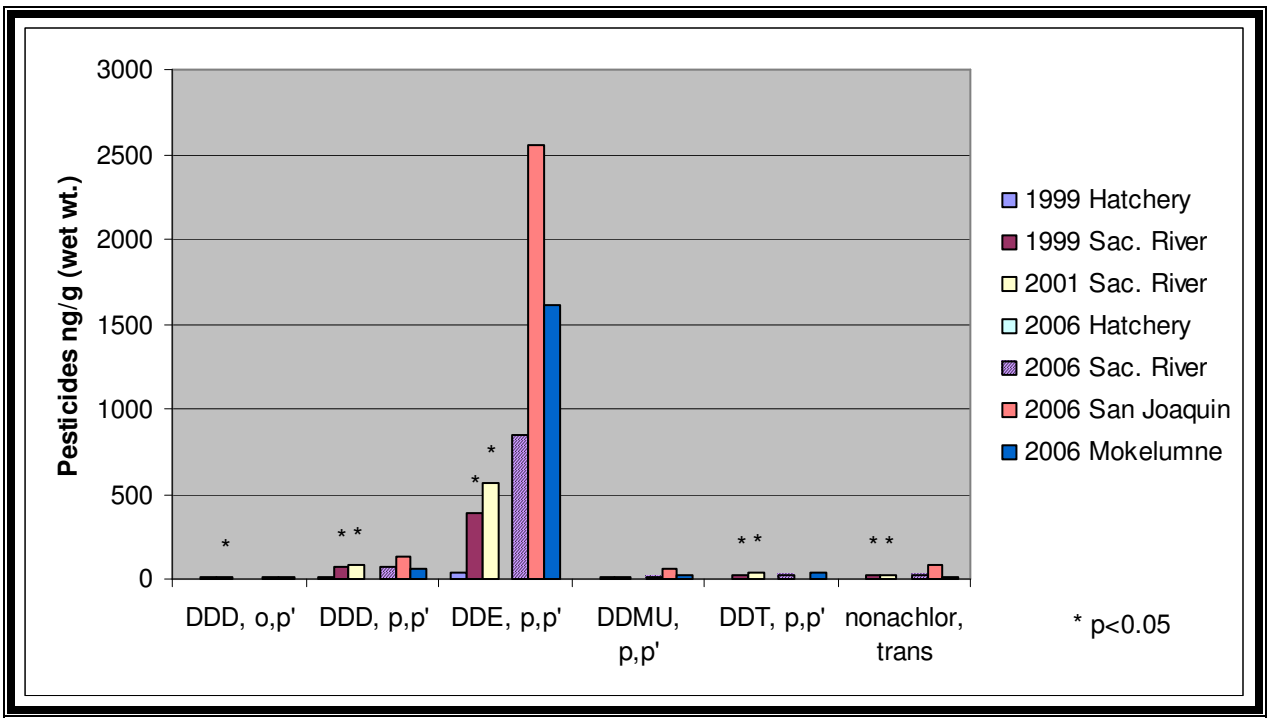


Figure 3c. Pesticide contamination in striped bass eggs from river collected females compared with hatchery eggs in 2006 and from the earlier maternal transfer studies conducted in 1999 & 2001.

Histopathological Confirmation of Gross lesions from 2006 developmental studies: 2006 Data unavailable during last reporting period.

In the 2006 developmental studies gross lesions were found in 95% of the larvae from river collected females and none of these lesions were seen in progeny from hatchery control female striped bass. Documentation of the lesions was provided in the year one final report. Histopathological analysis of a subset of the larvae was performed and provided confirmation of the gross lesions seen which included: Abdominal, brain and finfold edema; and necrosis of epithelium as shown below in Figures 4-6. In addition lesions were found in the brain of developing larvae from river collected females consistent with xenobiotic exposures and similar to those reported in earlier studies (Okihiro et al., 1990; Bailey et al., 1991; Bailey et al., 1994; Ostrach, 2006). The larvae from the river females exhibited necrotic lesions found mainly in the mid-brain undifferentiated neuroblasts and glial cells (Figure 7). The lesions were characterized by cytoplasmic pallor, nuclear fragmentation, areas of vacuolation and cellular pycnosis. The control larval brains appeared normal with no similar lesions present.

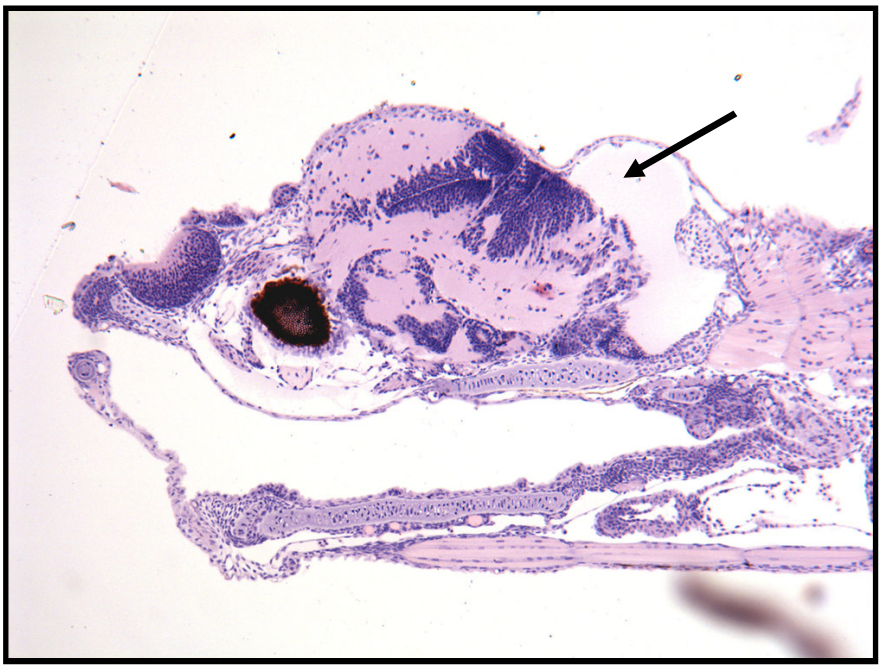


Figure 4. Brain edema (arrow) in 3 day post hatch striped bass larva the progeny of a river collected female.

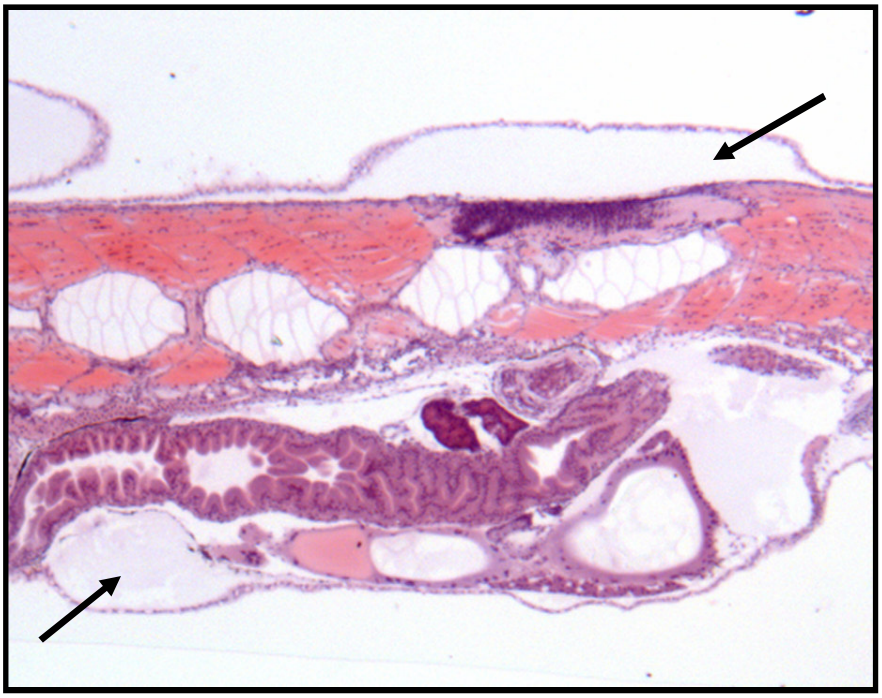


Figure 5. Finfold edema (top arrow) and abdominal edema (bottom arrow) in 3 day post hatch striped bass larva the progeny of a river collected female.

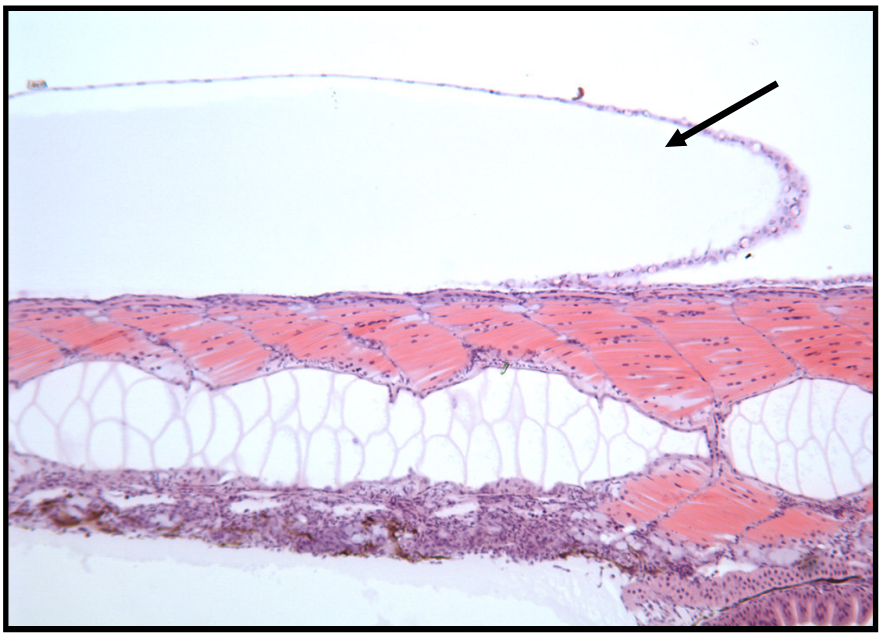


Figure 6. Finfold edema (arrow) and abdominal edema in 5 day post hatch striped bass larva the progeny of a river collected female.

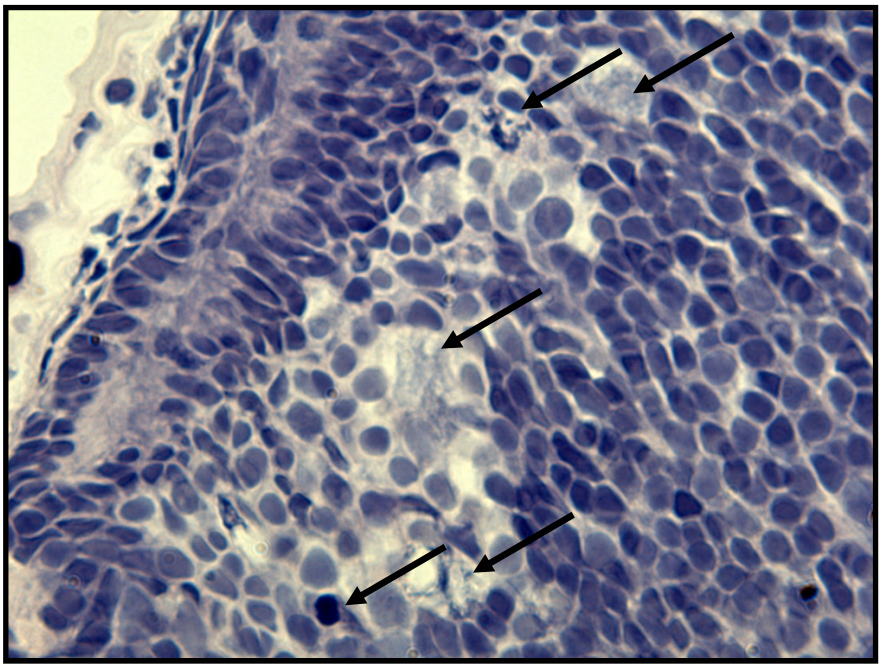


Figure 7. Brain lesion in 5 day post hatch striped bass larva the progeny of a river collected female. Arrows represent necrotic lesions of the undifferentiated neuroblasts and glial cells.

2007 Striped Bass Spawning and Developmental Studies

Three domestic broodstock female striped bass were spawned successfully. One female was spawned on May 2, 2007 and two females on May 9, 2007. Eggs and larval samples were collected from 12 hrs post fertilization through seven days post hatch for developmental and biochemical studies.

Six female striped bass were captured using electro-fishing on the Sacramento River on May 15, 2007 and were successfully spawned between May 16 & May 17, 2007. Of these six developmental samples were collected from three females (the other three crashed during development). Two of the six females were fertilized with sperm from three domestic hatchery striped bass to determine if any paternal effects might be affecting poor developmental/survival outcomes seen previously during maternal transfer studies conducted in 1999, 2001 & 2006. Of these one cross hatched successfully (31R07 x D) and developmental samples obtained. Egg and larval samples were collected throughout development from the 3 successful spawns/hatches through five days post hatching.

Four female striped bass were captured using electro-fishing on the Sacramento River on May 30, 2007 and two females were collected from DFG's fyke traps located just upstream of Knights Landing. Three of the females collected on the river were successfully spawned between May 31 & June 1, 2007. One of these was successfully fertilized with sperm from three domestic hatchery striped bass for the reasons as explained above. Attempts were made to spawn the two females collected from the fyke traps. One fish was not spawned as it was determined that the eggs were breaking down and in the process of being reabsorbed likely due to the stress of fyke trapping and transport. The other female was spawned but the eggs died during development with none surviving to hatching.

2007 Larval morphometric findings

Larvae produced from the three successful domestic spawns, larvae from six river collected female striped bass and larvae from the two domestic male/river female crosses were preserved in 10% formalin and photographed. Larvae produced from domestic females and river collected females were subjected to morphometric analyses to assess growth and development. This was the second time spawning and developmental experiments were performed at the UC Davis facility. For the maternal transfer study and in the previous 10 years all spawning and developmental work was performed at Professional Aquaculture Services in Chico California. Water temperatures at the Chico facility were approximately 2°C lower than those of the Davis facility. As such some differences in size and growth were seen between larvae reared at the different facilities (both domestic & river larvae were larger throughout the development period than in the maternal transfer study). However, results from 2006 & 2007 larval studies showed the same basic trends and problems with development as in the earlier maternal transfer study.

In the 2006 study larvae from one domestic female striped bass was compared to the progeny from five river collected striped bass. In 2007 the study was repeated with better spawning success. The 2007 study compared development of larvae from three domestic striped bass to the development of larvae from six river collected striped bass. In the 2006 study the river larvae were larger than the domestic counterparts but developmental abnormalities and growth were strikingly similar to results from the earlier the maternal transfer study. In both 2006 and 2007 one day post hatch river larvae were thinner and in appearance resembled a later stage than the domestic larvae as they did in the earlier study. Linear measurements are not the most accurate representation of growth and are why volume was used to evaluate growth in the maternal transfer study with a new technique rather than linear measurements (Ostrach 2006; Ostrach, et al. 2008). In 2006 and 2007 studies time and funding constraints prevented us from using the new volume measurement technique. Instead total surface area measurements were obtained as well as several linear measurements that can provide insight into growth and development. When total surface area (the sum of both lateral and dorsal surface areas) is analyzed an accurate representation of larval growth can be obtained (Figure 8b). In 2006 and 2007 the surface area results from the domestic females larvae indicate faster growth than their river counterparts between day 1 and day 3 and that growth continues from day 3 to day 5 post hatching (Table 1),. In contrast, although larvae from the river collected females surface area does increase between day 1 and day 3 it does so at a slower rate than the domestic larvae and surface area/growth of the river larvae decreases between day 3 and day 5 (Figure 8b & 8c and Table 1.). This indicates that the river larvae stop growing between day 3 and day 5 and they

begin to get smaller. This is a similar result to what was seen in the maternal transfer study. Results from measurements of the pectoral girdle also follow this trend. The domestic larvae's pectoral girdle measurement becomes smaller between day 1 and day 3 and then begins to show a slight increase in growth between day 3 and day 5 (Figure 9b & table 1). This follows normal striped bass developmental trends. When striped bass larvae are newly hatched they are a developing embryo early in organogenesis. Between day one and day three after hatching the embryonic larval shape develops into the larval form (longer and thinner more resembling a fish). In contrast the river larvae pectoral girdle measurement becomes smaller throughout the entire developmental period (Figure 9b). This is similar to what was seen in the maternal transfer study although in that study the river larvae did show slight positive growth at the pectoral girdle between day 3 and day 5. However in the first maternal transfer study (1999 & 2001) domestic larvae pectoral growth was over three times greater than the minimal growth seen in the river larvae (Table 1). The results from 2006 and 2007 studies indicate that as in the earlier maternal transfer study domestic striped bass larvae grow and develop in a normal manner whereas larvae produced from river collected females grow abnormally and more slowly during early development with negative growth seen during late development. In the 2006 study larvae from one of five river females spawned failed to survive beyond day four and in the 2007 study larvae from three of the six river females spawned failed to survive beyond day four. In the maternal transfer study, 2006 and 2007 larvae from all domestic females survived through day five as well as through first feeding (Day 6-7). These results indicate the vast majority of larvae from river collected females in all three studies are growing and developing abnormally adversely affecting subsequent survival, recruitment and likely adult population levels. The adverse results from the first maternal transfer study and the 2006 study are clearly a result of the xenobiotics transferred from river collected female fish to their progeny. The 2007 results are the same as in the previous two studies and also believed to be the result of maternal transfer of xenobiotics. This is a reasonable conclusion since all egg samples collected in 1999, 2001 & 2007 from Sacramento River spawning striped bass contained similar levels of xenobiotics (with some contaminant levels increasing between 1999-2006). However, a final definitive conclusion can not be made with out the chemical analysis of the eggs from the 2007 river collected female striped bass.

Growth	2007		2006		Maternal Transfer- 1999 & 2001	
	Hatchery	River	Hatchery	River	Hatchery	River
Body length Day1-3 (mm)	0.73	0.62	0.904	0.776	1.232	0.863
Body length Day3-5 (mm)	0.16	0.0101	0.032	0.04	0.376	0.376
Pectoral Girdle Day1-3 (mm)	-0.107	-0.068	-0.186	-0.109	-0.401	-0.174
Pectoral Girdle Day3-5 (mm)	0.0508	-0.052	0.003	-0.065	0.151	0.047
Surface area day1-3 (mm^2)	0.49	0.47	0.577	0.368	0.696	0.953
Surface area day3-5 (mm^2)	0.273	-0.27	0.163	-0.072	0.661	-0.151

Table 1. Growth in larvae from domestic (control) and river collected striped bass. 2006 and 2007 results confirm earlier results indicating that growth in larvae from river collected striped bass is abnormal and regresses between day 3 and 5 post hatching. Numbers represented in red indicate the significant abnormal growth detected in developing larvae from river collected striped bass.

Figure 8b. **2007 Larval Striped Bass Sum of Surface Areas**

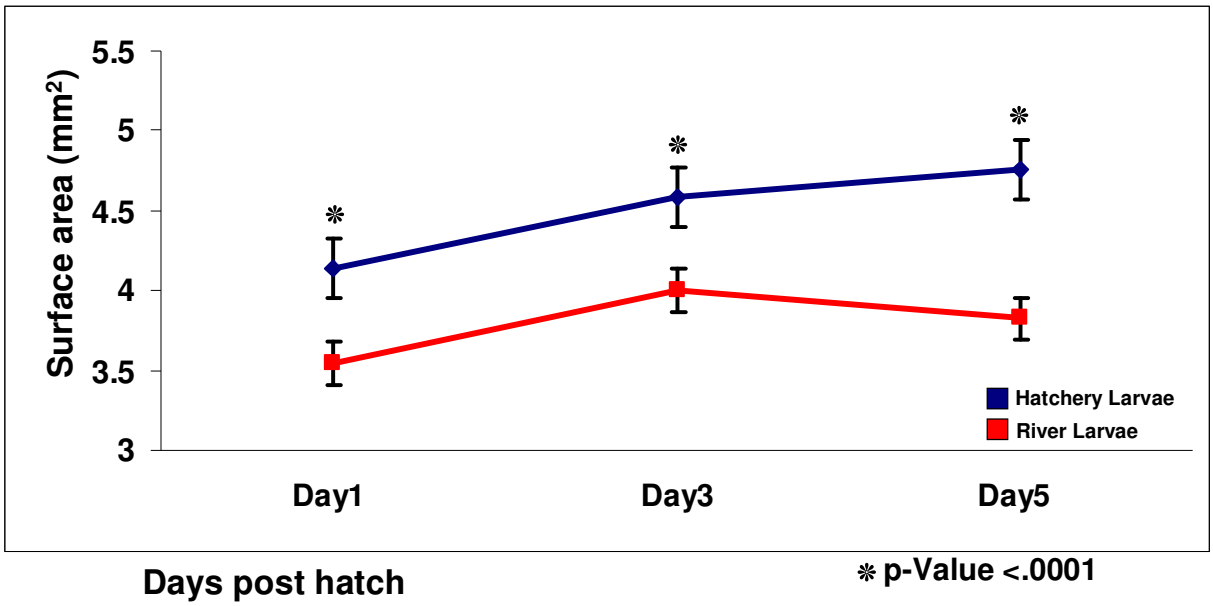


Figure 8b.Total surface area of developing striped bass larvae in 2007 indicating domestic larvae continue to grow throughout the developmental period in contrast to river larvae whose surface area increases between day1 – day 3 post-hatch but decreases between day 3 – day 5 post touch. N = 45 hatchery control larvae/data point (progeny of 3 control domestic striped bass); N=90 larvae/data point (days 1 & 3 progeny of 6 river collected striped bass) and N = 45 larvae/data point (progeny of 3 control domestic striped bass for day 5) due to no survival of river larvae from three of the river collected females to day five.

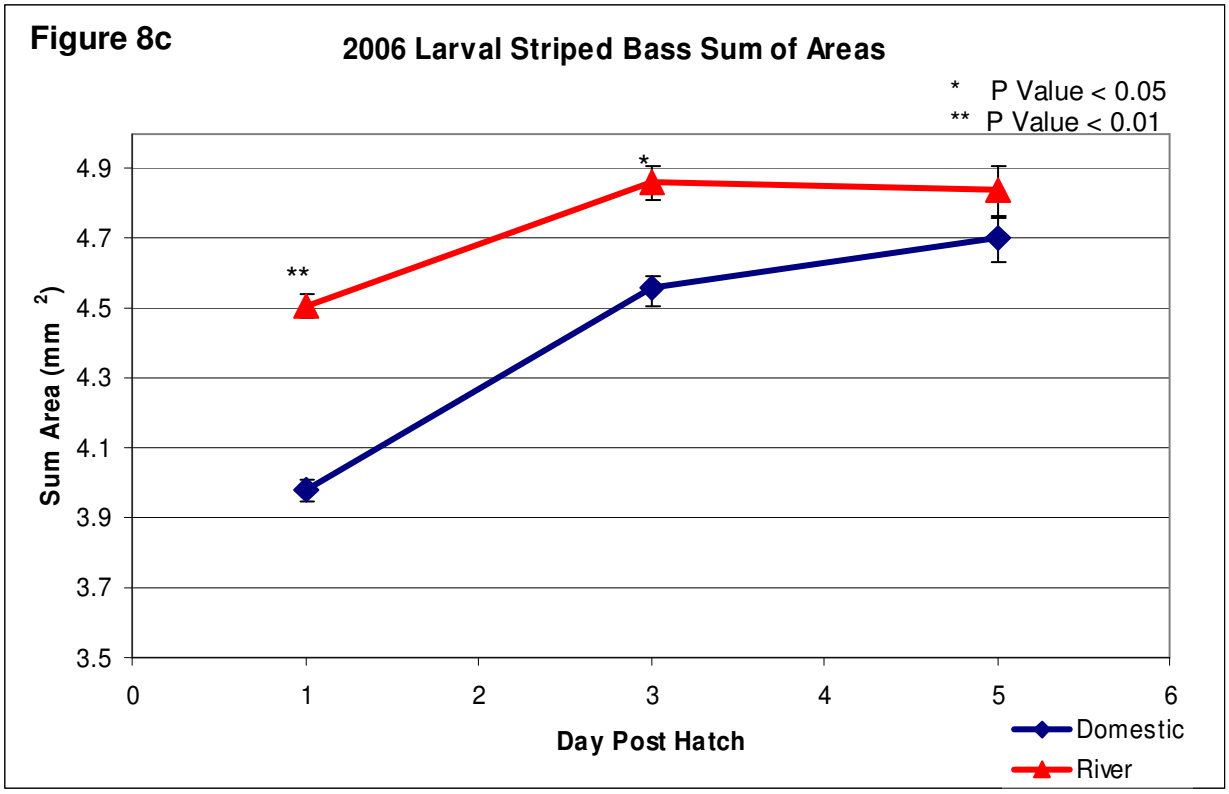


Figure 8c.Total surface area of developing striped bass larvae indicating domestic larvae continue to grow throughout the developmental period in contrast to River larvae whose surface area increases between day1 – day 3 post-hatch but decreases between day 3 – day 5 post touch. N=15 hatchery control larvae; N=60-75 river larvae from 4-5 river collected females.

Figure 9b

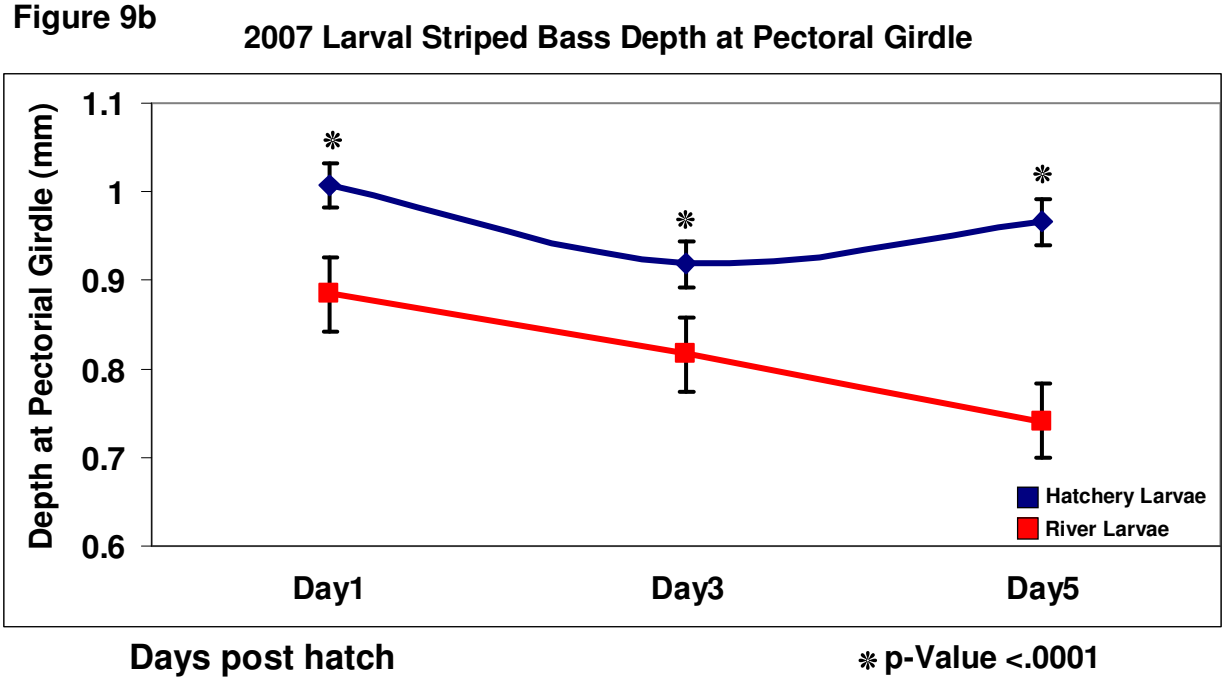


Figure 9b. Depth measurements at the pectoral girdle of developing striped bass larvae. Results indicate domestic larvae depth decreases between day 1 & day 3 (normal development as morphometry changes from an embryonic stage to the larval stage) after which the depth increases indicating continual growth whereas in the river larvae depth measurement continues to decline throughout the developmental period indicating deterioration of the larvae between Day 3-5 after hatching. N = 45 hatchery control larvae/data point (progeny of 3 controlled striped bass); N=90 larvae/data point (days 1 & 3 progeny of 6 river collected striped bass) and N = 45 larvae/data point (progeny of 3 control domestic striped bass for day 5) due to no survival of river larvae from three of the river collected females to day five.

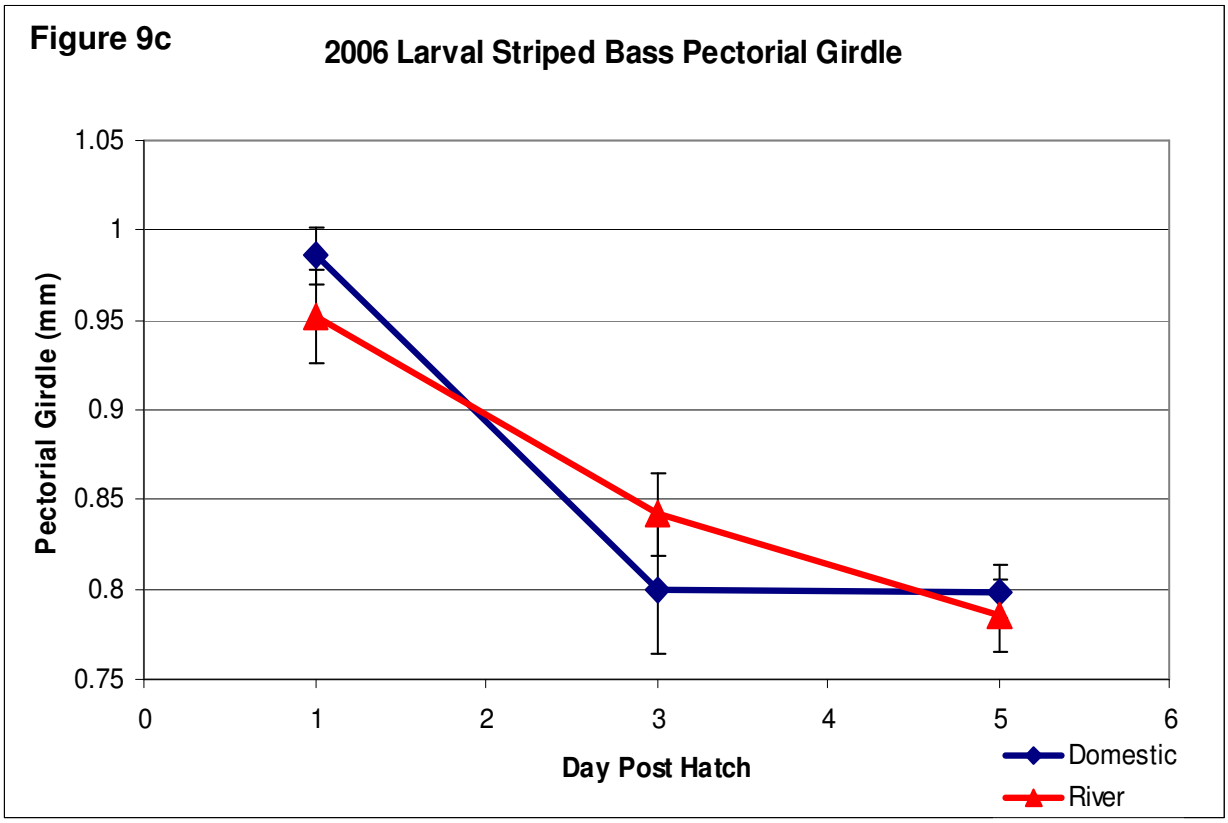


Figure 9c. Depth measurements at the pectoral girdle of developing striped bass larvae. Results indicate domestic larvae depth decreases between day 1 & day 3after which the depth stabilizes whereas in the river larvae depth measurement continues to decline throughout the developmental period. N=15 hatchery control larvae; N=60-75 river larvae from 4-5 river collected females.

Larval gross lesion results

In 2006 a review of the micrographs prior to morphometric analyses gross lesions were found in many larvae. This prompted a thorough evaluation of both groups of larvae using the micrographs of preserved larvae (n= 45 larvae from one domestic female and n=206 larvae from 5 river collected females). In 2007 the same evaluation was performed using the micrographs of preserved larvae (n = 135 larvae from three domestic females and n = 225 larvae from 6 or 3 {day 5} river collected females) Table 11a. The results from this analysis in both years were striking and indicated that river larvae had numerous gross lesions and that the majority of the lesions were either not seen or found at extremely low numbers in the domestic larvae (Figures 10b & 10c). Lesions encountered were abdominal edema, spinal deformities (lordosis & kyphosis), dorsal and ventral fin edema blistering and necrosis. A subset of micrographs was sent to Dr. Serge Dorsoshov for confirmation of the nature and severity of these lesions. Dr. Dorsoshov confirmed our findings and that these lesions are typically caused from extremely poor water quality or contaminants. Example micrographs of normal larvae throughout the developmental period are shown in Figures 130a-132a and examples of the lesions encountered in Figures 135a- 141a.

The lesions were scored on a scale of 0-3 with 0 meaning no lesion, 1 equaling a mild lesion, 2 a moderate lesion and 3 a severe lesion (Table 11a). The incidence of lesions in the domestic larvae in 2006 were minimal with 5 of 45 (11%) found to have spinal deformities, 3 of 45 (6%) had minimal abdominal edema and 0 of 45 had ventral fin edema blistering and necrosis. In contrast larvae from the five River collected females individually and collectively had a significantly higher incidence of the same lesions (Figure 10c). Collectively in river larvae the lesion incidence was 107 of 206 (52%) were found to have spinal deformities, 43 of 206 (21%) had abdominal edema and 197 of 206 (95%) were found to have ventral fin edema, blistering and necrosis (Figure 10c & Table 11a). In 2007 the results

were similar in comparison. The incidence of lesions in the domestic larvae were minimal with 7 of 135 (0.05%) found to have spinal deformities, none examined had abdominal edema and 6 of 135 (0.04%) had ventral fin edema, blistering or necrosis. As in the 2006 study, larvae from river collected female striped bass had a significantly higher incidence of the same lesions (Figure 10c). In the 2007 river larvae 56 of 225 (25%) were found to have spinal deformities, 192 of 225 (85%) had abdominal edema and 212 of 225 (94%) were found to have ventral fin edema, blistering and necrosis.

The most significant of these findings was abdominal edema, dorsal & ventral fin edema, blistering and necrosis of the epithelium. These types of lesions are caused by poor water quality or contaminant exposure (Westernhagen 1988; Marty, Heintz et al. 1997; Osmann 2007). The finfold damage (blistering and necrosis of epithelium) prominent in these larvae is a major problem for altricial pelagic larvae because it affects cutaneous respiration and always leads to lethal bacterial or fungal infections.

The lesions found in the river larvae are typically caused by poor water quality or contaminants. Larvae from both the domestic females and river females were spawned and reared in identical conditions using well water. River larvae in 2006 were the progeny from five female striped bass and two separate spawning events. In 2007 river larvae were the progeny from six female striped bass and two separate spawning events compared with progeny from three domestic/control striped bass. The absence of or very low incidence of these lesions in the domestic larvae and the preponderance of lesions found in the river larvae lead us to believe that the lesions are contaminant related. Histopathological evaluation of both groups of larvae (domestic versus river) confirmed the gross lesions and helped to further describe and characterized lesions encountered (Figures 4-7.).The only variables that differ between the two groups of larvae in both studies are the source of the females (domestic vs. river) and potential contaminant loads in the eggs. For the 2006 results we conclude that it was the xenobiotics contained in the eggs that caused the resulting gross and histological lesions. However, at this time a definitive statement cannot be made for the 2007 results as the egg analysis was unable to be completed due to lack of funding.

In addition to the lesions mentioned above, a review of micrographs taken of live larvae show “bubbles” and a frothy inconsistency in the yolk of many of the river larvae (Figure 131a). An example of a normal yolk from a domestic larva of the same age is seen in Figure 130a This condition was seen in the maternal transfer study and during histological evaluation these yolks did not stain well with eosin indicating a lack of protein (poor nutritional yolk quality).

In summary regardless of the lack of egg contaminant data from the 2007 in both the 2006 and 2007 the river larvae grew more slowly, growth ceased or regressed between day 3 and day 5 after hatching and river larvae had significant lesions not seen or seen at extremely low levels in the domestic larvae. Growth and developmental trends parallel that of the earlier maternal transfer study and indicate that serious problems with progeny from river collected striped bass continued to occur in 2006 and 2007. The combination of poor growth, abnormal development and lesions encountered lead to the conclusion that very few of the river larvae would make the transition from the larval to juvenile stage and potentially affect recruitment and adult population levels.

2007 Larval Gross Lesion Scores

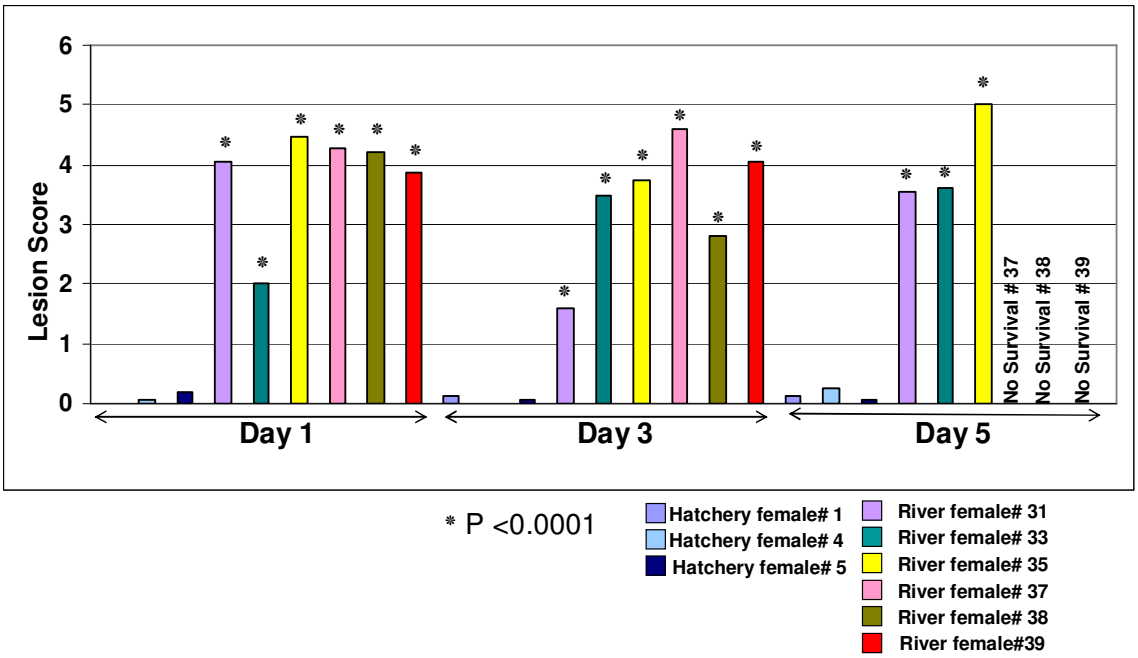


Figure 10b. Combined lesion scores for 2007 developing striped bass larvae. Lesions scored were: axial/spinal deformities, abdominal & finfold edema and skin blistering and necrosis. 0 = no lesion, 1 = minor lesion, 2 = moderate lesion & 3 = severe lesion. N=15 larvae/female/sample period.

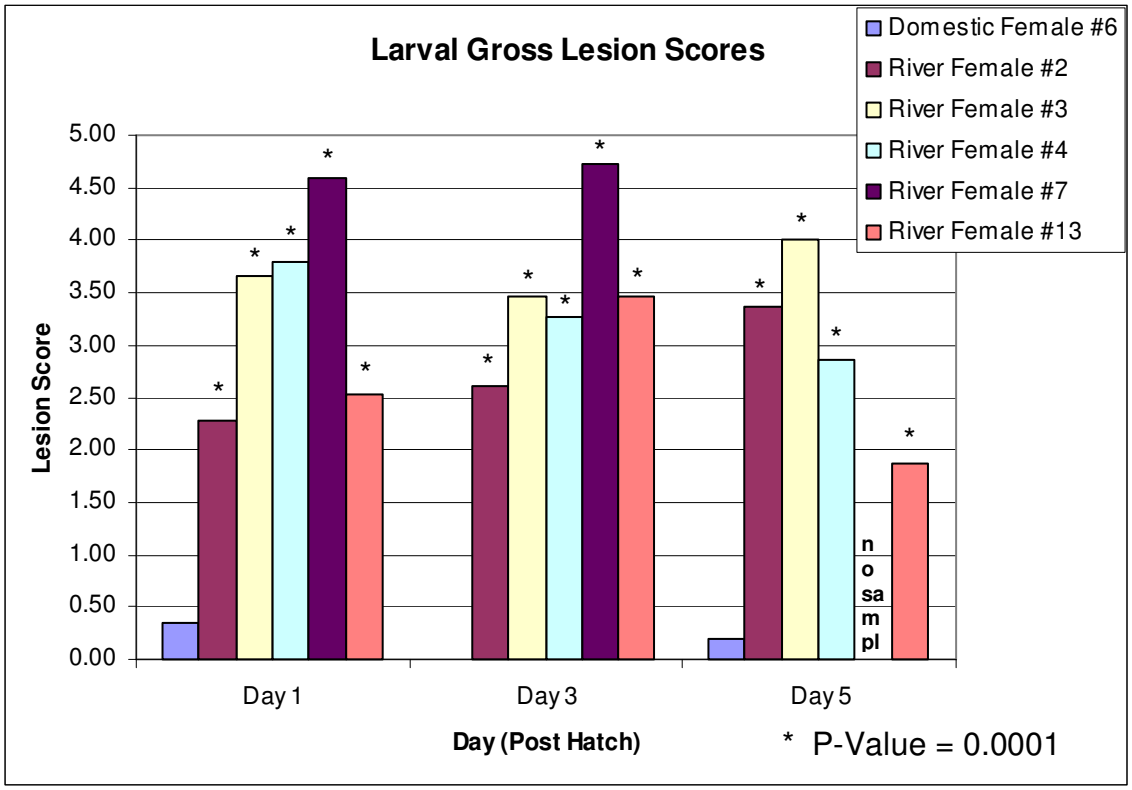


Figure 10c. Combined lesion scores for developing 2006 striped bass larvae. Lesions scored were: axial/spinal deformities, abdominal edema and skin blistering/edema and necrosis. 0 = no lesion, 1 = minor lesion, 2 = moderate lesion & 3 = severe lesion. N=15 larvae/female/sample period (with the exception of day 3 sample from female #7 where N=11).

Histopathological Confirmation of Gross lesions from 2007 developmental studies:

In the 2006 and 2007 developmental studies gross lesions were found in the vast majority of the larvae from river collected females and very few of these lesions were seen in progeny from hatchery control female striped bass. Histopathological analysis of a subset of the larvae was performed and provided confirmation of the gross lesions seen which included: Abdominal, brain and finfold edema; and necrosis of epithelium as shown above in Figures 4-7). In addition lesions were found in the brain of developing larvae from river collected females consistent with xenobiotic exposures and similar to those reported in earlier studies (Okihiro et al., 1990; Bailey et al., 1991; Bailey et al., 1994; Ostrach, 2006). The larvae from the river females exhibited necrotic lesions found mainly in the mid-brain undifferentiated neuroblasts and glial cells (Figure 7). The lesions were characterized by cytoplasmic pallor, nuclear fragmentation, areas of vacuolation and cellular pycnosis. These lesions were found in 42% of all river larvae that survived to day 5 after hatching. The control larval brains appeared normal with no similar lesions present.

Biochemical analysis of developing larvae

EROD assay for induction of Cytochrome P4501A1 activity:

Developing fish embryos and other early life forms are very sensitive to CYP1A-associated oxidative stress and toxicity (Guiney, Smolowitz et al. 1997; Wassenberg and Di Giulio 2004; Billiard, Timme-Laragy et al. 2006; Voelker, Vess et al. 2007). Results from our previous study on the maternal transfer of contaminants and effects on embryonic and larval striped bass indicated that chemical inducers of P4501A1 are being transferred in biologically relevant levels from river collected female striped bass (Ostrach 2006; Ostrach, et al., 2008) Therefore, we examined

EROD activity in developing larval striped bass to determine if this was one of the mechanisms involved in the developmental abnormalities seen in this and earlier studies. The EROD assay was not sensitive enough to detect differences in CYP1A levels in whole homogenized larvae so we have developed and verified a more sensitive competitive ELISA assay. The competitive ELISA method modified for use in developing striped bass larvae will allow us to detect extremely low (molar) levels of CYP1A in these extremely small larval samples. A similar method was developed and used to detect CYP1A as a pollution biomarker and to standardize CYP1A measurements such that better inter-laboratory comparisons and comparisons between species can be made (Tom et al. 2002). Following is the competitive ELISA method development and verification.

Cytochrome P4501A1 Competitive ELISA

We developed a CYP1A competitive-ELISA in order to quantify the induction of CYP1A as biomarker of contaminants exposure in larvae and juvenile striped bass. The principle of the competitive-ELISA is that the weaker the concentration of the protein of interest is, the stronger the signal is, which makes this technique especially sensitive. To proceed we obtained a recombinant bacteria strain expressing a fish CYP1A protein. We then cultured this bacteria strain and induced protein production. We isolated the CYP1A containing cytosol from the culture. Then, we purified a stock of CYP1A protein and determined its concentration. Finally we optimized the competitive-ELISA conditions in terms of standard curve, coating and antibody concentrations.

CYP1A protein production

A strain of *Escherichia coli* that contained an antigenic fragment of *Lithognathus mormyrus* CYP1A1 gene was obtained from Dr. Moshe Tom, Israel Oceanographic and Limnological Research. The bacterium was grown over night in Lysogeny Broth with 100 µg/mL ampicillin at 37 ºC overnight to produce a starter culture. The starter culture was used to inoculate one liter of Terrific Broth + 50 µg/mL ampicillin + 1.0 mM thiamine at 37 ºC. This was grown for four hours and then heme precursor δ-aminolevulinic acid and inducer isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM, and the culture was incubated at 28 ºC for 24 hours. After a first centrifugation at 5,000 x g for 15 minutes in 250ml flasks, bacteria were harvested and condensed into 50 mL centrifuge tubes after a second centrifugation. The cells were resuspended into 30 mL of 0.1 M potassium phosphate buffer + 20% glycerol + 0.1 mM DTT and EDTA (pH 7.4), 0.5 mg/ml of egg white lysozyme was added to the buffer and incubated for one hour at 4 ºC. A nonselective protease inhibitor (Sigma P8340) to a dilution of 1/10,000 and 1 µg/ml DNAase I was added. The resulted spheroplast suspension was then sonicated with a high powered probe sonicator at 25 Watts, 8 times for 30 seconds while being held on ice and then centrifuged at 30,000 g for 2 hours. The CYP1A containing cytosol was saved and the pellet was discarded.

CYP1A protein purification

The CYP1A containing cytosol was purified on a Ni^+ NTA Agarose (Qiagen) column equilibrated with 50 mM potassium phosphate buffer containing 0.2 M NaCl, 20 mM glycine, 20% glycerol and supported with 0.1 mM of both EDTA and DDT running buffer. The poly-His tail that was added on the recombinant protein fragment has a high affinity for the Ni^+ ion and allows for the crude extract to pass through and enrich the solution with the protein of interest. The protein was eluted from the column with running buffer containing 50 mM histidine. The fractions of protein were analyzed for total protein with the BioRad DC protein assay, and further analyzed with SDS polyacrylamide gel (InVitrogen NuPAGE) to determine the fractions that had high concentrations of CYP1A. These fractions were pooled, and analyzed with the BioRad DC protein assay to determine total protein content and by SDS gel to determine the CYP1A protein concentration. The results of the SDS gel showed a prominent band at 55kDa matching the molecular weight of the CYP1A protein fragment with a concentration of 0.18 µg/µL in the produced stock of approximately 10 mL.

CYP1A competitive-ELISA optimization:

The CYP1A protein faction was coated on high binding 96 well plates (Costar 3925, Black) at a level of 100, 200 ng/mL CYP1A1 protein fragment in Bicarbonate buffer, pH 9.6. This plate was then blocked with PBS buffer pH 7.5 containing 1% Hammerstein Casein, 1.0 mM Ethylene diamine tetra acetic acid (EDTA), and 0.05% Tween 20. Following this, the plate was assayed with several concentrations (1/5,000 and 1/10,000 dilutions) of Mouse Anti-

Trout CYP1A1 antibody (Biosense Laboratories) and then labeled with a marker antibody, Goat Anti Mouse Horse Radish Peroxidase (HRP) conjugate (Jackson Immuno Research), at 1/10,000 dilution. This reporter Ab-Enzyme conjugate catalyzed the reaction with the substrates ADHP or Amplex Red (Anaspec Inc.), 35.4 µM, and Urea Hydrogen Peroxide, 1.76 mM in 0.05 M Sodium Phosphate buffer, pH 7.4. Fluorescence was measured at 544 nm excitation with 590 nm emission on a Wallace Victor 2 Fluorescence Plate Reader. The results confirmed that the antigenic CYP1A protein fragment was present.

The assay has been optimized to contain a 200 ng/mL CYP1A coating step, 1/10,000 dilution of Mouse anti-striped bass CYP1A (Biosense Laboratories) competitive binding step, and a 1/10,000 dilution of Goat anti Mouse-HRP reporter Ab-enzyme step of the competitive ELISA assay. The experiments are now focused on optimizing the sample dilutions for larval homogenates and juvenile S9 liver fractions.

Larval homogenates from 2006 and 2007 developmental studies were analyzed using this new more sensitive assay to determine if the P4501A1 system is one of the mechanisms involved in the developmental abnormalities/lesions present during both years of larval developmental studies. The results were negative for both years indicating that the P4501A1 system is not a mechanism involved in these developmental abnormalities. This is not an unexpected finding as the liver and enzyme systems involved are not fully mature in striped bass larvae of this age. This is an important finding none the less as it eliminates one of the major routes of toxic action as the underlying mechanism(s) involved in developmental abnormalities reported in all 4 years studied (1999, 2001, 2006 & 2007). Further investigations are needed to fully understand how the complex mixture of xenobiotics is affecting larval striped bass development. Since larval striped bass are only available for use in developmental studies during a very limited time during the year (~2 months maximum) and because precise biochemical, molecular and genomic tools have not been worked out for this species so it may be necessary to use a surrogate species such as zebrafish (*Danio rerio*) to uncover the mechanisms involved in developmental abnormalities seen in larval striped bass. Zebrafish have the advantages of being easily reared and maintained in the laboratory year round, they are used as a surrogate species by NOAA and many other research groups investigating real world contaminant effects when the endemic species cant be used, they provide a model system for rapid high throughput screens of developmental toxicity in fish embryos and larvae, as well as genetic and molecular tools are well established (Hill, R. L., Jr. and D. M. Janz, 2003; Tiedeken et al., 2005; Incardona, J. P., H. L. Day, et al., 2006; Voelker, D., et al., 2007).

Another advantage of this assay is that it is directly applicable to at least 5 other genera of fish shown to be reactive with the antibody we use including rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), carp (*CYPrinus carpio*), turbot (*Scophthalmus maximus*) and Atlantic cod (*Gadus morhua*). It is likely that this assay will also be applicable to delta smelt, longfin smelt and most if not all fish species due to the highly conserved nature of the CYP1A gene and protein among fishes.

AChE assay

An important group of biomarkers for chemical exposure and effect are enzymes and the quantification of their activity in plants and animals. An example for a toxicant specific biomarker is the measurement of acetylcholinesterase (AChE) activity in response to exposure to organophosphates (OP) and carbamates, which represent the majority of insecticides currently in use in the world. Although a rapid shift in the last decade from Organophosphate pesticides to Pyrethroids has occurred in the San Francisco estuary watershed, AChE appears as a relevant mean of investigating biological effects of complex mixture of many neurotoxic contaminants on aquatic environments (Lionetto et al 2005). AChE inhibition is linked directly with the mechanism of toxic action. Results from our previous study on the maternal transfer of contaminants and effects on embryonic and larval striped bass indicated that chemicals that could affect nervous system development and AChE levels are being transferred in biologically relevant levels from river collected female striped bass (Ostrach 2006; Ostrach et al., 2008). Therefore, we examined AChE activity in developing larval striped bass to determine if this was one of the mechanisms involved in the developmental abnormalities seen in earlier studies.

Acetylcholinesterase (AChE) activity is one of the most common biomarkers of neurotoxicity used in aquatic organisms. Very few studies have analysed the effects of natural factors on AChE activity especially in estuarine fish. In the 2007 study we investigated the effects of natural factors on AChE activity such as development, size, water temperature and conductivity in larvae (i) and juveniles (ii) of striped bass (*Morone saxatilis*) a sentinel species in the San Francisco Estuary (results found below in Striped Bass Juvenile Biochemical Studies section). (i) We analyzed the change in AChE activity during early larval development of hatchery (control) *vs.* river females' progeny to test for potential effects caused by maternal transfer of contaminants.

We believe there are various contaminant based mechanisms involved in the abnormal growth and development of the river larvae. Also noticeable is the more than 3-fold increase of activity between day 1 and day 3, observable in domestic and river larvae alike. This increase correlates with the ongoing development of striped bass larvae's nervous and organ systems. Between day 1 and day 3 post-hatch organogenesis is ongoing and includes development of the eyes, mouth, gills, axial musculature, fins as well as development of the nervous system. The nervous system is developing and growing rapidly during this period to provide the -nerve pathways required to innervate musculature and sensory functions in the larvae. Findings of the 3-fold increase of activity between day 1 and day 3 in 2006 confirms the importance to monitor AChE activity in of these developing larvae.

Previously, we reported that 2006 river produced larvae had slightly lower AChE activity compared to domestic larvae during early development. This interpretation was based on the simple comparison between the mean AChE value of river produced larvae (without taking into account the variability in AChE individual values) and only AChE data from one domestic females progeny. Three larval domestic progenies were produced in the 2007 study that enabled us to statistically compared AChE activity between in river, river cross and control groups, i.e. taking into account the variability within each group. The 2007 developmental studies of larval striped bass AChE activity also displayed a strong significant increase throughout the developmental period. We plan to repeat this study in 2008 along with other techniques to attempt to determine and define the mechanisms responsible for the abnormal developmental results seen in 2006 & 2007 as well as in the earlier 1999 & 2001 developmental studies.

AChE activity in striped bass larvae:

AChE activity in striped bass larvae increased from approximately 0.02 $\mu mol.min^{-1}.mg\ protein^{-1}$ at 1d to approximately 0.13 $\mu mol.min^{-1}.mg\ protein^{-1}$ at 5d. No significant difference was detected between the control domestic ♀ – domestic ♂ (D), river ♀ – domestic ♂ (RD) and river ♀ – river ♂ (R) groups at each age 1, 3 and 5 days (Kruskal-Wallis test, $p>0.05$) (Figure 11). There was no significant difference in the change of AChE activity between groups (ANCOVA with age as co-variable, n=32, df=2, F=0.2, p=0.820).

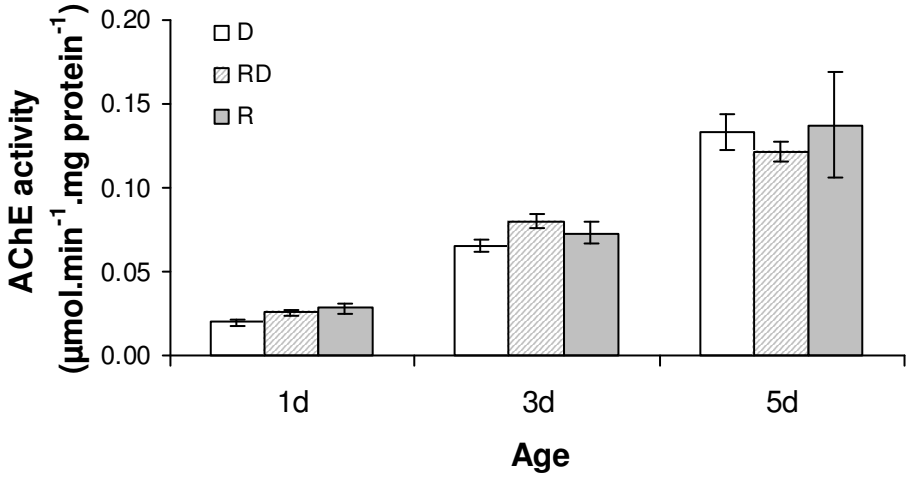


Figure 11: AChE activity in striped bass larvae from age 1d, 3d to 5d between the control domestic ♀ – domestic ♂ (D), river ♀ – domestic ♂ (RD) and river ♀ – river ♂ (R) groups.

Histopathology summary findings for fish collected in DFG surveys & POD special sampling events.

Striped bass from four surveys in 2007; Townet survey (TNS), Summer Kodial Trawl (SKT), Fall Mid-Water Trawl (FMWT) and POD special sampling surveys were examined histologically. The findings were similar to the findings in juvenile striped bass from surveys conducted in 2005 and 2006. A notable exception was the severity of the inflammation in the 2005 fish with trematodiasis in contrast to the general absence of inflammation in fish with trematodiasis from fish examined in the 2006 and 2007 surveys. The explanation for this difference in this finding is not clear, but may be related to the development of immunotolerance or adaptation to the parasitic infection in fish from 2006 and 2007 versus the fish from 2005. Another notable exception was the prevalence of the external (primarily the gill, but also the integument, oropharynx and opercular cavities) protozoan infections in the fish examined from 2006 and 2007 versus the fish from 2005. In this context, the prevalence of the protozoan infections was considered a conservative under estimate since the majority of fish examined from the FMWT and Townet Surveys in 2006 and 2007 did not include the heads and gills. Gill parasitism has been shown to adversely affect growth and interfere with respiration especially during the first year or two of life (Sadzikowski 1974). Regardless, the severity of the inflammation in the fish from 2005 with trematodiasis and the external protozoan infections in fish from 2006 and 2007 are considered a significant impact on the health status of the fish and subsequently the population that may result in morbidity and/or mortality in affected fish or may further compromise fish especially in juvenile fish. Viral isolation performed on selected fish from the 2007 surveys did not result in the isolation of any viral agents.

In addition, the vast majority of fish examined from 2005, 2006 and 2007 were positive for P450 expression by immunohistochemical and biochemical (EROD) analysis that indicated the similar exposure of fish to environmental toxicants regardless of location or age group. In addition a significant portion of fish examined in all three years were expressing vitellogenin indicating exposure to environmental estrogens and metallothionein indicating heavy metal exposure or severe oxidative stress. In the 2007 juvenile striped bass examined with these 3 biomarkers of contaminant exposure 33% were found to be under multiple types of sub-lethal contaminant exposure. Interactions between the physiological systems involved when multiple types of contaminant exposure such as these is complicated and can lead to an underestimation of exposure. Therefore these findings of positive single and multiple biomarkers are a conservative if not underestimate of the percentage of the juvenile striped bass population that are under sub-lethal contaminant exposure in the San Francisco Estuary causing physiological stress and likely immuno-suppression. These findings combined likely result in immuno-incompetence, morbidity and further compromise the health status of this population of juvenile striped bass during all 3 years of investigations.

Tow Net Survey 2007

Multiple whole juvenile striped bass (112) that were collected from the Sacramento-San Joaquin Delta in 2007 were submitted in formalin for microscopic examination (Table 6a).

The primary finding in these fish was the variable occurrence of occasional discrete verminous granulomas within the coelomic cavity of several fish that was not an unexpected finding, since trematode infections are not an uncommon finding in wild fishes. However, to have 34% of this group infected is a higher than normal incidence of infection and therefore constitutes a significant finding

The rare enteric coccidiosis in these fish was considered a conservative estimate since mild and/or early infections are often difficult to detect in histological sections. The enteric coccidiosis was not considered a significant finding due to the low incidence. However, severe infection may compromise enteric function in an affected fish and the finding should be considered since early infections are difficult to detect using these methods and given the high incidence of other infections seen in these fish.

The absence of branchial parasitic infections in these fish was due to the absence of the heads in the fish submitted for microscopic examination. Given the nature and severity of branchial parasitic infections seen in fish from other DFG

surveys (where branchial tissue was available) it must be noted that it is likely these fish also had high levels of branchial parasitic infections. The nature and severity of these infections are not considered to be a normal finding in a healthy population of juvenile fish. The juvenile striped bass evaluated are suffering from an abnormal incidence of parasitic infections and as such under significant physiological stress.

Summer Kodiak Trawl 2007

Diagnosis: Branchitis, mild, ciliated protozoan; striped bass (Fish#056, 320 and 326)

Tissues from 11 juvenile striped bass were examined microscopically; please refer to Table 4a that contains the identification numbers and data for each fish.

Three (3) fish (Fish#056, 320 and 326 had a mild branchial parasitic infection.

POD Special Survey 2007

Multiple whole juvenile striped bass (130) that were collected from the Sacramento-San Joaquin Delta in 2007 were submitted in formalin for microscopic examination (Table 8a).

The occurrence of occasional discrete verminous granulomas within the coelomic cavity of several fish was not an unexpected finding, since trematode infections are not an uncommon finding in wild fishes. The incidence of trematode infection in these fish is lower than what was seen in 2005 & 2006 but may mean conditions (host parasite occurrence) for trematode infection were less in this very dry year. However, other parasitic infections were detected in a large percentage of the fish evaluated (79%) such that these fish are under severe physiological stress.

The occurrence of enlarged lamellar epithelial cells in occasional fish that were further characterized by an abundance of dense basophilic cytoplasm, which was consistent with an intracellular bacterial (chlamydial) infection that is often referred to as epitheliocystis infection, was an interesting finding but was not considered a significant finding in these individual fish due to the rare or mild occurrence of these cells. Regardless of the significance, a definitive etiological diagnosis requires electron-microscopic examination of these cells, which can be performed for completeness as necessary.

The enteric coccidiosis in these fish was considered a conservative estimate since mild and/or early infections are often difficult to detect in histological sections. Although the enteric coccidiosis was not considered a significant finding, severe infection may compromise enteric function in an affected fish.

The most significant finding was the branchial protozoan infection detected in 79% of the juvenile striped bass evaluated that was consistent with a trichodinid infection, but also included other ciliated protozoan such as sessile ciliates. There was no significant difference among the various subgroups of these fish including the collection station or time of collection. It is important to understand that the antemortem severity of external parasitic infections cannot be definitively determined by histological examination, since external parasitic agents will generally leave the host following death of the host or will be removed from the tissue following fixation of the tissues. A more definitive determination of the severity of infection can only be determined by the cytological examination of branchial preparations using branchial tissue obtained from live fish or fish immediately following euthanasia. Definitive identification of the protozoan parasites cannot be performed on histological sections but also requires cytological preparations. Trichodiniasis can result in significant, chronic morbidity and mortality especially in juvenile fish and is often associated with factors or stressors (such as water quality or sub-lethal contaminant exposure) that further predispose fish to infection. To have 79% of this group infected is a higher than normal incidence of infection especially since this type of infection is underestimated in preserved fish. This constitutes a significant and disturbing finding and these juvenile striped bass evaluated are suffering from an abnormal incidence of parasitic infections and as such under significant physiological stress.

Fall Mid-Water Trawl 2007

Multiple juvenile striped bass (62) that were collected from the Sacramento-San Joaquin Delta in 2007 were submitted in formalin for microscopic examination (Table 7a).

The occurrence of occasional discrete verminous granulomas within the coelomic cavity of several fish was not an unexpected finding, since trematode infections are not an uncommon finding in wild fishes. The incidence of trematode infection in these fish is lower than what was seen in 2005 & 2006 but may mean conditions (host parasite occurrence) for trematode infection were less in this very dry year. However, other parasitic infections were detected in a large percentage of the fish evaluated (65%) such that these fish are under severe physiological stress.

The occurrence of enlarged lamellar epithelial cells in occasional fish that were further characterized by an abundance of dense basophilic cytoplasm, which was consistent with an intracellular bacterial (=chlamydial) infection that is often referred to as epitheliocystis infection, was an interesting finding but was not considered a significant finding due to the rare occurrence of these cells. Regardless of the significance, a definitive etiological diagnosis requires electron-microscopic examination of these cells, which can be performed for completeness as necessary.

The enteric coccidiosis in these fish was considered a conservative estimate since mild and/or early infections are often difficult to detect in histological sections. Although the enteric coccidiosis was not considered a significant finding, severe infection may compromise enteric function in an affected fish.

Findings included intracytoplasmic accumulation of eosinophilic droplets but are not an uncommon finding in various fishes (wild or captive and freshwater or marine species). In higher vertebrates (mammals), the presence of intracytoplasmic protein droplets is generally associated with protein absorption due to a glomerulopathy that results in the loss of protein in the glomerular filtrate, whereas in fishes the accumulation of eosinophilic droplets within the proximal renal tubules is generally not associated with glomerular lesions. However, some fish pathologists have considered that the loss of protein within the glomerular filtrate and the subsequent absorption of this protein in the proximal renal tubules may occasionally be associated with exposure to increased ammonia concentrations or exposure to toxicants. In this context, the observation of eosinophilic droplets within the proximal renal tubules of a few juvenile striped bass should not be dismissed as a normal finding, but should be considered as a possible indicator of a toxic or environmental insult in these fish especially considering the finding that the majority of these striped bass were found to be under one or several types of sub-lethal contaminant exposure.

The most significant finding in these fish was the branchial protozoan infection in 65% of the juvenile striped bass examined that was consistent with a trichodinid infection, but also included other ciliated protozoan such as sessile ciliates. There was no significant difference among the various subgroups of these fish including the collection station or time of collection. It is important to note that the antemortem severity of external parasitic infections cannot be definitively determined by histological examination, since external parasitic agents will generally leave the host following death of the host or will be removed from the tissue following fixation of the tissues. As such this finding is an underestimate of the actual level of infection in these fish. In this context, a more definitive determination of the severity of infection can only be determined by the cytological examination of branchial preparations using branchial tissue obtained from live fish or fish immediately following euthanasia. In addition, a definitive identification of the protozoan parasites cannot be performed on histological sections but also requires cytological preparations. However, Trichodiniasis can result in significant, chronic morbidity and mortality especially in juvenile fish and is often associated with factors or stressors that further predispose fish to infection. To have 65% of this group infected is a higher than normal incidence of infection especially since this type of infection is underestimated in preserved fish. This constitutes a significant and disturbing finding and these juvenile striped bass evaluated are suffering from an abnormal incidence of parasitic infections and as such under significant physiological stress.

Immunohistochemistry Summary Report-2007

Immunohistochemical (IHC) analysis for P450 expression using the CYP1-A antibody was performed on subsets of fish from the 2007 surveys. Positive controls and negative controls were performed and stained for P450 expression or lack of expression as anticipated validating each procedure. Positive staining generally involved occasional to multifocal staining of the hepatopancreatic vessels, heart (when present in sections) and the branchial lamellar vessels and/or the lamellar pillar cells. P4501A1 - IHC analysis was performed on 9 fish from the Summer Kodiak Trawl Survey, 48 fish from DFG'S Townet Survey; 47 fish from the Fall Midwater Trawl Survey; 159 fish from POD special collections. Throughout the entire sampling period and at all sites except one (site 508 ≥34% ≤ 50% positive) indicated that >50% of juvenile striped bass were positive for P4501A1 expression indicating sub lethal contaminant exposure (Figure 12 below). However, a significant number of the samples generally did not contain the gills (primarily from the DFG surveys) and, therefore, the negative results may not have indicated the absence of P450 expression. Furthermore, the major coelomic organs were often not included in the sections due to the small size of the fish and/or the previous sectioning for histological examination. Therefore, an underestimation of P450 expression is reflected in the numbers cited above. Heads including the gills and heart should be included with the samples in the future for a more definitive determination of P450 expression within this sampled population. This is the 3rd year in a row where IHC results indicate that the vast majority of juvenile striped bass are under sub lethal contaminant exposure throughout their entire range and first 6 months of life. This type of sub lethal contaminant exposure causes physiological stress and likely immuno-suppression which may explain the abnormal findings of parasitism and disease in these juvenile striped bass.

Positive P450 1A1 sites using Immunohistochemistry POD 2007

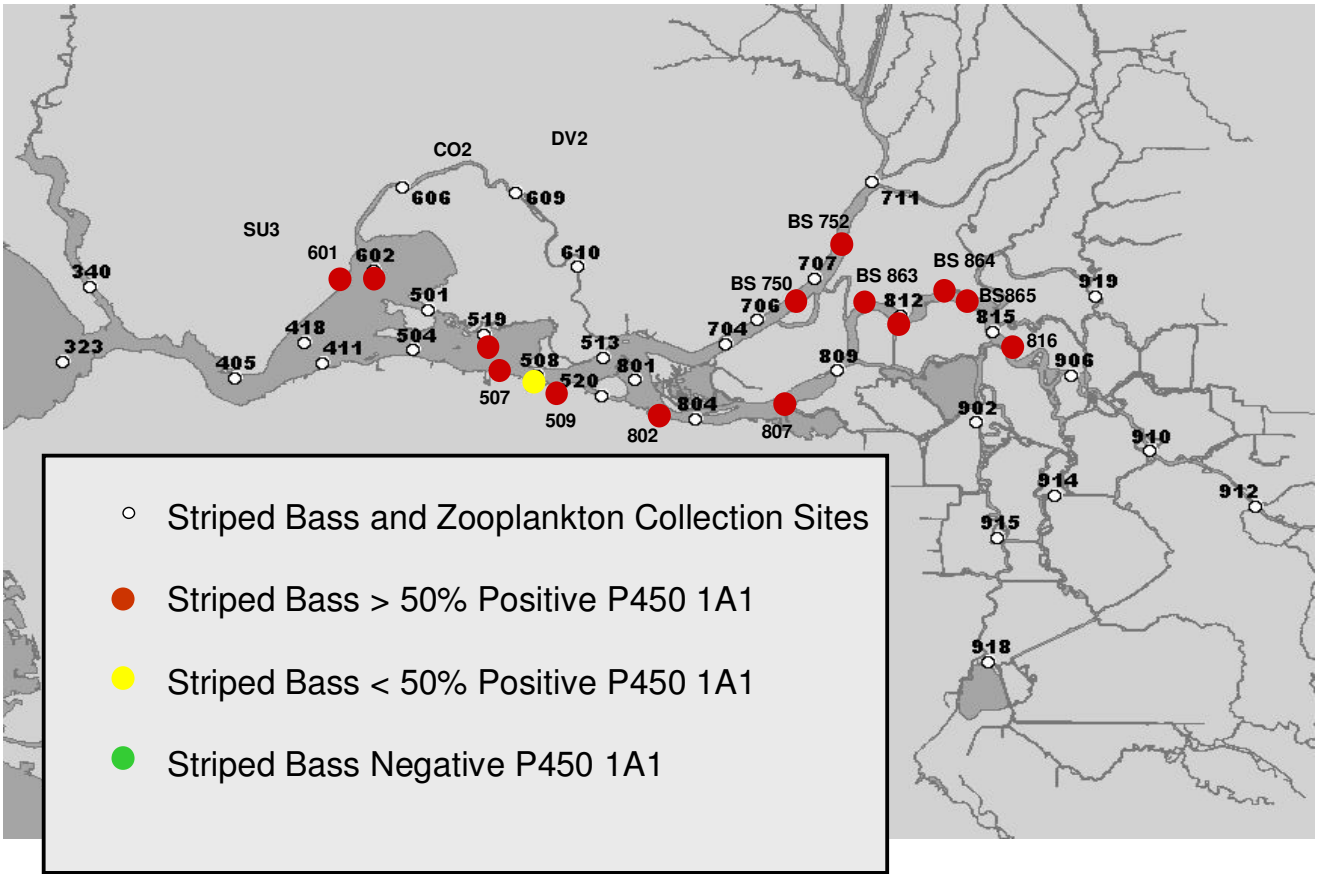


Figure 12.

Striped Bass Juvenile Biochemical Studies

Several methods have been developed for monitoring environmental exposures to environmental toxicants. Applicable endpoints include changes in liver and other tissue enzymes involved in metabolizing environmental toxicants, as well as, levels of proteins with reproductive and protective functions induced by exposure to environmental toxicants (Heppell, Denslow et al. 1995; Hodson, Efler et al. 1996; George, Gubbins et al. 2004; Sarkar, Ray et al. 2006).

EROD Assay

In aquatic species, the toxicity of polycyclic aromatic hydrocarbons (PAHs) is generally considered to be mediated by 2 different mechanisms. The non polar narcosis mechanism of PAH toxicity involves the non-specific partitioning of PAH into lipid bilayers following the hydrophobic-regulated tissue uptake of PAH (Incardona, Day et al. 2006). Another mechanism of PAH toxicity consists of a dioxin-like mode of action involving activation of aryl hydrocarbon receptor (AHR) and the subsequent induction of Cytochrome P4501A (CYP1A) and other genes involved in PAH metabolism. Although CYP1A is necessary for the metabolism of PAHs, this phase I enzyme activates PAHs and other planar hydrocarbons to produce active intermediates that form highly genotoxic covalent DNA adducts. These activated intermediates are highly reactive until they are further detoxified by phase II enzymes such as Glutathione S-transferase (GST) coupling them to glutathione so that they can be excreted.

Changes in liver microsomal 7-ethoxyresorufin-O-deethylase (EROD) activity are commonly used biomarkers for exposure of fish to a broad array of planar halogenated/polycyclic aromatic hydrocarbon environmental toxicants (Kruner and Westernhagen 1999; Whyte, Jung et al. 2000; Miller, Addison et al. 2004). The EROD assay detects the activity of CYP1A and other cytochrome P450 enzymes that can metabolize the ethoxy group on the Resorufin Ethyl ester to form a fluorescent resorufin product. The EROD assay provides a biomarker for environmental exposure to many such toxicants, including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated biphenyls (PCB), polychlorinated dibenzofurans (PCDFs) and polyaromatic hydrocarbons (PAH), and certain insecticides that induce the CYP1A1 enzyme and EROD activity (Kruner and Westernhagen 1999; Whyte, Jung et al. 2000). Induction of CYP1A protein and EROD activity by organic extractions of harbor sediments or SPMD collections has also provided methods for detecting PAH, and other EROD inducers in the environment (McArdle, McElroy et al. 2004). While induction of EROD activity has been extensively used as a biomarker for exposure to environmental toxicants, one should be aware that the induction of CYP 1A1/EROD activity can also be inhibited by environmentally relevant compounds, modulated by estrogenic agents, glucocorticoids, toxic metals and season (Kruner and Westernhagen 1999).

Although CYP1A is the main enzyme catalyzing the de-ethylation of ethoxyresorufin, other Cytochrome P450 enzymes can also contribute to EROD activity. In contrast, Benzoxyresorufin serves as a substrate for rat CYP 1A1, 1A2, 2B1, and to much lesser extents for 2C6, 2C11, 2C13, 2D1, 3A1 and 3A2 (Stresser, Turner et al. 2002). Benzoxyresorufin also serves as a substrate for human CYP1A1, 1A2, 2B1, and to lesser extents for 1A2, 2B6, 2D6, 3A4, 3A5 and 3A7 (Stresser, Turner et al. 2002). While the full spectrum of P450 enzymes that metabolize benzlyoxyresorufin in striped bass is unknown, measuring BROD activity provides an additional biomarker for environmental toxicant exposure.

Developing fish embryos and early life forms are very sensitive to CYP1A-associated oxidative stress and toxicity (Guiney, Smolowitz et al. 1997; Wassenberg and Di Giulio 2004; Billiard, Timme-Laragy et al. 2006; Voelker, Vess et al. 2007). Dioxins, PCBs and PAH induce CYP1A and CYP 2B1 to enable the organism to metabolize these environmental toxicants. However, CYP1A also activates these toxicants to their ultimate mutagenic/carcinogenic forms, and increase levels of Reactive Oxygen Species (ROS), oxidative stress, apoptosis and early developmental toxicity (Arzuaga, Wassenberg et al. 2006). Inhibiting CYP1A activity either by blocking CYP1A synthesis with morphilino antisense oligos or by using CYP1A inhibitors including alpha naphthoflavone (ANF), carbazole (CB) or dibenzothiophene (DBT) increases the toxicity of PAHs, indicating that CYP1A activity is critical for detoxifying PAHs (Wassenberg, Nerlinger et al. 2005; Billiard, Timme-Laragy et al. 2006). Exposure to CYP1A antagonists in

the environment and in body tissues may be inhibiting the induction and/or activity of CYP1A in fish and their young first generation offspring (Meyer, Nacci et al. 2002). For example, creosote-contaminated Elizabeth River Killifish, as well as, their first generation larval offspring were resistant to induction of CYP1A. In contrast, sensitivity to the induction of CYP1A was restored in first generation mature offspring, as well as, second and third generation larval offspring (Meyer, Nacci et al. 2002). The induction of EROD by TCDD was potently inhibited by several polybrominated diphenyl ether (PBDE) congeners (Kuiper, Bergman et al. 2004). EROD activity was also inhibited by exposure of fish to mercury and cadmium (Bozcaarmutlu and Arinc 2004) and to ketoconazole antifungals (Hegelund, Ottosson et al. 2004). Even common environmental and laboratory surfactants, including TritonX-100 and Tween 80, and organic solvent, methanol, markedly inhibits EROD activity (Kruner and Westernhagen 1999; da Silva and Meirelles 2004). These and other observations suggest that body burdens of environmentally relevant compounds are inhibiting the induction of CYP1A by other PAHs, thus potentiating PAH toxicity.

PAHs Dioxins and PCBs bind to the Aryl hydrocarbon receptor (AHR), which then dimerizes with AHR/aryl hydrocarbon receptor nuclear translocator (ARNT) to induce the transcription of many genes, including CYP1A (Carney, Peterson et al. 2004).
Blocking CYP1A synthesis by blocking the induction of AHR or CYP1A with morpholino oligos or by inhibiting CYPA1 activity with ANF generally reduces the toxicity of dioxins and PCBs to zebra fish (Carney, Peterson et al. 2004; Dong, Teraoka et al. 2004)**.**
In contrast, blocking fish AH receptor or CYP1A induction and/or activity fails to inhibit, and generally increases the toxicity and generation of developmental defects by PAH or weathered crude oil (Incardona, Carls et al. 2005; Wassenberg, Nerlinger et al. 2005; Billiard, Timme-Laragy et al. 2006). Collectively, these observations suggest that the induction of CYP1A, while adaptive to PAH exposures, can also enhance toxicity following exposure to dioxins and PCBs.

Endocrine disruptors can have complex effects and few studies have defined the many potential changes in signal transduction pathways following environmental toxicant exposure. For example, exposing juvenile salmon to 50 and 250 µg/L tributyltin (TBT) results in: decreased estrogen receptor alpha, decreased Vitellogenin, increased estrogen receptor Beta, increased androgen receptor, decreased CYP1A1, decreased glutathione S-transferase, increased CYP3A, increased uridine diphosphoglucuronosyl transferase (UGT) and AhRB mRNA expression (Mortensen and Arukwe 2007). TBT also resulted in a low dose induction and a high dose inhibition of aryl hydrocarbon receptor Alpha, ARNT and AhR repressor (AhRR) mRNA. Of the many effects of TBT, the induction of CYP3A and UGT likely serves to metabolize and detoxify TBT.

Thus, we examined EROD activity as a biomarker for exposure to these various environmental toxicants.

EROD assay for induction of Cytochrome P450 activity:

Livers from juvenile striped bass were dissected out and snap frozen in liquid nitrogen immediately after euthanasia. Livers were then stored at –80°C until they were prepared for EROD analysis according modifications of the procedures of (Hodson, Efler et al. 1996; Billiard, Bols et al. 2004). Briefly, livers were homogenized in 10 volumes of ice cold 0.02 M HEPES, 0.15 M KCl, pH 7.5 with 0.22 mM AEBSF and 1/200 Sigma Protease Inhibitor Cocktail. Following centrifugation at 10,000xG for 15 minutes at 4°C, the S9 supernatant fractions were stored at –80°C. The S9 fractions (40 µl) were then diluted 2-fold and added in triplicate to Costar #3915 black, 96-well plates. Then 40 µl of 10 µM Resorufin ethyl ether in 0.1 M HEPES, 0.1% BSA, pH 7.8 was added for the EROD assays. Forty µl of pre-incubated NADPH generating system was added to result in a final concentration of 0.8 mm $NADP^+$, 4.8 mM glucose-6-phosphate, 3.2 mM MgCl2 and 0.5 Units/ml glucose-6-Phosphate Dehydrogenase. EROD activity was then determined immediately by measuring fluorescence with Excitation 544 and emission 590 on a Perkin Elmer/Wallace Victor2 fluorescent plate reader. Plates were re-read every 2 minutes and the pmoles product formed estimated from resorufin standard curves. Previous studies (Radenac, Coteir et al. 2004), as well as, preliminary studies in our laboratory, showed that the Resorufin ethyl ether substrate has a slight overlap in fluorescence with that of the resorufin product, and also attenuated the excitation/emission of the product. Thus, resorufin standard curves were spiked on 40 µl of 10 µM substrate to correct for the interference by the initial concentration of substrate. Protein was

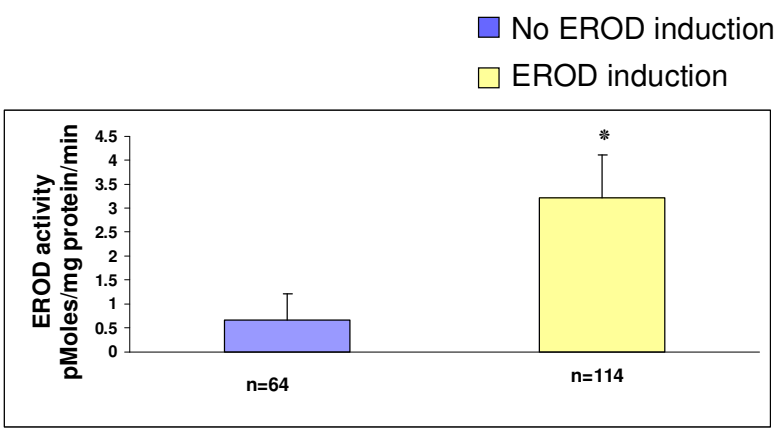
determined using Bio-Rad DC protein assay with BSA as standard. EROD activity over the 10-minute incubation was calculated as pmole resorufin formed per mg protein per minute.

EROD Activity in POD special sampling survey collected striped bass.

The majority of juvenile striped bass (~65%) collected for biochemical metrics from August 2007 through January 2008 were found to be under EROD induction (quantitative measure of P4501A1 induction/sublethal contaminant exposure). The percent of fish/date/site EROD expression/induction results are found in Table 9a. EROD induction in 114 of 178 of the juvenile striped bass was statistically significant ($p < 0.0001$) as shown in Figure 13. Maps showing temporal and spatial patterns of EROD induction are shown below in Figures 14-17. Samples with EROD induction are shown by the red dots on the maps. Additional studies are needed to determine the sources of the EROD inducers. Specifically is EROD being induced by xenobiotics coming down the Sacramento or San Joaquin rivers, from TCDDs, PCBs and other known contaminants from Travis AFB, from contaminants from Delta Industries, toxic paint from the mothball fleet, Bay Area refineries, or from other sources?

These data clearly show that EROD activity is induced in the vast majority of juvenile striped throughout the entire range and time period sampled. While only a limited number of striped bass fingerling samples were available for these EROD studies, these statistically significant observations, as well as our immunohistochemical findings of elevated CYP1A (P4501A1) corroborating the EROD results show that additional attention needs to be placed on defining the contaminant exposures responsible for elevating EROD and CYP1A activity. This data indicates that juvenile striped bass are under significant sublethal contaminant exposure throughout the entire range and time.

EROD Discussion: As discussed above, some environmental compounds have been shown to induce EROD activity while others can inhibit EROD through inhibiting the induction or enzymatic activity of CYP1A. Inhibition of PAH induced CYP1A gene expression and activity potentiates the early developmental toxicity of PAHs (Wassenberg, Nerlinger et al. 2005; Billiard, Timme-Laragy et al. 2006). In contrast, inhibition of CYP1A gene expression or activity reduces the early developmental toxicity of TCDDs. Since the majority of field samples showed marked elevations in CYP1A immunostaining, and the vast majority of field samples showed moderate to high EROD activity, additional studies are needed to more accurately define the environmental agents and locations in the Delta that are inducing EROD and CYP1A, as well as, their role in causing direct and indirect mortality of striped bass and other species in the San Francisco Estuary.



*p-Value <.0001, ***Dunnett method***

Figure 13. Statistical analysis of EROD induction in juvenile striped bass collected in the POD special sampling surveys.

August 2007 POD Sampling sites

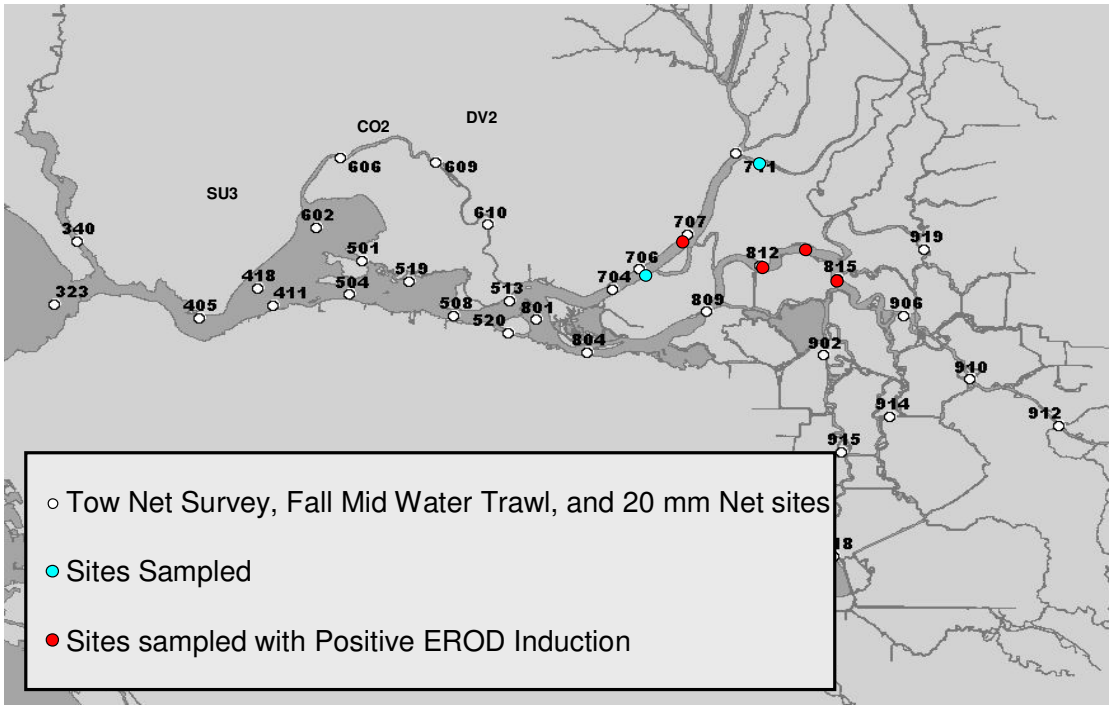


Figure 14. August 2007 EROD results map.

September – October 2007 POD Sampling sites

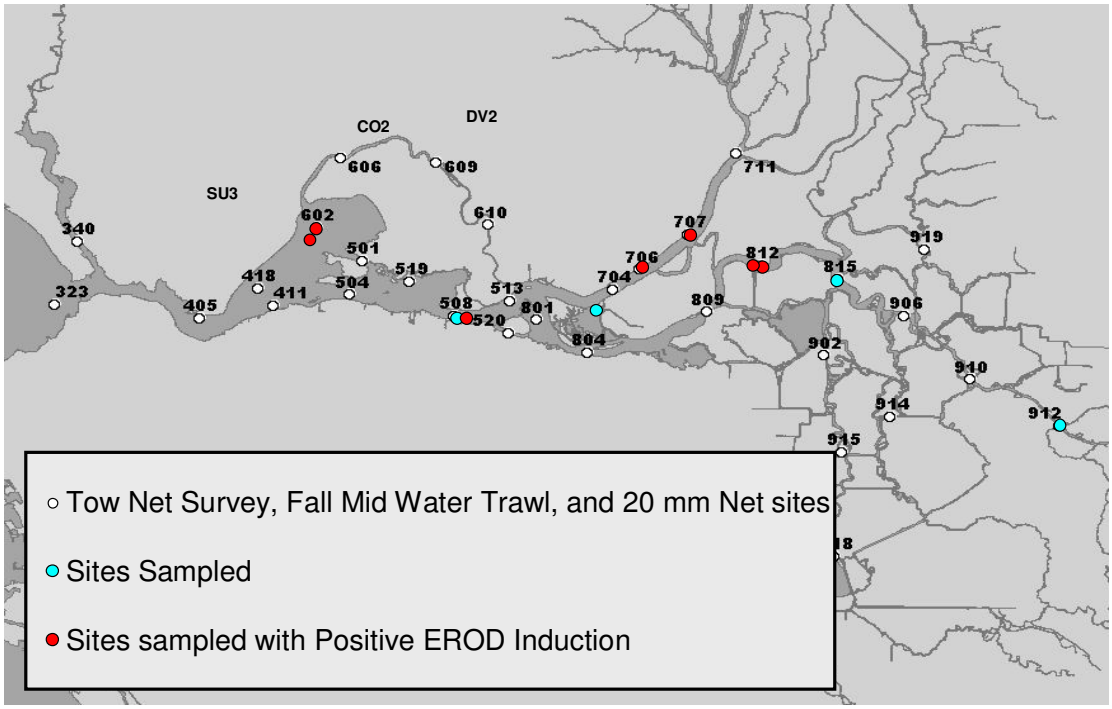


Figure 15. September – October 2007 EROD results map.

November - December 2007 POD Sampling sites

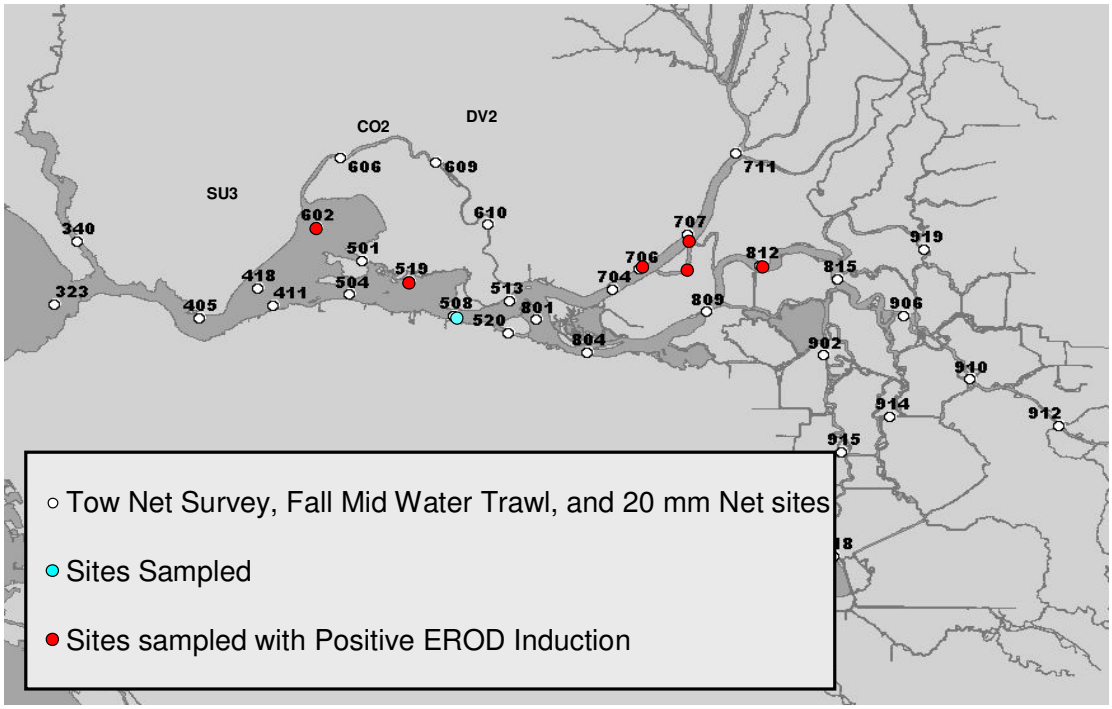


Figure 16. November – December 2007 EROD results map

January 2008 POD Sampling sites

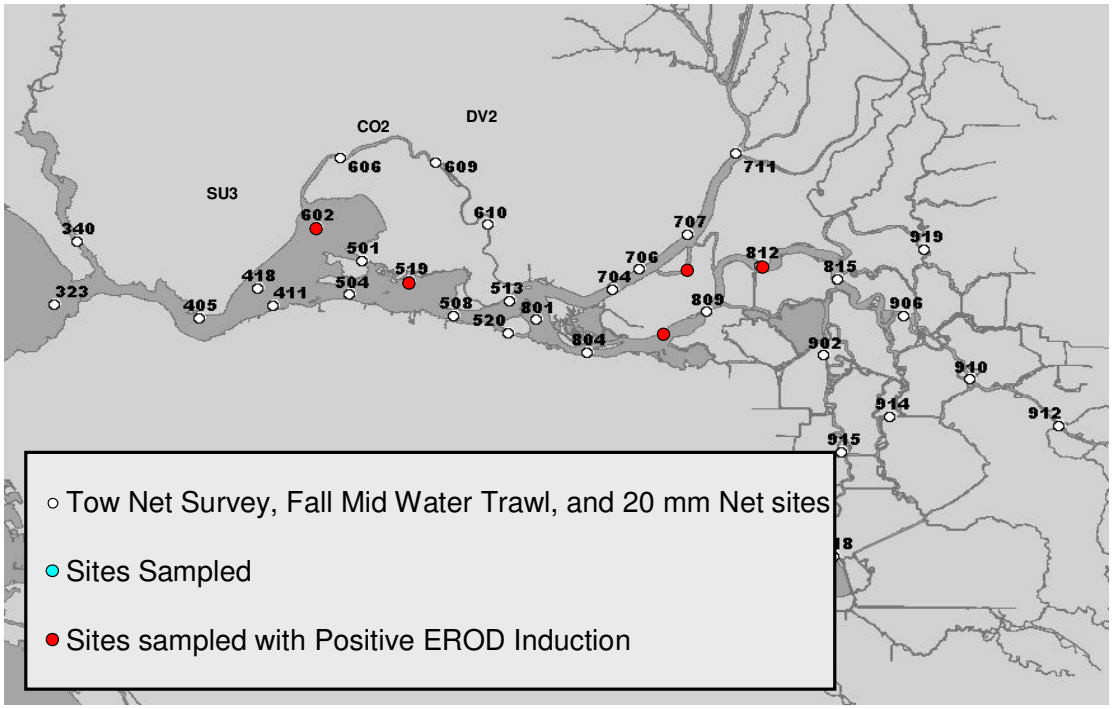


Figure 17. January 2007 EROD results map.

Metallothionein

Metallothionein is a heat stable, evolutionary conserved, low molecular weight protein with a high proportion of cysteine residues. Metallothionein has been implicated in diverse biological functions including metal ion homeostasis, binding and regulating the normal physiological levels of zinc and copper, while also protecting against toxic metals including cadmium and mercury (Cai, Klein et al. 2000; Lacorn, Lahrssen et al. 2001). The sequestering of toxic metals by MT markedly decreases their acute toxicity. More recent studies have shown that metallothionein also protects against oxidative stress (Viarengo, Burlando et al. 1999; Cai, Klein et al. 2000; Chung, Walker et al. 2005). Exposure to reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS) results in DNA strand breaks and increased apoptosis (Cai, Klein et al. 2000). As an antioxidant, MT functions to protect against oxidative damage of DNA, protein and lipids by RNS, and ROS including hydrogen peroxide, superoxide anion and hydroxyl radical (Cai, Klein et al. 2000).

The many thiol groups of MT provide reducing equivalents that protect against oxidative stress. Nevertheless, a detailed examination of the effects of increasing copper to zinc ratios reveals that MT can be an anti-oxidant or a pro-oxidant depending on the levels of zinc and copper (Suzuki, Rui et al. 1996). MT binds 7 atoms of Cd or Zn and 11-12 atoms of Cu per molecule, with a higher affinity for Cu than for Cd or Zn. Exposure to Cd results in an acute displacement of Zn and perhaps Cu, and a longer-term induction of MT (Lacorn, Lahrssen et al. 2001). MT acts as an antioxidant as long as Zinc is present in MT. However, once copper has displaced zinc from MT, MT acts as a pro-oxidant to release Cu (I) (Suzuki, Rui et al. 1996). In the presence of hydrogen peroxide, Cu (I) catalyzes the Fenton reaction producing hydroxyl radical and Cu (II). Since copper can undergo redox cycling, repeating this process results in large amounts of highly damaging hydroxyl radical and oxidative damage (Suzuki, Rui et al. 1996). Thus, MT levels and the ratio of Zn to Cu and other toxic metals play a critical role in inhibiting metal-induced toxicity / oxidative damage.

Metallothionein is induced by heavy metals including zinc, cadmium, and mercury (Schlenk, Zhang et al. 1995; Lacorn, Lahrssen et al. 2001; Chung, Walker et al. 2005). Other studies show that metallothionein is also induced by other chemical factors and oxidative stress (Viarengo, Burlando et al. 1999; Chung, Walker et al. 2005). The promoters of mammalian and picine metallothionein genes, MT1 and MT2, contain Metal Responsive Elements (MRE), glucocorticoid response elements, and oxidative stress-responsive elements (Chung, Walker et al. 2005). The promoters of other key genes involved in antioxidant defense including glucose-6-phosphate dehydrogenase (G6PD) and glutathione S-transferase (GST) also contain MRE. Such MRE containing promoters confer the ability of Metal Regulator Transcription factor-1 (MTF-1) to induce transcription of mRNA for MT, G6PD and GST (Chung, Walker et al. 2005). Oxidative stress also increases induction of MT, G6PD and GST, at least in part, by oxidizing MT cysteines, thereby releasing metal ions which likely act through MTF-1 and MRE to induce MT (Chung, Walker et al. 2005).

Induction of MT by pretreatment of the mussel Mytilus galloprivincialis with Cd resulted in decreased susceptibility to iron chloride and hydrogen peroxide-induced oxidative stress, oxyradical production, and cell mortality (Viarengo, Burlando et al. 1999). Subsequent studies revealed that pre-treatment of rainbow trout gill primary cultures to zinc not only induced MT A, MT B and many other antioxidants including, Glutathione S- Transferase (GST) and Glucose-6-phosphate dehydrogenase (G6PD), but also protected against hydrogen peroxide induced oxidative damage and apoptosis (Chung, Walker et al. 2005). Nevertheless, acute combined zinc and hydrogen peroxide exposure markedly increased the toxicity of hydrogen peroxide (Chung, Walker et al. 2005). These observations suggest that exposure to metals and oxidative stress has important roles in the induction of MT and many other antioxidants, as well as the ability to resist metal and hydrogen-peroxide-induced oxidative stress. The regulation of MT is complex, since it is induced by toxic metals, as well as, by oxidative stress. Furthermore, in gudgeon, exposure to toxic metals is more closely related to MT levels in liver than that of in gill or kidney (Van Campenhout, Bervoets et al. 2003). Thus, we examined liver MT levels as a biomarker of the exposure of striped bass to toxic metal and oxidative stress-inducting environmental toxicants.

Purification of Metallothionein Standards

Three days after the IP injection of Striped bass with 5 mg/kg zinc sulfate, fish were anesthesized with MS-222, euthanized and livers excised and stored frozen at –80°C. Livers were defrosted and homogenized with a potter-elvehjem homogenizer in 2 volumes of ice cold 10 mM Tris, 10 mM B-ME, 0.5 mm PMSF, pH 8.6. Following centrifugation at 21,000xG, metallothionein standards were purified from the supernatant using both heat precipitation and acetone precipitation methods (Thompson and Sutherland 1992; Lacorn, Lahrssen et al. 2001).

Heat treated precipitation method: The supernatant was heated at 90°C for 10 minutes, cooled on ice, spun at 13,000xG at 4°C, and the supernatant diluted 10-fold with 20 mM HEPES pH 8.1 and loaded on a 1x 10 cm DEAE Express ion exchange column (Sigma Chemical). Metallothionein was eluted using a 0 to 400 mM NaCl gradient in 20 mM HEPES, pH 8.1. The MT peak was quantitated by direct immunoassay following coating on Costar # 3925 black 96-well plates.

Acetone precipitation method: Supernatant was brought up to 50% acetone with ice-cold acetone. Following a one-hour incubation at –20°C and centrifugation at 13,000xG, at 4°C, the supernatant was recovered and brought up to 80% acetone. The resulting 50-80% acetone pellet was collected following centrifugation at 13,000xG, at 4°C, resuspended in 20 mM HEPES pH 8.1 and purified on the DEAE Express ion exchange column as described above (Lacorn, Lahrssen et al. 2001). Since the specific activity of DEAE purified MT was much higher following 50-80% acetone precipitation than following heat treatment/precipitation of other proteins, the 50-80% acetone precipitate purified MT was used as standard and the heat treated MT was used for coating in competitive immunoassays. While sufficient for defining peaks off a column, preliminary studies with direct assay of metallothionein after coating samples on 96 well plates showed poor linearity. Thus, metallothionein was assayed using a competitive immunoassay, which proved highly linear.

Exposure to Environmental Estrogens

Studies in several countries have revealed that reproductive development of fish is being disrupted by environmental exposures to Endocrine Disrupting Chemicals (EDC). Exposure to EDC in municipal and industrial effluents has been associated with an increased incidence of feminized males, intersexes and decreased fertility (Jobling, Coey et al. 2002; Jobling, Williams et al. 2006). Developmental exposure to estrogens, including 17-alpha ethinylestradiol, has been shown to feminize males, induce intersexes / ovotestes, decrease sperm counts, decrease fertility and even result in near extinction population declines in several piscine species (van Aerle, Pounds et al. 2002; Hill and Janz 2003; Jobling, Williams et al. 2006; Kidd, Blanchfield et al. 2007). Many natural and anthropogenic compounds in the environment have estrogenic activity, can induce vitellogenin (VTG) synthesis/levels, and can disrupt reproductive development of piscine species. Reproductive development has been shown to be disrupted by developmental exposure to: pesticides such as methoxychlor, and DDT metabolites, to common surfactants including 4-nonylphenol and 4-tert-octylphenol, to plastic monomers including Bis Phenol A and its metabolite 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP) and to estrogenic pharmaceuticals, including 17 alpha-ethinylestradiol (Hill and Janz 2003; Kang, Yokota et al. 2003; Ferreira, Antunes et al. 2004; Fenske, Maack et al. 2005; Ishibashi, Watanabe et al. 2005; Cionna, Maradonna et al. 2006; Mortensen, Tolfsen et al. 2006).

4-nonylphenol (NP) is a common sewage effluent contaminant. VTG levels were induced by long-term exposure to as little as 1.05 to 8.3 µg NP per L in rainbow trout (Harris, Santos et al. 2001; Thorpe, Hutchinson et al. 2001; Ackermann, Schwaiger et al. 2002) 30 µg NP per L in zebrafish (Hill and Janz 2003), and 50.9 µg NP per L in medaka (Kang, Yokota et al. 2003). Developmental exposure to 4-NP has been shown to reduce breeding success (egg viability, hatchability and swim-up success) feminize males, induce ovotestes formation in zebrafish (Hill and Janz 2003); decrease egg production, spermatogenesis and fertility in Medaka (Kang, Yokota et al. 2003); as well as, inhibit growth and IGF-I secretion in Atlantic Salmon (Arsenault, Fairchild et al. 2004; McCormick, O'Dea M et al. 2005). IGF-I plays a critical role in growth and enabling developing Salmon smolt to resist increased salinity during their migration to the ocean (Arsenault, Fairchild et al. 2004; Sakamoto and McCormick 2006). Thus, disruption of IFG-I secretion by exposure to estrogenic agents may decrease survival of developing anadromous fish species. Another commonly detected wastewater contaminant is the antibacterial agent, Triclosan. Studies in medaka show that Triclosan induces VTG in males, and that lower concentrations of Triclosan delays embryo hatching and decreases embryonic and larval survival (Ishibashi, Matsumura et al. 2004).

Cross Talk: To complicate matters, several studies have shown that estrogenic and EROD-inducing chemicals can interact. Estrogenic agents including EE2 and 4-NP, inhibit the induction of EROD activity by CYP1A agonists in European flounder and grey mullet (Vaccaro, Meucci et al. 2005; Cionna, Maradonna et al. 2006; Kirby, Smith et al. 2007). The inhibition CYP1A induction occurs at much lower concentrations of estrogenic agents than required to induce VTG in European flounder and grey mullet (Cionna, Maradonna et al. 2006; Kirby, Smith et al. 2007). Furthermore, induction of EROD by PAHs has also been shown to inhibit the induction of VTG by E2 in rainbow trout hepatocytes (Navas and Segner 2000). Thus, the true levels of estrogens in PAH contaminated sites may be underestimated due to high levels of CYP1A/EROD activity inhibiting estrogenic responses. While estrogenic compounds can act directly through estrogen receptors to induce VTG levels, other chemicals can alter VTG and other estrogenic responses by affecting estrogen receptor levels, aromatase levels or metabolism of estrogens. For example, TBT decreases VTG apparently by decreasing E2 receptor alpha (Mortensen and Arukwe 2007). Hydroxylated PCBs can also be estrogenic, at least in part by inhibiting estrogen sulfotransferase and therefore the metabolism of estrogens (Kester, Bulduk et al. 2002). Thus, hydroxylated PCB's are likely to induce both EROD and VTG. These observations show significant cross talk between the induction of EROD by the aryl hydrocarbon receptor pathway and the induction of Vitellogenin and reproduction by the estrogen receptor pathway. These observations also raise concerns that monitoring EROD activity alone may underestimate effects of environmental agents on the induction of CYP1A and EROD activity in watersheds containing estrogenic contaminants.

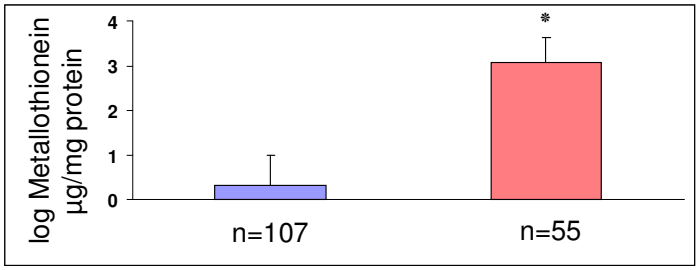
Genetic differences in sensitivity to estrogenic agents are found in diverse species ranging from fish to mammals (Spearow, Doemeny et al. 1999). For example, Sunshine Bass were much more sensitive than mummichogs to

induction of VTG by New York sewage effluent (McArdle, Elskus et al. 2000). Thus, we also need to be concerned about species and populations differences in sensitivity to EDC.

Metallothionein & Vitellogenin Results

Metallothionein (n=162) and vitellogenin (n=178) assays are completed for juvenile striped bass collected during POD special sampling surveys. Results indicate that a substantial number of juvenile striped bass are expressing metallothionein and vitellogenin in significantly elevated levels. Metallothionein was expressed in 52 of 162 fish evaluated (34%) as shown in Figure 19 and vitellogenin in 39 of 174 fish evaluated (22%) as indicated in Figure 21. Expression of these two biomarkers was both spatially and temporally correlated Figures 19, 21 & 22. Vitellogenin expression indicates exposure to estrogenic compounds/estrogenic mimics and alone can cause significant adverse effects on juvenile fish such as developmental problems associated with sexual differentiation, sex reversal and excessive physiological stress. Metallothionein is induced by heavy metals including zinc, cadmium, and mercury as well as by other chemical factors, and by severe oxidative stress. In this case expression of metallothionein at levels reported indicates these fish are under severe physiological stress whether due to metals or oxidative stress alone.

These findings coupled with the IHC and EROD findings indicate these juvenile striped bass are under several types of sublethal contaminant exposure. In fish evaluated for multiple biomarkers of contaminant exposure 18 fish were positive for both EROD & metallothionein, 17 for EROD & vitellogenin, 6 for vitellogenin & metallothionein and 13 fish were positive for all three biomarkers (EROD, Vtg &MT). These results indicate that 54 of 162 (33%) juvenile striped bass evaluated for biochemical biomarkers were under multiple types of sub-lethal contaminant exposure. Interactions between the physiological systems involved when multiple types of contaminant exposure such as these is complicated, as described above in the estrogenic exposure cross talk section, and can lead to an underestimation of exposure. Therefore these findings of positive single and multiple biomarkers are a conservative if not underestimate of the percentage of the juvenile striped bass population that are under sub-lethal contaminant exposure in the SFE causing physiological stress and likely immuno-suppression.



p-Value <.0001, *Dunnett method

Figure 18. Statistical analysis of metallothionein expression in juvenile striped bass collected in the POD special sampling surveys.

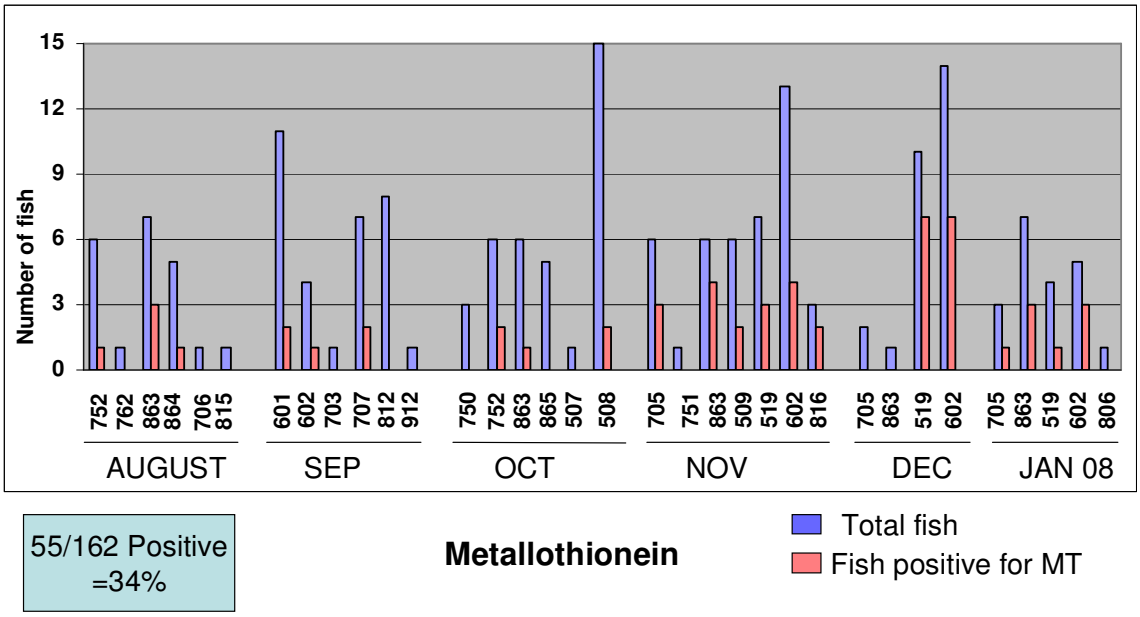
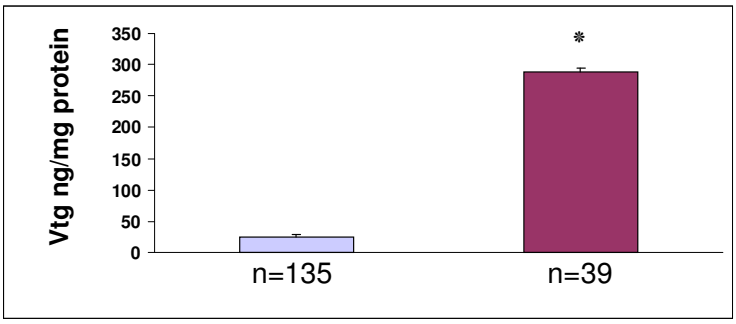


Figure 19. Metallothionein expression by location and month.



*p-Value <.0001, ***Dunnett method***

Figure 20. Statistical analysis of vitellogenin expression in juvenile striped bass collected in the POD special sampling surveys

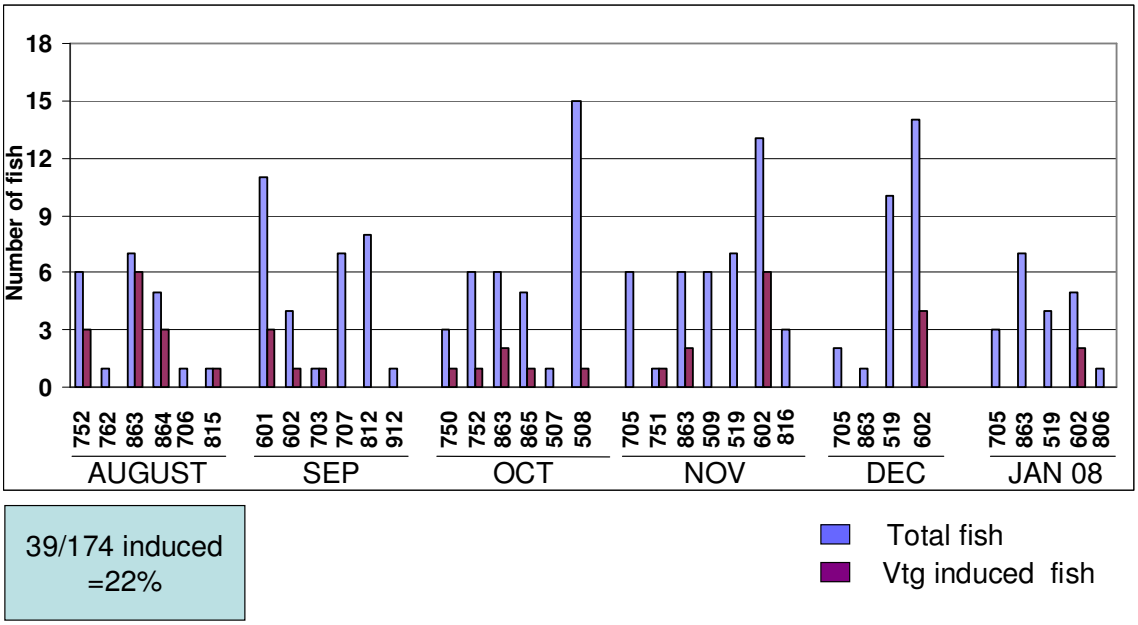


Figure 21. Vitellogenin expression by location and month.

2007 POD Sampling sites indicating positive Metallothionein and Vitellogenin

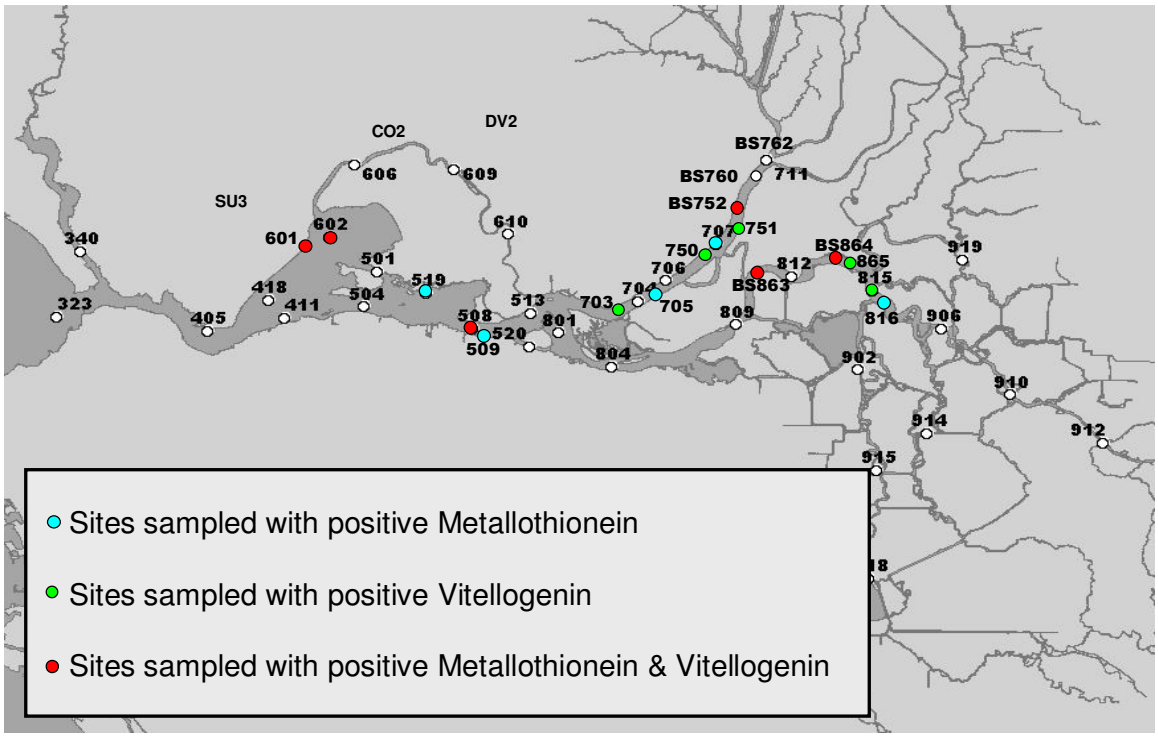


Figure 22. Metallothionein & Vitellogenin results map.

AChE Summary

An important group of biomarkers for chemical exposure and effect are enzymes and the quantification of their activity in plants and animals. An example for a toxicant specific biomarker is the measurement of acetylcholinesterase (AChE) activity in response to exposure to organophosphates (OP) and carbamates, which represent the majority of insecticides currently in use. AChE inhibition is linked directly with the mechanism of toxic action.(Ellmann, 1961) developed a relatively simple method for quantifying AChE activity in vertebrate tissues, since then numerous studies have shown that exposure to OPs or carbamates results in a concentration-dependent inhibition of AChE activity in various tissues. AChE is one of the two broad classes of choline esterases, and responsible for the removal of the neurotransmitter acetylcholine (ACh) from the synaptic cleft through hydrolyzing ACh into cholin and acetic acid (Fulton & Key 2001). The irreversible or reversible binding of OPs and carbamates, respectively, to the esteratic site of AChE potentiates the cholinergic effect, leading to the disruption of neurotransmitter processes.

For vertebrates, these enzymes are vital for the somatic nervous system, the parasympathetic nervous system, the sympathetic nervous system and the central nervous system (CNS) (Fulton & Key 2001). The measurement of enzyme activity emerged as a diagnostic tool in laboratory and field studies with lower and higher vertebrates (Mayer FL 1992). Monitoring AChE inhibition has been widely used in freshwater aquatic ecosystems as an indicator of OP insecticide exposure and effect. One of the advantages of this method is that measuring significant AChE inhibition provides evidence that a sufficient dose of compound has reached the target site to produce a physiological effect. In addition, enzyme inhibition often times persists for an extended period of time, while OP insecticide detection in the environment may prove unsuccessful due to rapid degradation (Fulton & Key, 2001)

AChE activity has been measured in various tissues of several fish species, and a number of studies have examined the relationship between specific levels of OP-induced AChE inhibition and lethality. Relatively high OP concentrations, such as those resulting from an accidental spill, cause hyperactivity, muscle twitching, loss of equilibrium and ultimately death (Zinkl et al., 1991). However, environmentally realistic concentrations encountered by fish are typically much lower, and recent studies have focused on investigating the relationship between OP exposure, AChE inhibition and sublethal effects (Sandahl et al., 2005). Sandahl et al. (2005) for example, specifically related observed changes in fish behavior to AChE inhibition in brain and muscle; thus, linking the degree of enzyme inhibition to behavioral impairments such as compromised predator avoidance, territory defense, and reduced schooling and feeding success, which may, in turn, reduce survival or reproductive success.

AChE Assay

We analyzed AChE activity in brain of Striped Bass similar to the method described by (Wheelock 2005) for Chinook salmon. Entire brains from sacrificed bass were removed, flash-frozen in liquid nitrogen and stored at -80°C. Samples were weighed, diluted 1:10 (mg:µl) in 0.1 M sodium phosphate buffer (pH 8.0) with 0.5 % Triton X-100, and homogenized (1 min, glass on glass) on ice. Homogenates were centrifuged at 4°C for 10 min at 7000 g to remove large particulate material. The supernatant fraction was transferred to separate tubes and the total protein concentration was determined with the Bio-Rad DC Protein Assay, also described in (Wheelock 2005). Average protein concentration in brain from all fish analyzed was 12.2 µg/µl.

The AChE assay was performed using optimized conditions developed in this laboratory for Striped Bass brain. Sodium phosphate buffer (0.1 M, pH 8.0) with 0.5 % Triton X-100 (Sigma-Aldrich) was added to the supernatant fraction for a final dilution of 1:200 or 1:250(mg:µl). 30 µl of diluted supernatant were transferred to microplate wells containing 250 µl of 0.1 M sodium phosphate buffer (pH 8.0), 10 µl of 10.3 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), and 30 µl of 21.4 mM acetylthiocholine iodide (AtChI). Final assay concentrations were 0.32 mM DTNB and 2 mM AtChI. Samples were run in triplicates and absorbance at 405 nm was measured at 2 min intervals for 10 min at 25°C with an automated microplate reader. Substrate blanks were included on each plate and all samples were corrected for background hydrolysis. All activities were calculated as $\mu mol^{-1}.min^{-1}.g$ wet $weight^{-1}$, then normalized to protein content (µmol/min/mg protein).

2007 POD samples: None of the individuals sampled in the field indicate a significant inhibition of AChE. This is not an unexpected finding. AChE inhibition occurs when fish are being exposed to significant levels of pesticides and levels required are not typically seen where the fish were collected. However, it is known through RMP data (SFEI data available on their website) and maternal transfer studies in this lab that striped bass are found to have a chemical body burden of legacy and current use pesticides. Results from this study indicate that these contaminants are not being bioaccumulated through the water or food sources of these juvenile fish but are likely being bioaccumulated by striped bass of an older age utilizing a different and contaminated food source or habitat.

In a laboratory experiment using juvenile striped bass an inhibition of AChE of up to 61 % was seen after an intra-peritoneal exposure of the organophosphate Diazinon at a dose of 100 $mg.kg^{-1}$. AChE activity was measured in juveniles collected in the estuary sampled monthly from August 2007 to January 2008. Here we show that AChE activity was strongly positively correlated to water temperature. The spatial-temporal variability of AChE is currently analysed relative to water temperature and conductivity, and fish size. This later statistical analysis will enable to unravel the relative effects of these different natural factors on the activity of this important neurotoxicity biomarker. Taking into account these natural factors when using AChE as a neurotoxicity biomarker can help to determine and understand the role of contaminants, and manage their effects on pelagic fish populations in the San Francisco Estuary.

Positive control experiment inducing AChE activity inhibition in striped bass:

The positive control injected with 50 and 100 $mg.kg^{-1}$ dose of the organophosphate Diazinon showed significant AChE inhibition of approximately 55 to 61 % respectively (Table 2).

Treatment/dose	AChE Activity (±SE) ($\mu mol.min^{-1}.mg\ protein^{-1}$)
Solvent control	0,137 ± 0.011
Diazinon 50 $mg.kg^{-1}$	0,062 ± 0.003
Diazinon 100 $mg.kg^{-1}$	0,053 ± 0.004

Table 2. Summary of AChE mean activity (± Standard Error) for each group of the positive control experiment: *i.e.* Solvent control (saline buffer), Diazinon 50 $mg.kg^{-1}$ and Diazinon 100 $mg.kg^{-1}$

AChE activity in striped bass YOY juveniles from the POD 2007 survey:

AChE activity in striped bass juveniles remained around 0.25 $\mu mol^{-1}.min^{-1}.mg\ protein^{-1}$ from August to October, then dropped significantly to reach approximately 0.1 $\mu mol^{-1}.min^{-1}.mg\ protein^{-1}$ in January onwards. No significant difference in AChE activity was detected between fish from the three different sampled areas in September, November and December (Kruskal-Wallis test, $p>0.05$). Fish sampled in the San Joaquin river had lower AChE activity than fish from the Sacramento River in August (Mann-Whitney U test, $p=0.034$). In October and January fish caught in Suisun Bay had lower AChE activity than fish sampled in the Sacramento and San Joaquin rivers (Kruskal-Wallis test, $p=0.016$ and $p=0.015$ respectively) (Figure 23). The lower levels observed may be due to slower growth at those sites and times or temperature effects but further investigation is required. We observed a strong significant positive correlation between water temperature and AChE activity (Pearson correlation, $n=171$, $R=0.764$, $p<0.001$) (Figure 24).

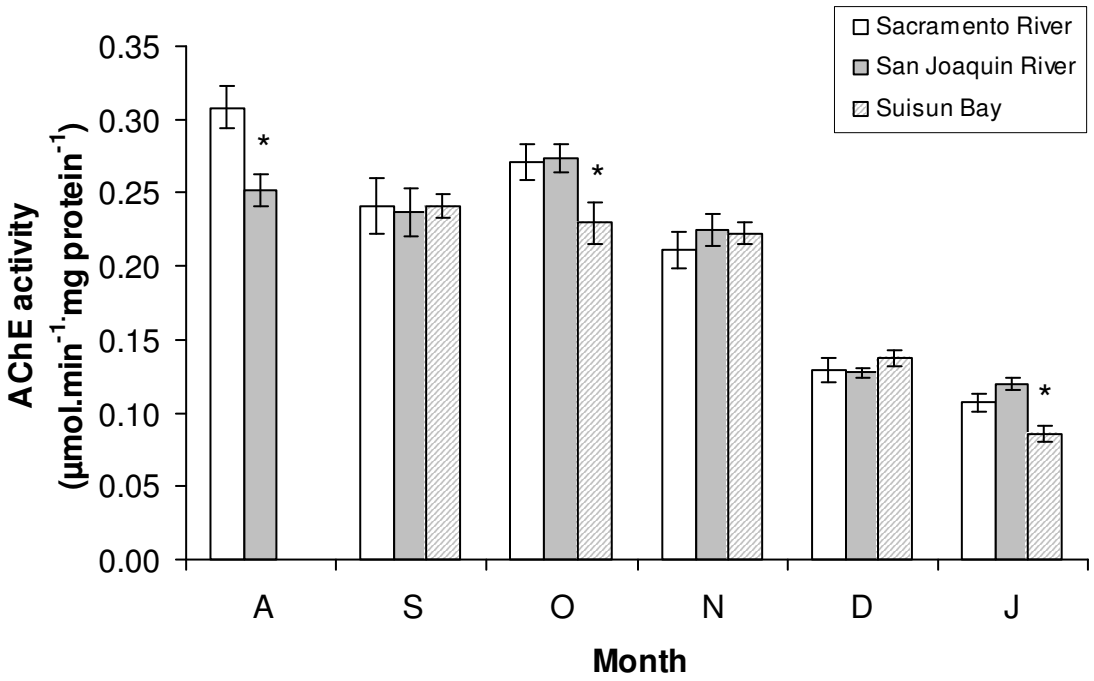


Figure 23. Temporal change in ACHE activity for YOY juvenile striped bass sampled from August 2007 to January 2008 in the three regions of the San Francisco estuary. Error bars are Standard Error (SE). The star Indicates significant differences between groups using the non-parametric Kruskal-Wallis test with $p<0.05$.

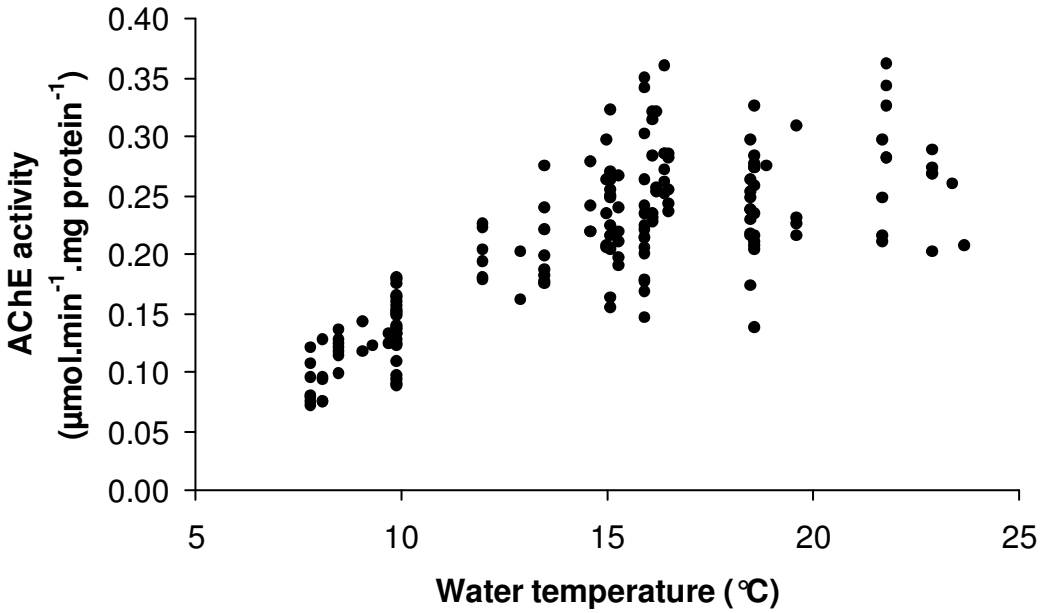


Figure 24. Relationship between water temperature and AChE activity in YOY juvenile striped bass sampled from August 2007 to January 2008 in the San Francisco estuary (n = 171).

Juvenile striped bass otolith age estimation and growth

Juvenile striped bass samples were received from fish collected by the California Department of Fish and Game Summer Kodiak Survey, Townet Survey, Fall Midwater Trawl Survey and special collections during the months of June 2007 - January 2008. The surveys sampled a wide are in the delta system including sites in Montezuma Slough, the confluence of the Sacramento San Joaquin Rivers, the Sacramento River and the Stockton deep water channel. The fish heads were removed and stored in 70% ethanol (Tables 4a-8a).

Sagittal otoliths were extracted under a dissecting scope and embedded into blocks of Eponate resin, then incubated at 80˚ for 12 hours. The choice of sagittal otolith comes from the significant size difference over the asterisci or lapilli, thus being easier to handle, prepare and analyze and are the otoliths used in other age/growth studies in striped bass (Secor 1991; Secor and Dean 1992). After hardening the resin blocks containing the otoliths were sectioned in the transverse plane using a low speed diamond saw. The decision to use the transverse plane for age estimation came from a review of what previous striped bass researchers used (Jody Callihan personal communication; Secor 1991; Secor & Dean 1992) as well as experimentation with the quality of differing planes. Crystal Bond™ affixed the sectioned blocks to a glass microscope slide and otoliths thinned with the core brought into view by polishing with wet sandpaper of three grits: 300, 600 and 1500. The closer the core to the surface, the finer the grit was applied to the block. Alumina slurry applied to a patch of nylon cloth brought the prepared otolith to a fine polish and removed scratching that resulted from the sandpaper.

Finished slides were photographed and recorded digitally for further analysis. Cover slips placed over a drop of immersion oil provided a slight increase in clarity and helped to smooth out any remaining scratches on the otolith surface. A Qimaging 5.0 MP digital microscope camera linked to a Leica DM 1000 compound microscope using a 40X objective was used to photograph the otoliths. Images were captured by the Qimaging software CapturePro ver. 5.1 (Media Cybernetics). All photographs were taken at 400x magnification. Two to three photographs were taken of each otolith to insure that a complete path could be traced from core to edge in at least two separate directions.

Images were imported into ImagePro ver. 6.2 (Media Cybernetics) for counting of daily increments. Counting began at the second check mark from the core where banding becomes consistent and exogenous feeding begins (Secor 1991). Otoliths were coded and read blindly a minimum of three times by a single observer, with two out of the three being counted in opposite directions on the otolith. An average of the three counts was taken as the final age estimation.

Counts for all areas were graphed against fork length in Microsoft Excel on a scatter plot and fitted with both a simple linear regression ($L= a+bx$) and curvilinear ($L=ae^{GX}$) line of best fit (Campana 1992). The curvilinear model produced the higher R^2 value was deemed the better fit for the data and is the model used in other striped bass growth studies (Secor and Houde 1995). From this model instantaneous growth was determined for the entire sampling area and individual areas by plotting them in a scatter plot with L representing fork length, X the final count/age, fitting them with a curvilinear line and taking the G (instantaneous growth) value of that line.

Juvenile otolith results

The age and growth of otoliths from 95 juvenile striped bass collected in 2007 were evaluated from samples provided by DFG's monitoring surveys. Otoliths evaluated were from 4 locations in the estuary: The confluence of the Sacramento and San Joaquin Rivers, n = 16; Montezuma Slough, n = 31; Sacramento River, n = 24 and the Stockton Deep Water Channel, n = 24. In 2006 the age and growth of otoliths from 65 juvenile striped bass were evaluated from 6 locations in the estuary: Montezuma Slough, n = 11; Suisun Bay, n = 7; Suisun Marsh, n = 33; Sacramento River; n = 4, Grizzly Bay, n = 6 and Napa River n = 4. We had hoped to compare age and growth results by region for these two years since 2006 was a relatively wet year and 2007 was a very dry year. However, there were not enough samples collected from the same locations to compare by region. We did compare the two years using samples from

all delta locations combined. The instantaneous growth rate calculated for all juvenile striped bass otoliths analyzed from the various regions of the delta in 2007 was 0.250 d^{-1} with an r^2 value of 0.705 (Figure 26). In 2006 the instantaneous growth rate from all locations sampled was 0.235 d^{-1} with an r^2 value of 0.718 (Figure 25) indicating very similar growth in both years despite the difference in climate and that the samples were not from all the same locations. In comparison to a striped bass study performed on the East Coast in the Chesapeake Bay the instantaneous growth rate calculated from the SFE was found to be at the lower end of the range found in that study. In the Chesapeake Bay study the range of instantaneous growth rates were found to be 0.26 d^{-1} – 0.33 d^{-1} with a mean of 0.29 d^{-1} (Secor and Houde 1995). In the Chesapeake Bay study the temperatures were similar to those found in the San Francisco Estuary in 2006 and 2007 ranging from 19°C-20 5°C throughout the same developmental period. In that study it was determined that the major factors affecting juvenile striped bass growth after~25 days post hatching were temperature and zooplankton abundance. The San Francisco Estuary primary productivity has historically been significantly lower than that of the Chesapeake Bay system therefore the instantaneous growth rates determined in this study in 2006 and 2007 seem reasonable and comparable. It does not appear that this age cohort of fish from two years of investigations is growing at a significantly slower or faster rate than counterparts in the East Coast study. There are relatively few other East Coast studies that are comparable to our study of this estuary system. Laboratory studies on the East Coast have shown differences in instantaneous growth rates between South Carolina strains of striped bass (Santee-Cooper -0.54 d^{-1} – 0.85 d^{-1}) and Maryland strains (Chesapeake Bay-0.57 d^{-1}– 0.109 d^{-1}). However comparisons of instantaneous growth rates between field collected specimens and laboratory studies must be viewed with caution, as there are many variables in the field not found in a controlled laboratory setting.

When juvenile striped bass otolith results are viewed by region some differences in instantaneous growth rates are seen. In Montezuma Slough the instantaneous growth rate was determined to be 0.313 d^{-1} with an r^2 value of 0.68 and sample size of 31 (Figure 28); at the confluence of the Sacramento and San Joaquin Rivers instantaneous growth rate was found to be 0.232 d^{-1} with an r^2 of 0.69 and sample size of 16 (Figure 27); in the Sacramento River the instantaneous growth rate was found to be 0.208 d^{-1} with an r^2 of 0. and sample size of 24(Figure 29); in the Stockton Deep Water Channel the instantaneous growth rate was found to be 0.112 d^{-1} with an r^2 of 0.22 and sample size of 24 (Figure 30). The instantaneous growth rates in Montezuma Slough were found to be larger than the average of all regions and statistically larger than all other regions sampled in 2007 (Figure 31), the confluence of Sacramento and San Joaquin Rivers values were near the average with Sacramento River And Stockton Deep Water Channel smaller than the average for all regions. However, the sample size when viewed by region is relatively small so additional samples from all regions are needed to verify any apparent differences in regional instantaneous growth rates observed.

In summary, instantaneous growth rates determined for juvenile striped bass from all regions sampled from 2006 and 2007 are similar to minimum growth rates found in striped bass from the Chesapeake Bay study. Although 2006 was considered a wet/high flow year and historically striped bass are found to be in better condition and higher numbers during this type of climactic condition the growth rates from all regions in 2007 a very dry year were similar. Only two regions were sampled in both 2006 and 2007 Montezuma Slough and the Sacramento River. The growth rate in Montezuma Slough was much less in 2006 at 0.161 d^{-1} than in 2007 where it was 0.313 d^{-1} and the reverse true for the Sacramento River samples where in 2006 the growth rate was 0.304 d^{-1} and in 2007 was 0.208 d^{-1}. However, the sample size reported in 2006 from these two is relatively small (n= 11 and n = 4 respectively) compared to 2007 so this comparison must be viewed with caution. To better determine age and growth differences in juvenile striped bass collected from various regions and climactic conditions in differing years a more consistent yearly sampling protocol must be put in place. It is likely that during dry versus wet years juvenile striped bass are found in different abundance and in different habitats with some being more suitable for better growth and survival. In these two years of investigations due to the relatively low numbers collected we cannot make any definitive statements about growth and survival by region during these very different climatic years. In addition, due to collection methods very few juvenile striped bass less than 40 days of age were included in this otolith analysis. So the finding of "normal" instantaneous growth rates needs to be viewed with caution. In conclusion we can state that from the limited samples collected in 2006 and 2007 across all regions growth was very similar in these two very different climactic years.

Without evaluating larval/juvenile striped bass at the more sensitive life stage, the metamorphosis of larvae into juveniles and young juveniles, current findings may be that of robust survivors. This study does not provide any insight on growth rates or health due to lack of histological samples from the late larvae to early juvenile age category and any potential problems affecting these earlier more sensitive life stages. We recommend that sampling of smaller and earlier life stage striped bass is incorporated into future sampling efforts such the data can be obtained and evaluated in a more complete manner.

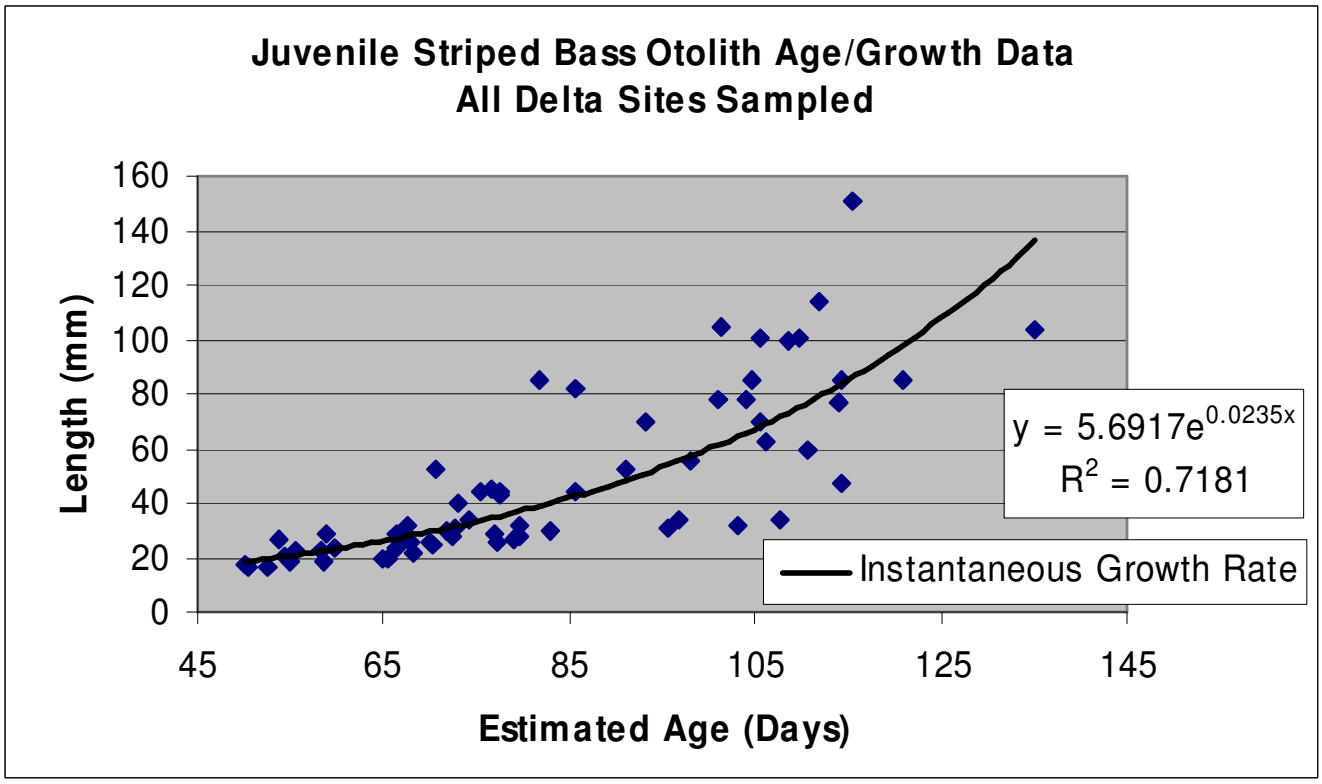


Figure 25. Juvenile striped bass otolith age/growth data 2006:
All locations combined, n = 61.

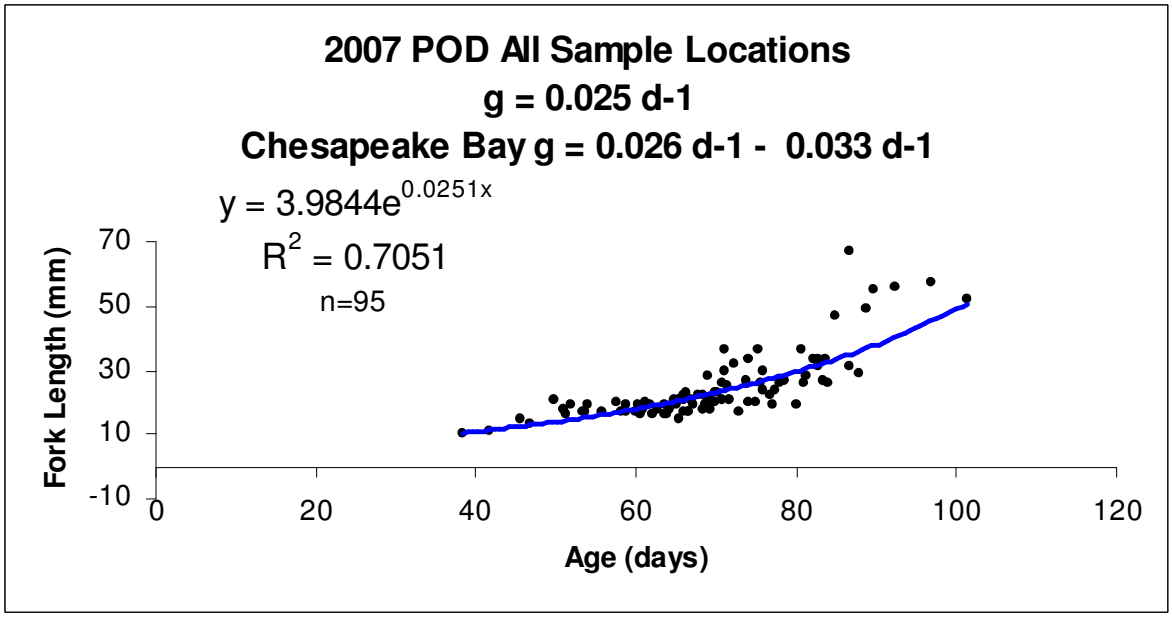


Figure 26. Juvenile striped bass otolith age/growth data 2007:
All locations combined, n = 95.

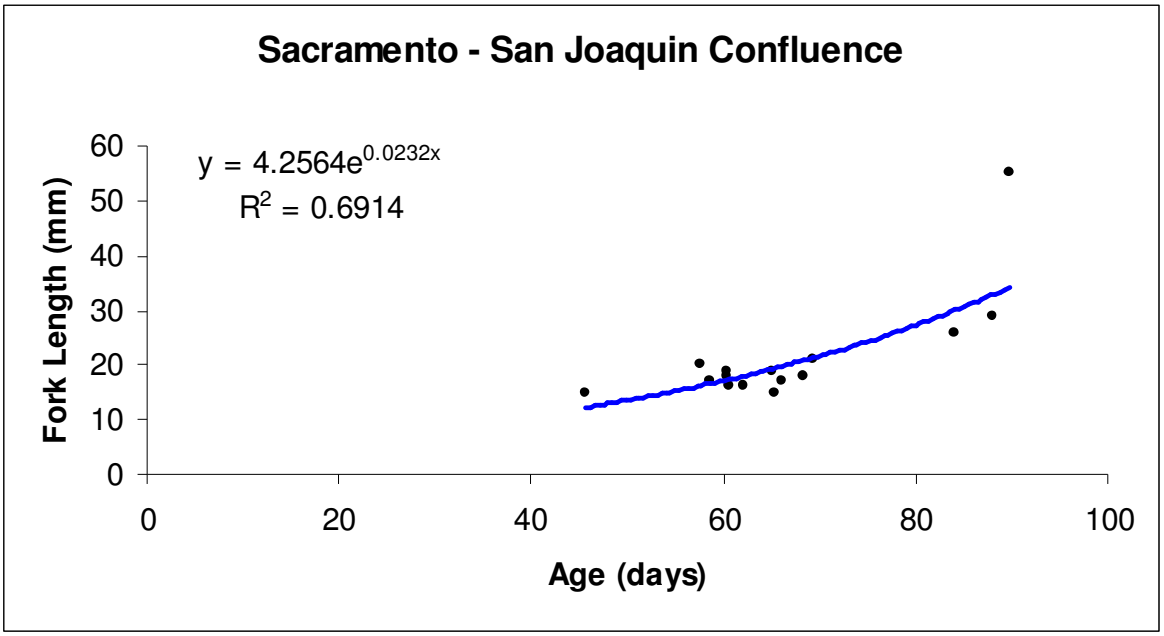


Figure 27. Juvenile striped bass otolith age/growth data 2007: Confluence of the Sacramento San Joaquin Rivers, n = 16.

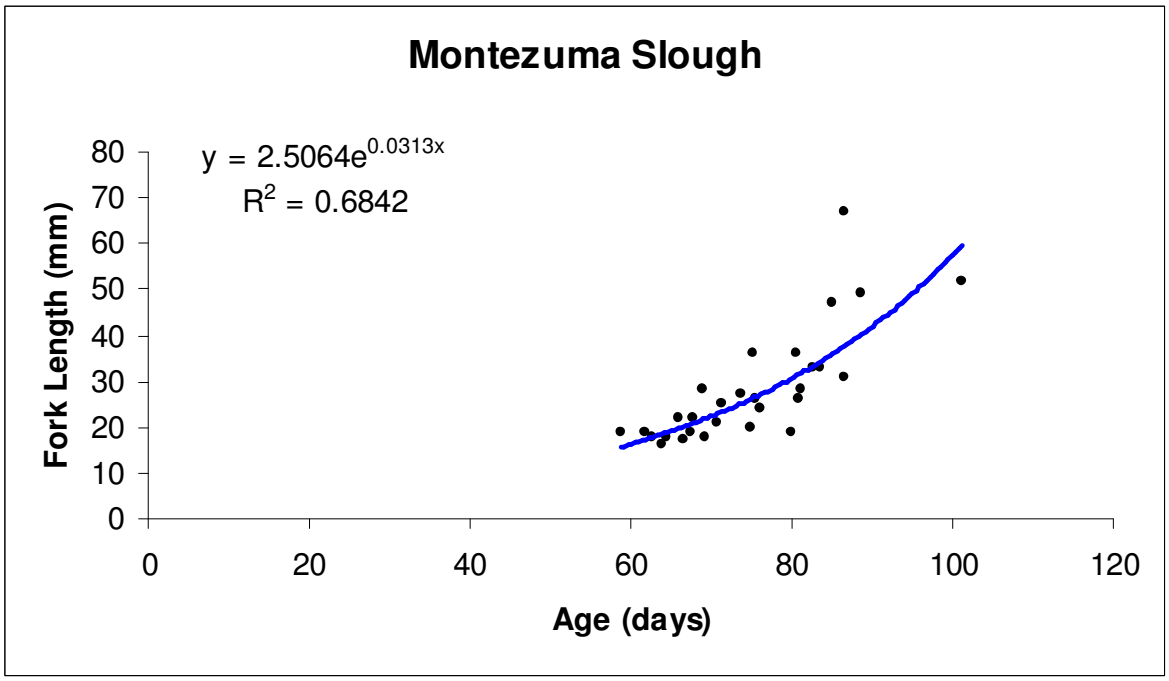


Figure 28. Juvenile striped bass otolith age/growth data 2007: Montezuma Slough, n = 31

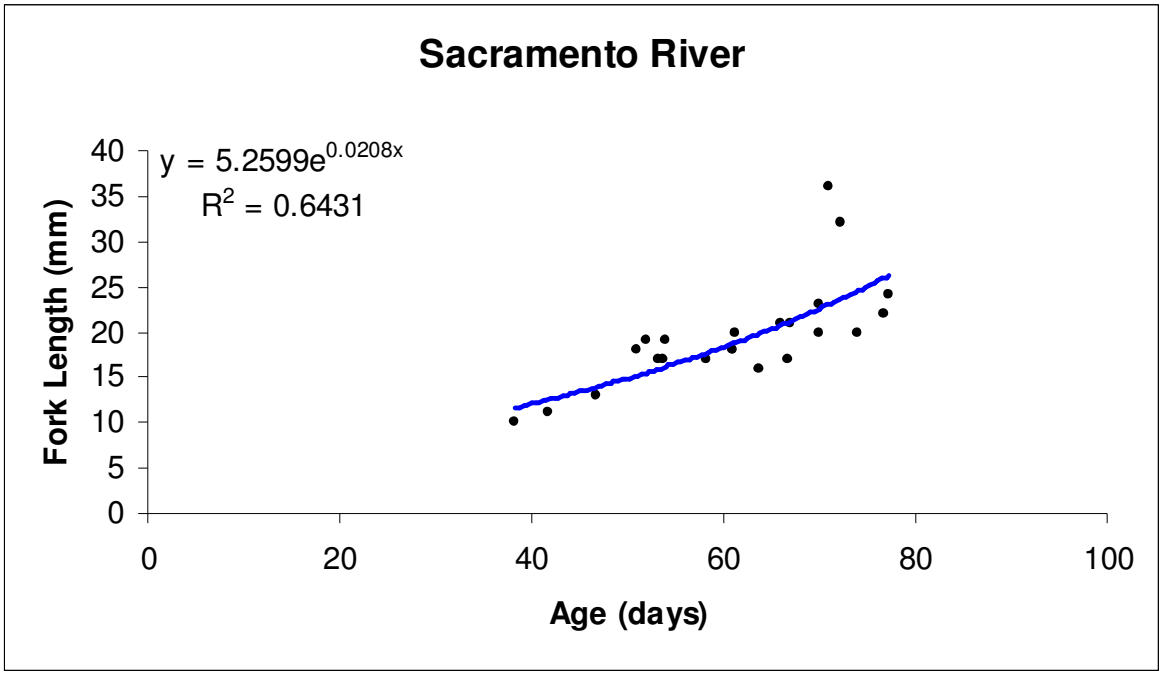


Figure 29. Juvenile striped bass otolith age/growth data 2007: Sacramento River, n = 24

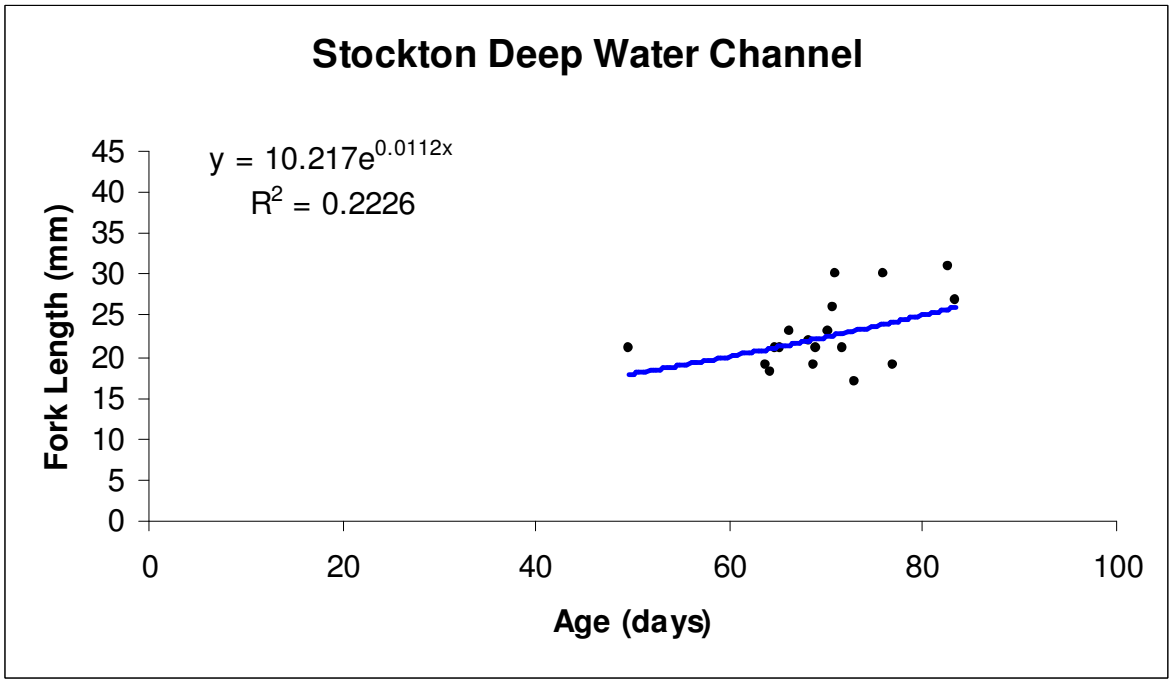
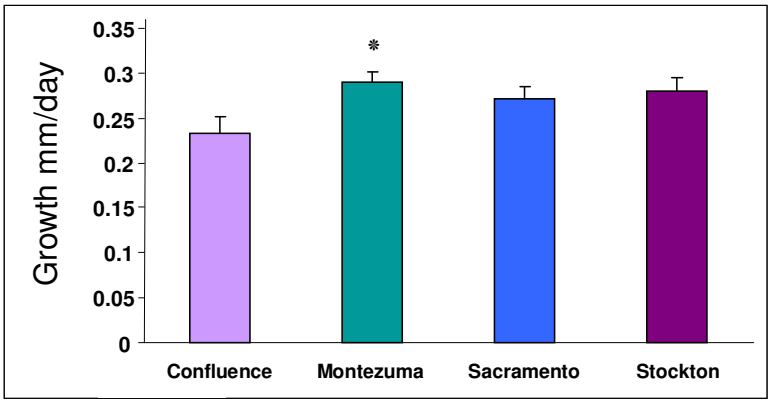


Figure 30. Juvenile striped bass otolith age/growth data 2007: Stockton Deep Water Channel, n = 24



*p-Value <.005, *Dunnett method*

Figure 31. Comparison of juvenile striped bass growth through 55 days of age by location in 2007. Growth in Montezuma Slough was significantly higher than in the other locations.

Age and microgeochemical analyses of Adult Otoliths

Methods

Strontium isotopic composition was analyzed using a Multi-Collector ICP Mass Spectrometer (Nu Plasma HR manufactured by Nu Instruments Ltd., UK). The otoliths were microsampled with a New Wave Research UP-213 laser ablation system equipped with an Nd:YAG deep UV (213nm) laser (New Wave Research, Inc 48660 Kato Road, Fremont CA 94538). Spot transects from core to rim were assayed using a laser beam size 60µm, 80-100% laser power, and 10 Hz repetition rate. Helium was used as the carrier gas at a flow rate of 0.85 L/min and mixed with Ar at a flow rate of ~1.0 L/min prior to introduction to the mass spectrometer. Typical ^{88}Sr signals of 2-6 volts were obtained during the analyses. Gas blank and background signals were monitored until ^{84}Kr and ^{86}Kr stabilized after the sample change (i.e. exposing sample cell to the air) and were measured for 30 seconds. Then the laser was turned on typically for 30-60 seconds. Background signals were subtracted from the measured signals automatically. The $^{86}Sr/^{88}Sr = 0.1194$ was used to correct for instrumental fractionation in accordance with the exponential law. The peak intensities for ^{88}Sr, ^{87}Sr, ^{86}Sr, ^{85}Rb, and ^{84}Sr are measured simultaneously. The ^{85}Rb peak is monitored to correct for any ^{87}Rb interference on ^{87}Sr. The accuracy of the measured $^{87}Sr/^{86}Sr$ ratios were verified by measuring NIST Sr standard and in-house carbonate standard throughout the analytical session. To allow for comparisons between otoliths run during different sessions, and to allow for interlaboratory comparisons between otolith and water data, all $^{87}Sr/^{86}Sr$ ratios are expressed as $\Delta^{87}Sr$, where:

$$\Delta^{87}Sr = (^{87}Sr/^{86}Sr_{sample}) - (^{87}Sr/^{86}Sr_{seawater\ standard}) \text{ X } 100{,}000. \quad \textit{(eq. 1)}$$

Mixing Models

Strontium composition of the San Francisco Estuary, and its two principle freshwater inputs, the Sacramento and San Joaquin Rivers, has been well studied. Ingram & Sloan (1992) developed a mixing curve for the San Francisco Estuary using measured salinity, water strontium concentration and strontium isotopes:

$$Salinity = 35\,ppt \times \frac{\Delta^{87}Sr_{otolith} \times [Sr]_{FW} - \Delta^{87}Sr_{FW} \times [Sr]_{FW}}{\Delta^{87}Sr_{Mar} \times [Sr]_{Mar} - \Delta^{87}Sr_{FW} \times [Sr]_{FW} - \Delta^{87}Sr_{otolith}([Sr]_{FW} - [Sr]_{Mar})}$$

(eq. 2)

Where $\Delta^{87}Sr_{Mar}$ (0, by definition) and $[Sr]_{Mar}$ (7.9 ppm) are the global marine end-members and $\Delta^{87}Sr_{FW}$ (-292) and $[Sr]_{FW}$ (0.093 ppm) are the locally measured freshwater end-members.

Results from 10 otoliths collected on the Mokelumne and 1 on the San Joaquin River in 2006.

Striped Bass life-histories for 11 adult striped bass were analyzed on the Multi Collector LA-ICPMS at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry (Figures 1a – 11a). Salinities are calculated from measured $^{87}Sr/^{86}Sr$ using the mixing-model presented in Ingram & Sloan, 1992. Mean annual salinities at four sites within the San Francisco Bay Estuary are plotted for reference.

The one six year old female fish collected on the San Joaquin River during the spawning run lived the vast majority of its life in freshwater with the exception that between year 3 – 4 where it made one migration to higher salinities (Figure 11a). The contaminant profile for the eggs from this female was similar to or higher than those of Sacramento River collected females where maternal transfer has been shown to adversely affect larval development and survival (Figures 1, 2, 3b, 3c and Tables 1a-3a).

The results from the 10 fish (8 females & 2 males) collected from the Mokelumne River in 2006 were interesting and indicated that there were both resident and nonresident fish collected at the location. Of the eight females collected six were determined to be resident fish living their entire lives in the Mokelumne River were nonresident fish. One of the two nonresident females lived in relatively high salinity during its early life and then spent the remainder in the habitat between Chipps Island and Suisun Bay (Figure 1a). The other nonresident female lived the majority of its juvenile life up through sexual maturity (1.5-4.5 years of age) in the relatively high salinity habitat between San Pablo Bay and the ocean after which it appeared to make yearly migrations from high salinity to freshwater presumably to spawn (Figure 3a). The contaminant profiles of the eggs from both of these females was similar to those of Sacramento River collected fish where maternal transfer has been shown to adversely affect larval development and survival (Table 1a-3a). Findings of differing contaminant levels in the eggs of resident female fish collected on the Mokelumne River are interesting and deserving of further investigation. Two of the resident females' contaminant profiles were similar to those of fish collected on the Sacramento River, two had contaminant profiles that were significant but less than those collected from the Sacramento River and two of the females had contaminant profiles similar to or less than those found in hatchery controls (Table 1a-3a).

The strontium isotopic signatures of the resident fish are clearly a Mokelumne River signature. The difference seen in the resident fish eggs may be due to differing diets of the individual fish. In addition, findings from the Mokelumne River resident fish indicate larvae from the females' with low/no contaminants in their eggs would have a much higher probability of developing normally and higher survival than larvae produced from females exploiting the delta habitat. Although the Mokelumne River is not the preferred spawning habitat for striped bass these results indicate that sub-groups of resident fish living in relatively uncontaminated sites within the delta system may be contributing more to the striped bass population than originally considered.

Results from 11 otoliths provided by DFG's Creel Census from the Pacific Ocean off the coast of Stinson Beach.

Habitat use for 11 striped bass was analyzed on the Multi Collector LA-ICPMS at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry using methods described above (Figures 29a – 39a). Despite being collected in the ocean environment off the coast of Beach all but two of these fish live the vast majority of their life in freshwater through the low salinity habitat of Suisun Bay. Two fish (SB6 - Figure 34a & SB9 - Figure 37a) lived in the slightly more saline habitats in between Suisun Bay in San Francisco bay. Results indicate that these ocean collected fish were only making brief excursions to the marine environment presumably to feed during the summer and that they were not spending any significant portion of their life in the ocean environment. This corroborates earlier findings of ocean collected fish from various regions between Stinson Beach and Monterey Bay.

Results from 90 otoliths provided by SFEI's RMP & FMP Collections from various regions within the San Francisco Estuary.

In collaboration with SFEI 90 adult male and female striped bass adult striped bass otoliths were provided from the RMP & FMP surveys in support of our research from various areas in the estuary system including: San Pablo Bay (n=23); Berkeley/Central Bay(1); South Bay (n=1); Rio Vista Fish Derby (n=2); Cache Slough (n=1); Cosumnes River (n=1); Miner Slough (n=1); Clifton Court Forebay (n=20) collected outside the radial gates); Liberty Island (n=21); Toe Drain (n=3); and Knights Landing (n=16). We reported results of twelve of these in our last report but have included all 90 here.

Liberty Island Results Figures 40a – 60a.

Twenty one adult striped bass were collected near Liberty Island. Of these six fish lived the vast majority of their life in freshwater, three live mainly between Chipps Island and Suisun Bay, three spent the majority of their time in the habitat between Chipps Island and San Francisco Bay, four fish exhibited migration patterns between fresh and salt water during the latter part of their lives and five fish had variable life history patterns spending varying amounts in the delta and more saline habitat.

Knights Landing Results Figures 61a – 76a.

Sixteen adult striped bass were collected on the Sacramento River at Knights Landing. Ten fish live the vast majority of their life in freshwater habitat, two exhibited life history patterns that varied spending time in both freshwater and more saline habitat, three exhibited migration patterns between salt and fresh water during the latter part of their lives and one fish lived the majority of its life between Chipps Island and Suisun Bay.

Clifton Court Results Figures 77a -96a.

Twenty adult striped bass were collected at Clifton Court outside the radial gates. Sixteen fish lived their entire lives as residents of Clifton Court or in freshwater, three spent the majority of their life in freshwater with the latter part of life spent between Chipps Island and Suisun Bay and one fish exhibited migration patterns between Suisun Bay and salt water after its first year of life (Figure 79a).

Cosumnes River, Rio Vista, Toe Drain, Miner Slough and Cache Slough Results Figures 97a – 104a.

One to three fish were collected at each of these sites with the habitat use results as follows: the one female striped bass collected on the Cosumnes River (Figure 97a) spent its entire life in freshwater with the exception of a brief excursion into day more saline habitat during its first year of life; of the two male fish from the Rio Vista fishing derby one resided in freshwater during its first two years of life and thereafter utilized the habitat between Chipps Island and San Francisco Bay (Figure 99a) and the other striped bass spent approximately one year/20% of its life in the marine environment with the remainder between freshwater and Suisun Bay; three male striped bass were collected at the Toe Drain of these two spent their entire lives in freshwater (Figures 100a-101a) and the third fish exhibited migration patterns between fresh and salt water after the second year of life (Figure 102a); one female striped bass was collected at Miner Slough and it lived in freshwater during the first two years and exhibited migration patterns between fresh and salt water and thereafter (Figure 103a); one female striped bass was collected at Cache Slough that lived in freshwater its first three years and exhibited migration patterns between salt in freshwater thereafter (Figure 104a).

San Pablo Bay, South Bay & Central Bay Results Figures 105a – 129a.

Twenty three adult striped bass were collected from San Pablo Bay and one striped bass from South and Central Bay were also examined. As of this report the conversion from a number of spots to age is not yet completed for these 25 fish. The conversion and final analysis should be completed within two weeks. Note that the distance between rings on adult otoliths get smaller/more compressed as the fish gets older such that the number of spots shown on the x-axis is not a linear representation of age. These fish collected in the Outer Bays appeared as a group to be more migratory and live in more saline habitats than those collected in other regions of the estuary. Ten striped bass collected in San Pablo Bay exhibited migratory patterns between fresh and salt water, three fish resided mainly in the marine environment, five fish spent the majority of their time between Chipps Island and San Francisco Bay, two spent the majority of their lives between Chipps Island and Suisun Bay and two fish lived the majority of their life in freshwater habitat. The one striped bass collected in South Bay live the majority of its life in freshwater with approximately the last two years residing between freshwater and San Pablo Bay (Figure 114a). The striped bass collected in the Central Bay exhibited migratory patterns between fresh and salt water after approximately the first six months of life (Figure 129a).

Striped bass habitat use findings.

Habitat use has been shown to be an important factor in the bioaccumulation of contaminants in striped bass. This study examines habitat use in relation to the bioaccumulation and maternal transfer of xenobiotics to progeny in the San Francisco Estuary system as well as examines adult habitat patterns to better understand factors relating to the POD. Otolith microchemistry has been used to measure habitat utilization and migration patterns of striped bass on the East Coast in the Chesapeake Bay (Secor and Dean 1992) and Hudson River (Secor and Piccoli 1996). However, this approach has only recently been applied to striped bass on the West Coast. In this study, habitat use and residence time over the life of adult striped bass was studied by analyzing otolith Strontium isotopes using laser ablation MC-ICPMS analyses. The last two years of female striped bass' life was examined more intensively as female striped bass transfer their entire body burden of lipophilic contaminants to the eggs at spawning. Otolith 87Sr/86Sr values indicate that the vast majority of these striped bass are residing primarily in meso-haline waters and not the ocean, which is in agreement with the salinity/habitat use data.

It is common knowledge that striped bass are captured by sport fishermen in the Pacific Ocean both North and South of this estuary system. In order to determine if there are subgroups or subpopulations of striped bass utilizing the ocean habitat and not the estuary as shown by the fish collected on the upper Sacramento River attempts were made to collect otoliths from ocean captured striped bass. Previously we reported habitat use patterns of 4 ocean collected fish with results indicating that the majority evaluated thus far are utilizing the freshwater and Delta habitats and not spending a significant time exploiting the Pacific Ocean habitat. We obtained from DFG's Creel census an additional 11 adult striped bass collected in the Pacific Ocean off the coast of Stinson Beach. Results indicate that these ocean

collected fish were only making brief excursions to the marine environment presumably to feed during the summer and that they were not spending any significant portion of their life in the ocean environment (Figures 29a -39a). This corroborates earlier findings of ocean collected fish from various regions between Stinson Beach and Monterey Bay. However, we plan to evaluate more striped bass collected from various regions in the Pacific Ocean to increase the numbers of fish evaluated and to better determine if there is a subgroup exploiting ocean habitat rather than the Delta.

The majority of adult striped bass otoliths evaluated thus far have been from collections on the upper Sacramento River comprised mainly of spawning females. In an effort to provide a broader spatial coverage of the Delta habitat use by striped bass and to include more adult male fish we obtained otoliths from 90 adult male and female striped bass collected from various regions of the Delta by SFEI as part of their regional monitoring programs (Figures 40a – 129a). Location of capture and numbers collected at each site are listed above in the results section. The 65 striped bass collected from Liberty Island, Knights Landing, Clifton Court, Cosumnes River, Rio Vista, Toe Drain, Miner Slough and Cache Slough habitat use data indicates that 35 fish lived in freshwater, 11 lived in a meso-haline habitat between freshwater and Suisun Bay, 9 fish had variable life histories spending time in both fresh and saline habitat but not exhibiting typical migration patterns and 10 fish exhibited migration patterns between fresh and salt water habitats during their adult life. Results from these 65 fish corroborate earlier findings indicating that the majority of these adult striped bass are utilizing the freshwater and meso-haline habitat east of San Francisco Bay. Histograms of all striped bass evaluated thus far (with the exception of the 25 fish from the outer bays that have not been converted to age data) illustrating lifetime habitat use, the last two years habitat use by location and sex (when available) is found below in Figure 32 & 33.

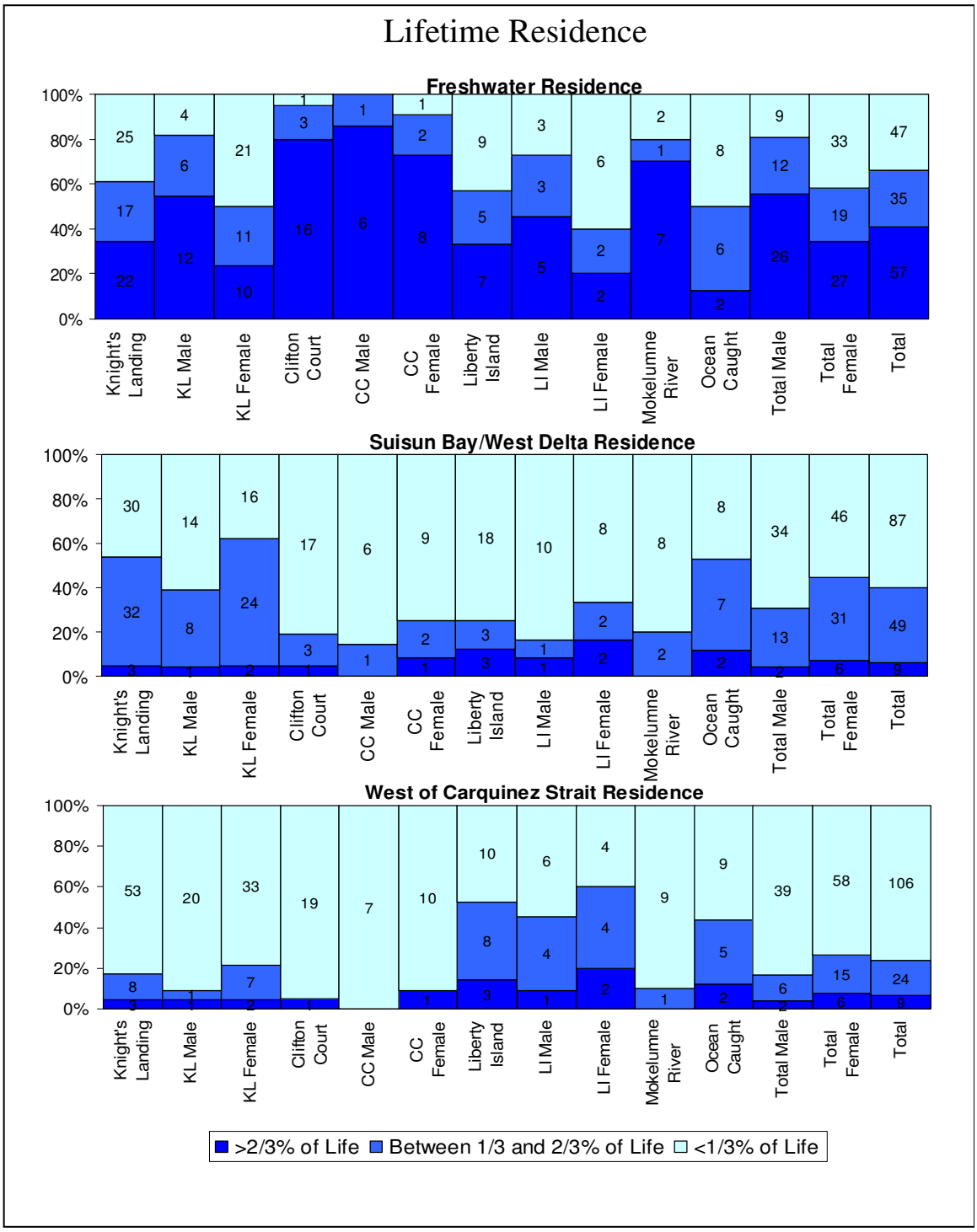


Figure 32. Lifetime habitat use of adult striped bass by location of capture and sex (when available). Each histogram represents habitat use by percent residence time of the same group of fish for each habitat type (Freshwater, Suisun Bay/West Delta & West of Carquinez Strait) and numbers in each bar represent the number of adult striped bass.

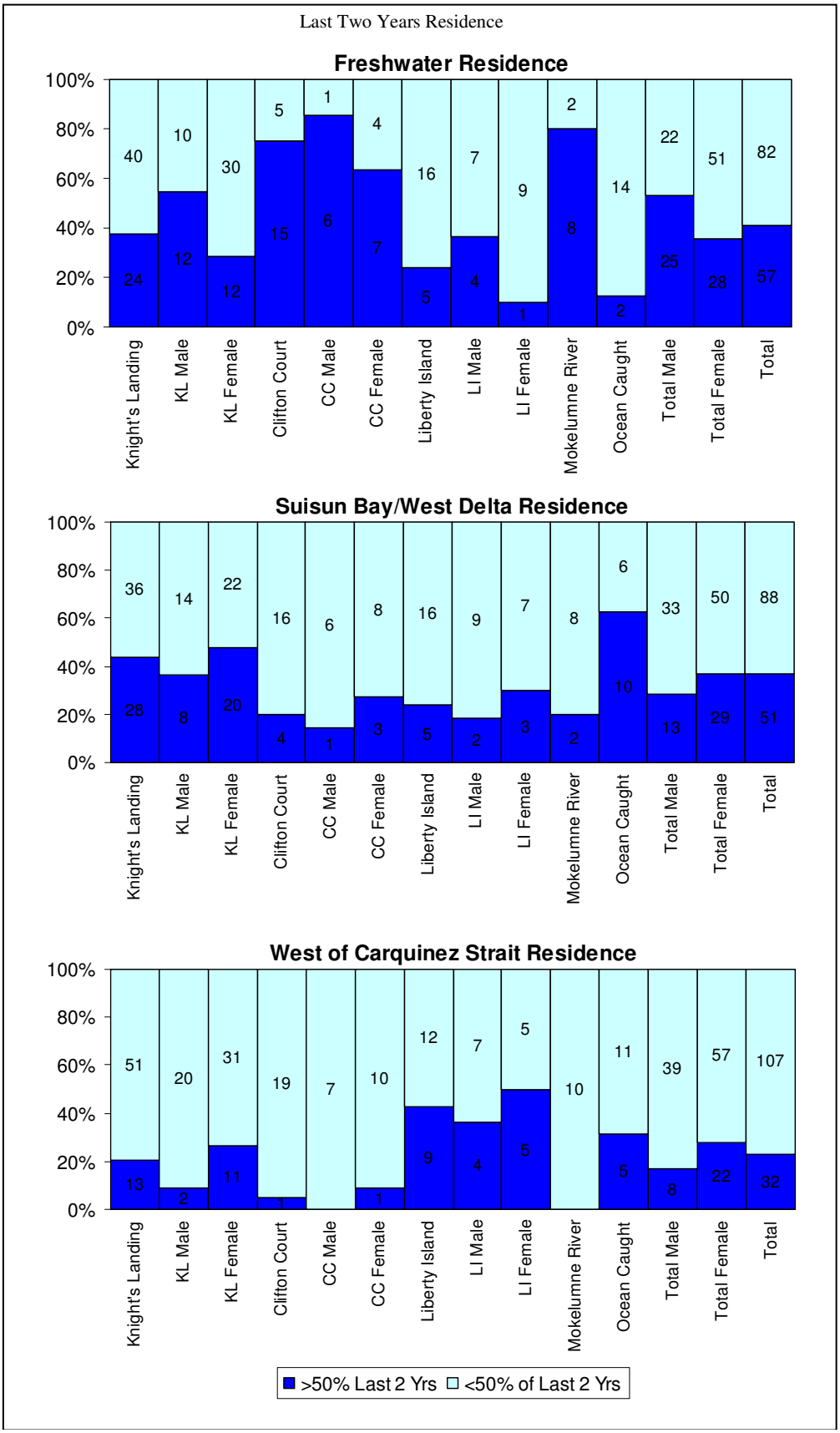


Figure 33. Habitat use of adult striped bass during the last two years of life by location of capture and sex (when available). Each histogram represents habitat use by percent residence time of the same group of fish for each habitat type (Freshwater, Suisun Bay/West Delta & West of Carquinez Strait) and numbers in each bar represent the number of adult striped bass.

As the numbers of striped bass habitat use patterns analyzed increases it appears that some resident sub-groups in various areas of the estuary within the population may exist. In addition to a subgroup of resident striped bass collected on the Mokelumne River it appears that other subgroups of striped bass with differing life history patterns may exist within this population that could affect bioaccumulation of contaminants and reproduction. Fourteen of 20 striped bass collected at Clifton Court appear to be resident fish living outside the radial gates likely due to a plentiful food supply brought to them by the pumping operations. The strontium isotopic signature from these 14 fish is consistent with that of the water in the location of capture and indicates no movement during the majority of their lives (Figures 77a, 78a, 84a, 86a – 96a). Striped bass collected in the outer bays (San Pablo Bay, South Bay and Central Bay) seem to have life history patterns different than those of fish collected elsewhere in the estuary and may also represent a subgroup within the population. Although final conversion of laser ablation spots to age has not been completed for the fish from the outer bays these fish appeared as a group lived in regions of higher salinity and were more migratory than those collected in other regions of the estuary. Three fish spent the majority of their life in the marine environment and represent the only striped bass marine residents evaluated thus far, 10 striped bass exhibited migration patterns between fresh and salt water habitats during their adult life, 11 had habitat use patterns indicating variable use of fresh and saline environments but not exhibiting typical migration patterns, 5 lived the majority of their lives in the habitat between Chipps Island and Suisun Bay (one lived between Chipps and SF Bay) and only 2 fish captured in the outer bays lived the majority of their lives in freshwater.

Depending on the location contaminant bioaccumulation and effects on reproduction may be different between some of the subgroups. For example as mentioned above some female striped bass collected on the Mokelumne River were lifetime residents and showed extremely low contaminant levels in her eggs. The majority of the striped bass collected at Clifton Court also appeared to be freshwater resident fish. Although egg samples for contaminant analysis were not obtained for these fish if they did spawn in the area of the radial gates their eggs/larvae would be lost either by entrainment or by succumbing to the effects of the pumps. Contaminant levels in tissues for some of the striped bass collected at Clifton Court should be available by the next reporting period and may provide additional information on bioaccumulation contaminants in different regions of the estuary. Striped bass collected in the outer bays appeared to be fish that live in more saline environments and contained higher numbers of migratory individuals. If these fish are spawning it is unlikely they are doing so on the Sacramento River. None of the female fish collected from Knights Landing during the spawning runs (1999, 2001 & 2006) have shown habitat use patterns similar to those found from these striped bass collected in the outer bays exhibiting migratory or more saline habitat use. Contaminant levels for some of these outer bay fish should be available by the end of the next reporting period in December 2008. In summary the vast majority of adult female and male striped bass evaluated in this study encompassing a very broad area of the San Francisco Bay Estuary system including fifteen fish collected in the Pacific Ocean are exploiting the freshwater and delta habitats while not spending any appreciable time in the Pacific Ocean environment. A few fish make periodic trips to the ocean presumably to feed. Although subgroups may exist within the three locations evaluated thus far (Mokelumne River, Clifton Court & the outer bays) other striped bass collected at each of these locations appear to be nonresident fish exploiting other areas of the estuary as well as the location of capture.

To determine if habitat use of adult female striped bass was correlated with contaminant loads found in the eggs we examined PCB & PBDE levels of 31 females collected in 1999, 2001 and 2006 and examined their habitat use (Figure 32 & 33). All female striped bass collected in this study spawning on the Sacramento River contained biologically significant levels of PBDE & PCB in their eggs (Table 1a-3a). In our last report on the 15 females collected in 1999 and 2001 a positive correlation was found for both lifetime and the last two years Delta residence time and high levels of PBDE measured in the eggs of female striped bass. No correlation was found for either lifetime or last two years Delta residence time and PCB levels in female striped bass eggs. However, with the addition of the 16 females egg samples from 2006 which included samples from the Mokelumne River and San Joaquin River findings have changed and indicate less of a correlation (no significant correlation) of PBDE and Delta residence time (Figure 34). Analysis of PBDE levels and residence time of striped bass between Carquinez Strait and the ocean also showed no correlation (Figure 35). However, when PBDE levels were examined versus fork length a positive correlation was found (Figure 36). This is a new finding that may indicate that the bioaccumulation of PBDE's and maternal transfer is different as

compared to other lipophilic contaminants such as PCBs. In the case of PCBs (and pesticides) female striped bass maternally transfer the vast majority of the contaminant load to their eggs such that they have extremely low levels of PCBs subsequent to spawning and there is no correlation between PCBs and fork length in female striped bass (Figure 36). The correlation between PBDE levels and fork length suggests that female striped bass to not maternally transfer the entire PBDE load to their eggs but retain and continue to bioaccumulate higher levels as the fish get larger/older. It may also suggest that the metabolism of PBDE in striped bass and transfer to the eggs is different in this relatively new class of compounds than in other lipophilic contaminants. These results indicate further investigation of PBDE bioaccumulation and physiology is necessary to fully understand these results. Habitat use and PCB correlation was re-analyzed with the addition of the 16 female striped bass at results from 2006. The PCB findings are consistent with our earlier reports in that there is no correlation between PCB accumulation and freshwater habitat use (Figure 37) and a slight positive correlation between PCB bioaccumulation and residence time of striped bass between Carquinez Strait and the Pacific Ocean (Figure 38). These results are consistent with sediment sampling that indicates higher PCB levels in the outer bays and higher PBDE levels in the Delta. These preliminary results linking chemical burden data to habitat use contribute to a better understanding of the role habitat residence plays in the bioaccumulation and maternal transfer of xenobiotics in San Francisco Estuary fish**.** Samples collected and analyzed thus far have provided new and important information in both of these areas. However, it is essential to increase the numbers and locations of striped bass sampled and analyzed using these methods to better understand striped habitat use in general and as it relates to the bioaccumulation of contaminants. Managers can benefit by gaining a better understanding of the individual variability in the movement patterns of the striped bass population in the San Francisco Estuary and ocean environment, and the migration patterns subjecting the fish to the greatest risk of exposure to xenobiotics.

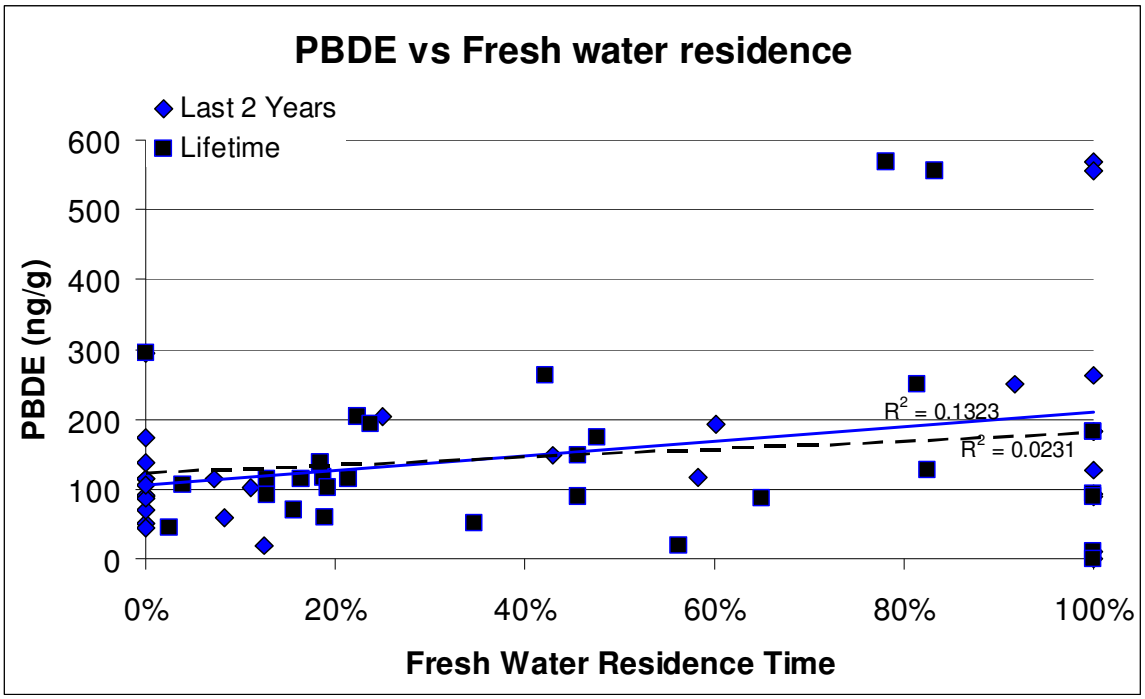


Figure 34. PBDE contamination in eggs of 31 river collected female striped bass correlated with freshwater residence time.

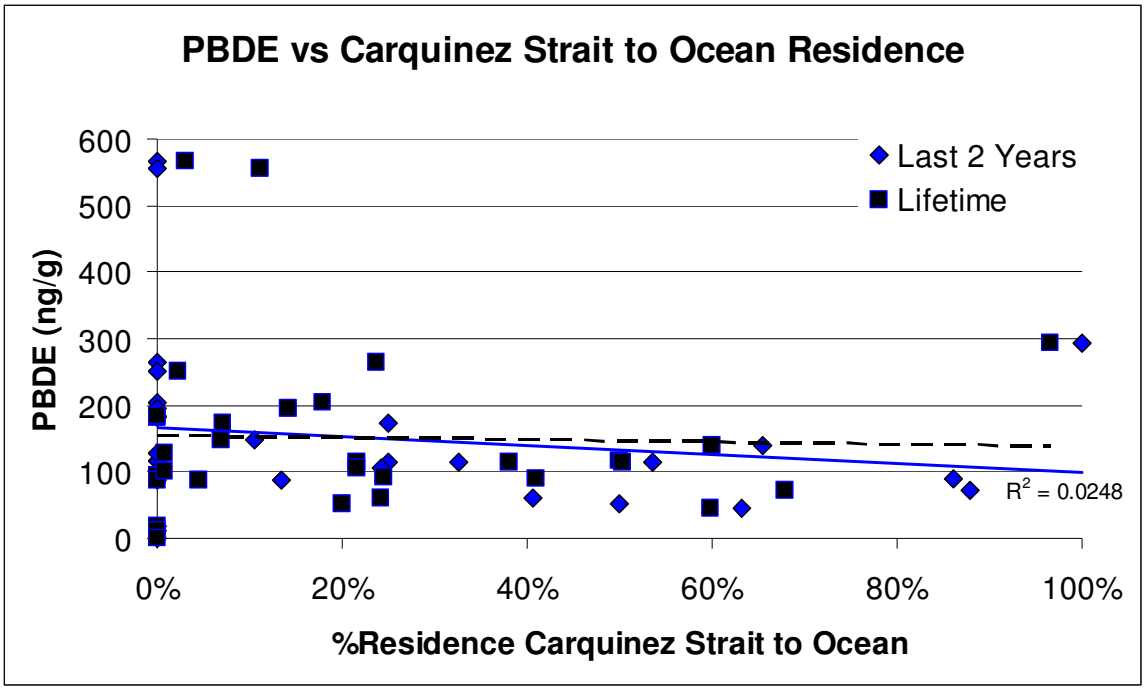


Figure 35. PBDE contamination in eggs of 31 river collected female striped bass correlated with residence time between Carquinez Strait and the Pacific Ocean.

PCB & PBDE Levels in Female Striped Bass Eggs vs. Fork Length

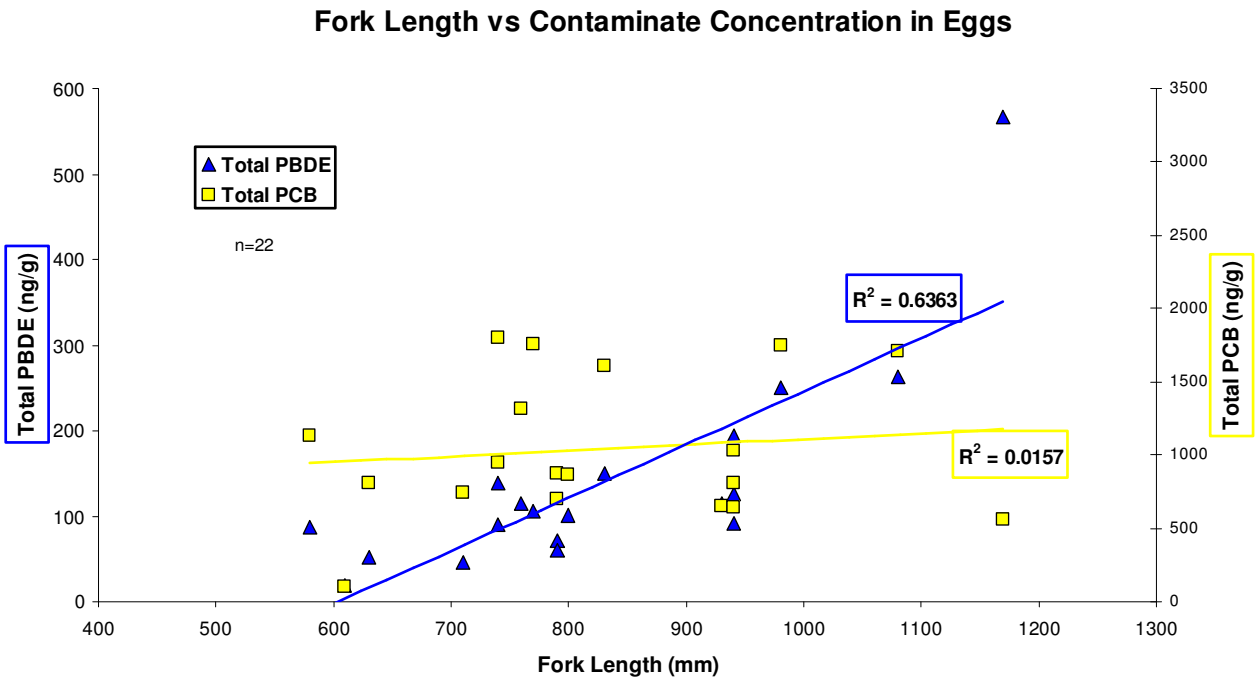


Figure 36. PCB & PBDE contamination in eggs of 31 river collected female striped bass correlated to fork length

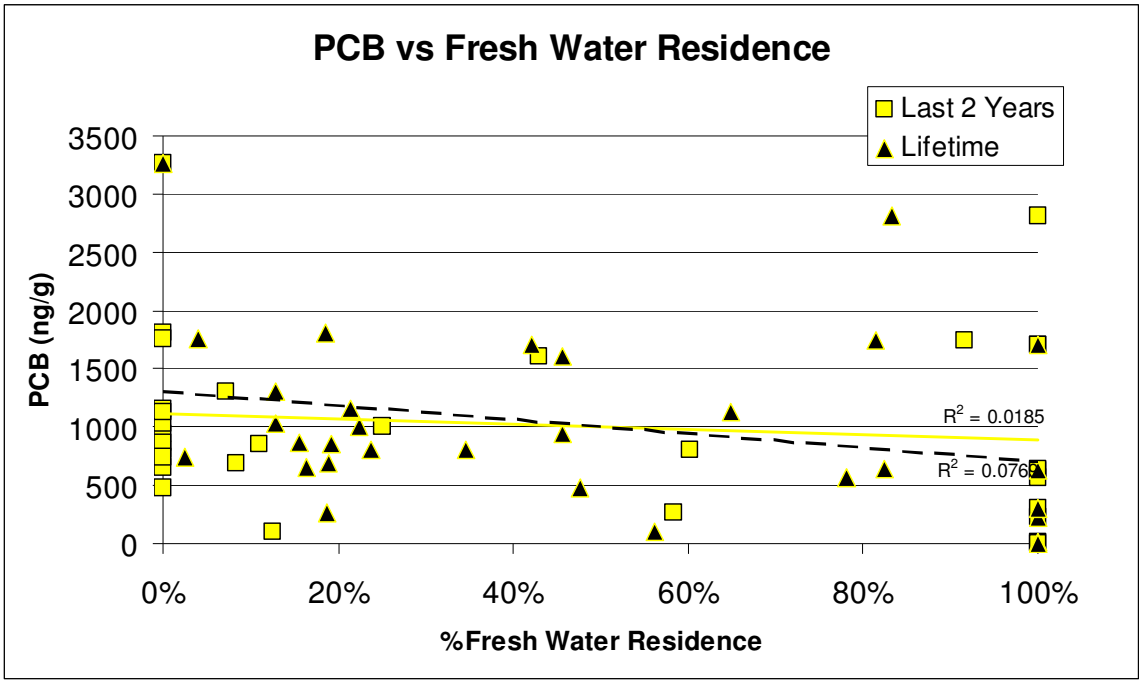


Figure 37. PCB contamination in eggs of 31 river collected female striped bass correlated with freshwater residence time.

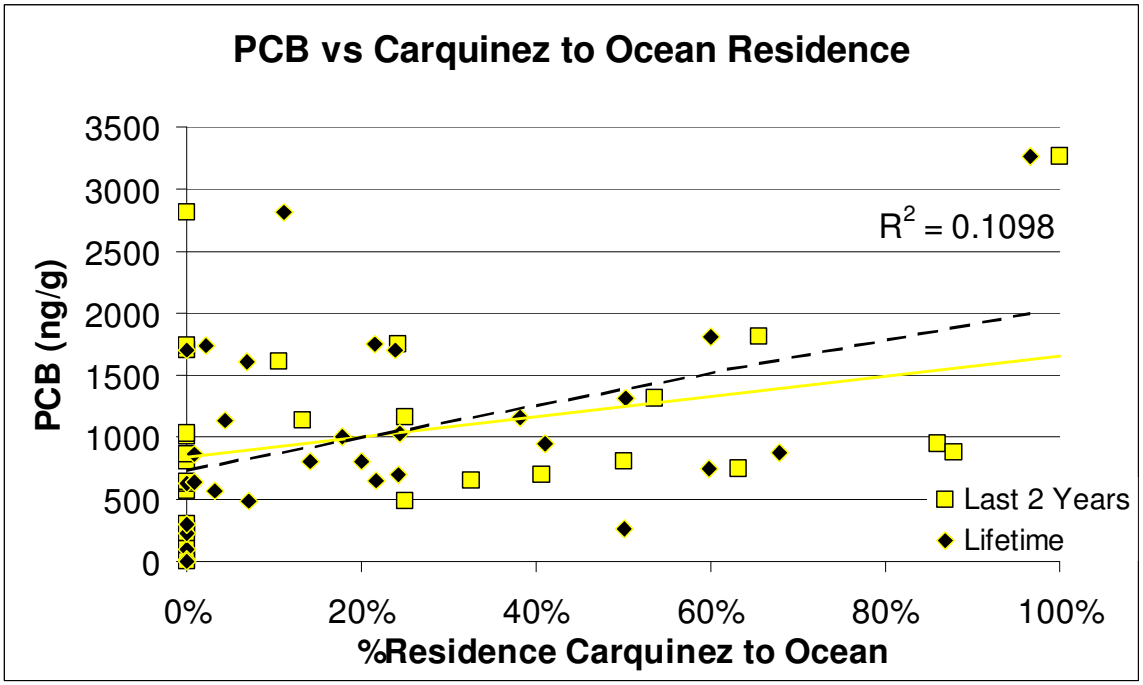


Figure 38. PCB contamination in eggs of 31 river collected female striped bass correlated with residence time between Carquinez Strait and the Pacific Ocean

Appendix A

	PBDE 017	PBDE 047	PBDE 066	PBDE 100	Total PBDE
2H06	ND	5.06 DNQ	ND	ND	**0**
4H06	ND	3.52 DNQ	ND	ND	**0**
1R06	ND	197	12.4	69.1	**278.5**
3R06	ND	94.3	ND	19.7	**114**
4R06	ND	194	8.58	46.6	**249.18**
5R06	ND	142	4.18 DNQ	31.2	**173.2**
7R06	ND	507	5.06 DNQ	60.4	**567.4**
9R06	ND	51.5	ND	ND	**51.5**
11R06	ND	117	8.69	20.7	**146.39**
12R06	ND	359	8.91	45.7	**413.61**
13R06	ND	198	6.71 DNQ	65.1	**263.1**
14R06	ND	18.9	3.3 DNQ	ND	**18.9**
060516-1SJ	2.42 DNQ	455	2.75 DNQ	101	**556**
060516-2 Moko	0	162	8.14 DNQ	41.3	**203.3**
060516-3 Moko	ND	11.6	ND	2.2 DNQ	**11.6**
060523-4 Moko	ND	104	ND	11.9	**115.9**
060523-8 Moko	ND	2.31 DNQ	ND	ND	**0**
060523-9 Moko	ND	77.6	ND	10.5	**88.1**
060523-5 Moko	ND	159	ND	23.2	**182.2**
060523-6 Moko	ND	159	ND	24.3	**183.3**
060523-7 Moko	ND	81.4	ND	12.4	**93.8**

Table 1a: PBDE levels (µg/g wet weight) by fish

	2H06	4H06	1R06	3R06	4R06	5R06	7R06	9R06	11R06	12R06	13R06	14R06	060516-1SJ	060516-2Moko	060516-3Moko	060523-4Moko	060523-8Moko	060523-9Moko	060523-5Moko	060523-6Moko	060523-7Moko
18	ND	ND	ND	ND	ND	ND	ND	ND	5.45	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
28	ND	ND	4.56	1.5 DNQ	2.72 DNQ	1.41 DNQ	2.39 DNQ	ND	12.7	1.66 DNQ	3.09	ND	3.02	ND	ND	1.35 DNQ	ND	1.67 DNQ	2.16 DNQ	2.38 DNQ	ND
31	ND	ND	2.81	ND	ND	ND	1.38 DNQ	ND	9.35	ND	1.6 DNQ	ND	1.44 DNQ	ND	ND	ND	ND	ND	ND	ND	ND
33	ND	ND	ND	ND	ND	ND	ND	ND	1.68 DNQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
44	ND	ND	3.06	2.13 DNQ	5.95	ND	2.14 DNQ	ND	16.7	2.17 DNQ	4.35	ND	4.36	ND	ND	ND	ND	ND	3.92	2.7 DNQ	ND
49	ND	ND	5.51	4.10	13.2	3.03	5.01	ND	32.2	3.85	7.98	ND	9.21	2.27 DNQ	ND	2.07 DNQ	ND	2.35 DNQ	6	4.42	ND
52	2 DNQ	1.5 DNQ	7.17	6.71	14.2	4.00	6.81	ND	44.4	6.71	10.9	ND	13.3	3.82	ND	4.78	ND	4.2	8.79	7.87	2.88
56	ND	ND	ND	ND	ND	ND	ND	ND	3.54	ND	1.61 DNQ	ND	2.33 DNQ	ND	ND	ND	ND	ND	ND	ND	ND
60	ND	ND	ND	ND	ND	ND	ND	ND	4.98	ND	2.03 DNQ	ND	2.17 DNQ	ND	ND	ND	ND	ND	ND	ND	ND
66	ND	ND	2.86	3.97	10.4	2.92	4.4	ND	31	6	10.6	ND	14	2.52 DNQ	ND	3.44	ND	3.11	5.85	7.02	ND
70	ND	ND	4.26	2.99	5.96	2.25 DNQ	5.10	ND	28.4	5.71	11.4	ND	12.6	2.2 DNQ	ND	3.89	ND	3.36	4.07	6.4	1.37 DNQ
74	ND	ND	2.52 DNQ	1.75 DNQ	4.54	1.48 DNQ	2.44 DNQ	ND	19.7	3.12	8.28	ND	7.27	1.71 DNQ	ND	1.99 DNQ	ND	1.53 DNQ	2.3 DNQ	2.91	ND
87	2.06 DNQ	ND	5.02	6.17	13.7	4.06	7.01	9.5 DNQ	16.9	6.78	12.9	2.2 DNQ	13.4	6.92	1.61 DNQ	3.39	ND	2.77	7.85	4.39	2.91
95	2.51 DNQ	1.89 DNQ	8.26	8.93	32.2	6.69	6.65	7.64 DNQ	25	11.4	13.6	1.67 DNQ	25.3	5.02	ND	4.31	ND	4.12	11.6	7.43	3.05
97	ND	ND	4.24	5.52	15	2.46 DNQ	4.72	ND	13.6	5.43	12	ND	14.2	5.31	ND	1.91 DNQ	ND	2.51 DNQ	7.94	3.51	1.62 DNQ
99	3.06	2.17 DNQ	9.48	21.5	59.8	10.6	10.2	18.3	57.1	19.7	20.6	3.08	46.7	15.3	ND	5.4	ND	7.48	26.4	12	4.41
101	4.49	3.22	19.3	33.9	96.3	13.2	19.9	30.1	84.5	32.4	43.9	4.34	88.2	22	ND	8.73	ND	10.2	40.8	18.5	7.36
105	ND	ND	4.78	6.23	13.8	4.13	2.64 DNQ	ND	17.8	8.28	18.1	ND	23.1	5.66	ND	2.44 DNQ	ND	2.32 DNQ	7.22	5.06	3.56
110	3.36	2.61 DNQ	16.2	16.0	45.3	11.4	16	15	35.7	16.5	27.3	3.28	41.1	13.1	ND	6.1	ND	7.19	20.9	9.83	5.33
118	2.67 DNQ	2.04 DNQ	13.3	20.5	56.1	11.6	13.8	14.3	50.2	23.1	50.8	3.77	58	19.1	ND	5.39	ND	5.59	25.3	12.4	8.4
128	ND	ND	5.26	7.76	21.8	3.68	5.93	8.15 DNQ	19.6	7.66	13.1	ND	21.7	8.79	ND	2.36 DNQ	ND	3.13	10.9	4.36	2.1 DNQ
137	ND	ND	ND	1.47 DNQ	5.49	ND	ND	ND	5.02	1.99 DNQ	3.82	ND	4.53	2 DNQ	ND	ND	ND	ND	ND	ND	ND
138	4.5	3.92	27.4	44.8	127	24.7	29.3	58.5	122	50.6	83.1	5.23	121	51.6	2.4 DNQ	13.6	ND	17.3	62.4	29.3	10.4
141	ND	ND	3.04	7.38	19.8	2.49 DNQ	2.69 DNQ	ND	13.8	6.24	9.07	ND	16.9	5.84	ND	1.45 DNQ	ND	ND	10.3	3.15	ND
149	3.84	3.24	22.3	34.4	134	15.2	23.6	48.9	59.6	23.5	36.1	2.86	74.8	25.3	ND	5.17	ND	8.21	53.5	11.8	4.04
151	ND	ND	5.83	15.4	50.8	6.24	7.36	19.8	28.4	8.71	11.2	ND	26.6	11.1	ND	1.97 DNQ	ND	3.08	21	3.8	ND
153	6.35	6.00	48.7	95.8	297	37.9	34.7	64.5	182	72	128	7.8	241	84.1	3.20	15.5	ND	17.1	142	30.2	15.7
156	ND	ND	2.03 DNQ	3.62	8.65	2.22 DNQ	2.11 DNQ	ND	7.64	3.5	7.56	ND	9.47	4.4	ND	ND	ND	ND	4.94	1.91 DNQ	ND
157	ND	ND	ND	ND	2.16 DNQ	ND	ND	ND	1.63 DNQ	ND	ND	ND	2.09 DNQ	ND	ND	ND	ND	ND	ND	ND	ND
158	ND	ND	2.59 DNQ	4.54	15.1	2.96	4.84	ND	10.9	4.41	5.39	ND	14.3	6.1	ND	1.29 DNQ	ND	ND	7.39	2.57 DNQ	ND
170	ND	ND	3.06	9.73	19.4	2.09 DNQ	2.07 DNQ	ND	8.66	8.07	9.85	ND	11.8	7.35	ND	ND	ND	ND	20.2	4.54	ND
174	ND	ND	3.43	7.76	22.4	ND	ND	ND	8.17	4.21	6.36	ND	14.8	1.81 DNQ	ND	ND	ND	ND	13.2	2.35 DNQ	ND
177	ND	ND	6.54	11.6	35.9	3.9	2.9	8.68 DNQ	19.4	6.17	10.2	ND	23.9	7.51	ND	ND	ND	ND	20.2	2.53 DNQ	ND
180	1.92 DNQ	ND	16.8	38.8	98.1	13.6	17.9	28.1	49.5	26.4	36.8	ND	66.8	30.8	2.34 DNQ	5.39	ND	4.37	58.8	13.5	4.74
183	ND	ND	6.52	13.1	36.2	4.43	7.74	14.1	20.1	9.1	10.6	ND	23.3	11.3	ND	1.69 DNQ	ND	2.16 DNQ	20.4	3.45	ND
187	ND	ND	18.7	36.7	117	12.1	21	32.5	64.1	25.8	34.8	ND	85.9	29.4	ND	4.96	ND	6.2	63.5	14.3	3.18
194	ND	ND	2.73 DNQ	3.91	12.3	ND	ND	ND	4.84	3.28	3.74	ND	6.94	2.96	ND	ND	ND	ND	8.03	ND	ND
195	ND	ND	ND	ND	3.54	ND	ND	ND	2.01 DNQ	1.41 DNQ	2.05 DNQ	ND	2.46 DNQ	ND	ND	ND	ND	ND	2.03 DNQ	ND	ND
200	ND	ND	ND	ND	5	ND	ND	ND	1.68 DNQ	ND	ND	ND	3.15	ND	ND	ND	ND	ND	1.85 DNQ	ND	ND
201	ND	ND	2.78	5.12	11.3	ND	ND	ND	3.22	5.89	5.7	ND	6.94	3.55	ND	ND	ND	ND	13.5	3.62	ND
203	ND	ND	ND	4.5	10.7	ND	ND	ND	3.38	5.27	7.12	ND	5.54	2.77	ND	ND	ND	ND	11.8	3.74	ND
206	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.45 DNQ	ND	ND
PCB 1248	ND	ND	ND	ND	ND	ND	ND	ND	280 DNQ	ND	ND	ND	ND	510	ND	ND	ND	ND	ND	ND	ND
PCB 1254	ND	ND	260	520	1400	270	290	430 DNQ	1400	520	810	65 DNQ	1400	98 DNQ	ND	150	ND	180	610	300	140
PCB 1260	ND	ND	ND	150	420	ND	ND	ND	160	170	210	ND	250	ND	ND	ND	ND	ND	370	90 DNQ	ND
Total % Lipid	21.4	21.9	27.9	24.3	22.8	25.1	26.6	27.89	20.1	19.6	24.5	22.9	19.6	24.8	20.8	30.2	21.36	24.2	25.3	21.3	29.1

Table 2a: PCB levels (µg/g wet weight) by fish

	chlordane, cis	chlordane, trans	chlorpyrifos	dacthal	DDD, o,p'	DDD, p,p'	DDE, o,p'	DDE, p,p'	DDMU, p,p'	DDT, o,p'	DDT, p,p'	dieldrin	hexachlorobenzene	nonachlor, cis	nonachlor, trans	oxadiazon	oxychlordane	Total % Lipid
2H06	ND	ND	ND	0.815 DNQ	ND	ND	ND	24.6 DNQ	ND	ND	ND	4.08	ND	ND	ND	ND	ND	21.4
4H06	ND	ND	ND	ND	ND	ND	ND	24.2 DNQ	ND	ND	ND	2.97	ND	ND	ND	ND	ND	21.9
1R06	11 DNQ	ND	47.8	1.05 DNQ	12.3 DNQ	61.9	ND	469	24.4 DNQ	ND	ND	7.89	ND	ND	18.6	4.43	ND	27.9
3R06	12.3 DNQ	ND	ND	0.926 DNQ	13.3 DNQ	92.2	ND	2340	49.7	ND	42.4 DNQ	12.1	1.56 DNQ	ND	21.6	ND	ND	24.3
4R06	16.6	ND	ND	ND	ND	45.4	ND	279	22.4 DNQ	ND	ND	8.87	ND	13.8	29.0	ND	ND	22.8
5R06	14.5	ND	ND	ND	ND	60.2	ND	721	19.8 DNQ	ND	ND	10.9	1.52 DNQ	ND	30.8	ND	ND	25.1
7R06	23.9	6.99 DNQ	ND	4.79	18.0	118	21.1 DNQ	2460	49.9	15 DNQ	121	12.3	1.92 DNQ	17.1	46.0	ND	ND	26.6
9R06	ND	ND	ND	ND	ND	ND	ND	120 DNQ	ND	ND	ND	3.90	ND	ND	ND	ND	ND	27.89
11R06	15.5	ND	ND	ND	20.1	140	ND	286	42.4	17.1 DNQ	69.8	18.3	ND	ND	21.3	ND	ND	20.1
12R06	18.4	ND	ND	0.943 DNQ	ND	79.8	ND	1190	38.9 DNQ	ND	44.9 DNQ	8.53	1.97 DNQ	17.1	43.3	ND	ND	19.6
13R06	ND	ND	ND	ND	ND	44.7	ND	570	21.1 DNQ	ND	ND	4.58	ND	ND	27.1	ND	ND	24.5
14R06	ND	ND	ND	ND	ND	21.2	ND	127	ND	ND	ND	6.42	ND	ND	5.69 DNQ	ND	ND	22.9
060516-1SJ	36.4	ND	ND	4.08	17.3	130	ND	2550	61.8	ND	57.4 DNQ	23.0	3.24 DNQ	46.6	85.0	ND	7.72 DNQ	19.6
060516-2Moko	ND	ND	ND	2.22	ND	17.4	ND	312	ND	ND	ND	16.1	2 DNQ	ND	17.9	ND	ND	24.8
060516-3Moko	ND	ND	ND	ND	ND	ND	ND	42.8	ND	ND	ND	1.96	ND	ND	ND	ND	ND	20.80
060523-4Moko	15.2	ND	ND	2.31	ND	37.4	ND	303	16.1 DNQ	ND	ND	10.4	2.05 DNQ	13.8	27.9	ND	ND	30.2
060523-8Moko	ND	ND	ND	ND	ND	ND	ND	15.7 DNQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	21.36
060523-9Moko	22.9	ND	ND	3.61	33.5	252	26.3 DNQ	8290	119	35.1 DNQ	289	33.3	2.99 DNQ	26.0	36.0	2.27	ND	24.2
060523-5Moko	17.0	ND	ND	3.46	20.0	117	ND	2760	59.2	ND	55.9 DNQ	28.3	4.13	14.5	24.5	3.59	ND	25.3
060523-6Moko	15.3	ND	ND	2.03	ND	59.6	ND	821	21.7 DNQ	ND	35.8 DNQ	12.6	1.88 DNQ	ND	28.1	ND	ND	21.3
060523-7Moko	ND	ND	ND	2.06	ND	24.1	ND	353	ND	ND	ND	17.9	ND	ND	13.3 DNQ	ND	ND	29.1

Table 3a: Pesticides (µg/g wet weight) by fish

2007 SKT Survey									
Sample Date	Station Code	Fork Length	Serial Year	Serial Number	IHC	Otolith	Viral	Biochem	Area
1/9/2007	706	80	2007	0028	*				SR
2/8/2007	508	63	2007	0231					SB
3/9/2007	602	109	2007	0320	*				GB
3/9/2007	602	85	2007	0321	*				GB
3/9/2007	602	88	2007	0322	*				GB
3/9/2007	602	127	2007	0323	*				GB
3/9/2007	602	101	2007	0324	*				GB
3/9/2007	602	67	2007	0325	*				GB
3/9/2007	602	87	2007	0326	*				GB
3/9/2007	602	77	2007	0327	*				GB

Table 4a. Juvenile striped bass collected in the Summer Kodiak Trawl survey.

Abbreviation	Area
SM	Suisun Marsh
SB	Suisun Bay
GB	Grizzly Bay
SR	Sacramento River
D	Delta
M	Montezuma

Table 5a: Legend for location abbreviations used for the special samples surveys

Table 6a - 2007 TNS Survey

Sample Date	Station Code	Fork Length	Serial Year	Serial Number	IHC	Otolith	Viral	Biochem	Area
6/11/2007	902	16	2007	0001					D
6/11/2007	902	14	2007	0002					D
6/11/2007	915	17	2007	0003					D
6/11/2007	912	33	2007	004					D
6/11/2007	912	27	2007	005					D
6/11/2007	912	21	2007	006					D
6/11/2007	912	27	2007	0007					D
6/11/2007	912	17	2007	0008					D
6/11/2007	912	18	2007	0009					D
6/11/2007	912	19	2007	0010					D
6/11/2007	912	19	2007	0011					D
6/12/2007	812	14	2007	0012					D
6/12/2007	812	26	2007	0013					D
6/12/2007	707	11	2007	0014					SR
6/12/2007	704	17	2007	0015					SR
6/12/2007	704	17	2007	0016					SR
6/12/2007	704	18	2007	0018					SR
6/13/2007	801	29	2007	0019					SB
6/13/2007	801	21	2007	0020					SB
6/13/2007	504	22	2007	0024					SB
6/14/2007	610	28	2007	0025					SM
6/14/2007	610	19	2007	0026					SM
6/14/2007	610	26	2007	0027					SM
6/14/2007	610	18	2007	0028					SM
6/14/2007	610	17	2007	0029					SM
6/14/2007	610	16	2007	0030					SM
6/14/2007	610	18	2007	0031					SM
6/14/2007	610	34	2007	0032					SM
6/14/2007	610	22	2007	0033	*				SM
6/14/2007	610	25	2007	0034					SM
6/14/2007	609	21	2007	0035					M
6/14/2007	609	18	2007	0036	*				M
6/14/2007	609	18	2007	0038					M
6/14/2007	606	20	2007	0039					M
6/15/2007	520	18	2007	0043					SB
6/15/2007	520	17	2007	0044					SB
6/15/2007	520	21	2007	0045					SB
6/15/2007	520	16	2007	0046					SB
6/25/2007	809	17	2007	0049					D
6/25/2007	912	41	2007	0050					D
6/25/2007	912	30	2007	0051	*				D

Table 6a - 2007 TNS Survey

Sample Date	Station Code	Fork Length	Serial Year	Serial Number	IHC	Otolith	Viral	Biochem	Area
6/25/2007	912	21	2007	0052	*				D
6/25/2007	912	30	2007	0053	*				D
6/25/2007	912	21	2007	0054	*				D
6/25/2007	912	32	2007	0055	*				D
6/25/2007	912	22	2007	0056	*				D
6/25/2007	912	21	2007	0057	*				D
6/25/2007	912	21	2007	0058	*				D
6/25/2007	912	26	2007	0059	*				D
6/25/2007	912	31	2007	0060	*				D
6/25/2007	912	23	2007	0061	*				D
6/25/2007	912	26	2007	0062	*				D
6/25/2007	912	19	2007	0063	*				D
6/25/2007	912	0	2007	0064	*				D
6/26/2007	707	17	2007	0065					SR
6/26/2007	707	18	2007	0066	*				SR
6/26/2007	707	11	2007	0067					SR
6/26/2007	706	20	2007	0068					SR
6/26/2007	707	10	2007	0069					SR
6/26/2007	706	23	2007	0070					SR
6/26/2007	706	17	2007	0071					SR
6/26/2007	706	18	2007	0072					SR
6/26/2007	706	13	2007	0073					SR
6/26/2007	704	18	2007	0074					SR
6/26/2007	704	19	2007	0075					SR
6/26/2007	704	19	2007	0079					SR
6/26/2007	704	21	2007	0083					SR
6/26/2007	704	20	2007	0084					SR
6/27/2007	804	18	2007	0088					D
6/27/2007	804	17	2007	0089					D
6/27/2007	804	19	2007	0090					D
6/27/2007	801	16	2007	0091					SB
6/27/2007	801	15	2007	0092					SB
6/27/2007	801	19	2007	0093					SB
6/27/2007	513	16	2007	0094					SB
6/27/2007	513	15	2007	0095					SB
6/28/2007	610	28	2007	0096					SM
6/28/2007	610	33	2007	0097					SM
6/28/2007	610	22	2007	0098					SM
6/28/2007	610	24	2007	0099					SM
6/28/2007	610	19	2007	0100					SM
6/28/2007	610	19	2007	0101					SM
6/28/2007	610	22	2007	0102					SM

Table 6a -2007 TNS Survey									
Sample Date	**Station Code**	**Fork Length**	**Serial Year**	**Serial Number**	**IHC**	**Otolith**	**Viral**	**Biochem**	**Area**
6/28/2007	609	36	2007	0103					M
6/28/2007	609	26	2007	0104	*				M
6/28/2007	609	31	2007	0105					M
6/28/2007	609	19	2007	0106	*				M
6/28/2007	609	27	2007	0107	*				M
6/28/2007	609	24	2007	0108	*				M
6/28/2007	609	26	2007	0109	*				M
6/28/2007	609	33	2007	0110	*				M
6/28/2007	606	36	2007	0111	*				M
6/28/2007	602	27	2007	0112	*				GB
6/28/2007	602	33	2007	0113	*				GB
6/28/2007	602	33	2007	0114	*				GB
7/9/2007	914	16	2007	0116	*				D
7/10/2007	912	23	2007	0117	*				D
7/11/2007	704	32	2007	0118	*				SR
7/11/2007	704	22	2007	0119	*				SR
7/11/2007	704	20	2007	0120	*				SR
7/11/2007	704	21	2007	0121	*				SR
7/11/2007	704	36	2007	0122	*				SR
7/11/2007	704	16	2007	0123	*				SR
7/11/2007	704	24	2007	0124	*				SR
7/11/2007	704	16	2007	0125	*				SR
7/11/2007	513	17	2007	0126	*				SB
7/11/2007	513	26	2007	0137					SB
7/11/2007	513	20	2007	0140	*				SB
7/11/2007	513	55	2007	0141	*				SB
7/13/2007	610	24	2007	0147	*				SM
7/14/2007	411	56	2007	0149	*				SB
7/25/2007	804	45	2007	0150					D
7/26/2007	609	59	2007	0152					M
7/13/2007	609	47	2007	0181					M
7/13/2007	610	52	2007	0182	*				SB
7/13/2007	610	49	2007	0183	*				SB
8/7/2007	704	17	2007	0159	*				SR
8/8/2007	504	76	2007	0160	*				SB
8/8/2007	504	57	2007	0161	*				SB
8/8/2007	504	63	2007	0162	*				SB
8/9/2007	609	67	2007	0163	*				M

Table 6a: Juvenile striped bass collected in the Townet Survey.

Table 7a. - 2007 FMWT Survey

Sample Date	Station Code	Fork Length	Serial Year	Serial Number	IHC	Otolith	Viral	Biochem	Area
09/10/2007	418	92	2007	0005	*				SM
09/10/2007	418	82	2007	0006	*				SM
09/10/2007	601	82	2007	0007	*				SM
09/10/2007	601	92	2007	0008	*				SM
09/10/2007	601	95	2007	0009	*				SM
09/10/2007	601	107	2007	0010	*				SM
09/10/2007	601	95	2007	0011	*				SM
09/10/2007	601	88	2007	0012	*				SM
09/10/2007	601	118	2007	0013	*				SM
09/10/2007	601	112	2007	0014	*				SM
09/10/2007	601	95	2007	0015					SM
09/10/2007	601	91	2007	0016					SM
09/10/2007	602	83	2007	0017	*				GB
09/11/2007	605	88	2007	0022	*				SM
09/11/2007	605	103	2007	0023	*				SM
09/11/2007	516	72	2007	0024	*				SB
09/11/2007	516	73	2007	0025	*				SB
09/11/2007	517	99	2007	0026	*				SB
09/12/2007	507	91	2007	0027	*				SB
09/12/2007	507	98	2007	0028	*				SB
09/12/2007	509	108	2007	0029	*				SB
09/12/2007	509	95	2007	0030	*				SB
09/12/2007	509	82	2007	0031	*				SB
09/12/2007	509	87	2007	0032	*				SB
09/12/2007	509	103	2007	0033					SB
09/12/2007	510	103	2007	0034	*				SB
09/12/2007	510	89	2007	0035	*				SB
09/12/2007	510	92	2007	0036					SB
09/12/2007	511	82	2007	0037	*				SB
09/12/2007	608	68	2007	0038	*				SM
09/12/2007	608	72	2007	0039	*				SM
09/12/2007	608	86	2007	0040	*				SM
10/03/2007	414	123	2007	0041	*				SB
10/03/2007	416	127	2007	0042	*				SB
10/03/2007	416	109	2007	0043	*				SB
10/03/2007	602	80	2007	0044					GB
10/03/2007	602	103	2007	0045	*				GB
10/03/2007	602	101	2007	0046	*				GB
10/03/2007	601	102	2007	0048	*				SM
10/03/2007	601	104	2007	0049	*				SM
10/03/2007	603	96	2007	0050	*				SM

Table 7a. - 2007 FMWT Survey									
Sample Date	**Station Code**	**Fork Length**	**Serial Year**	**Serial Number**	**IHC**	**Otolith**	**Viral**	**Biochem**	**Area**
10/04/2007	410	94	2007	0051					SB
10/04/2007	410	104	2007	0052	*				SB
10/04/2007	410	107	2007	0053					SB
10/04/2007	410	93	2007	0054					SB
10/04/2007	515	88	2007	0055	*				SB
10/04/2007	515	104	2007	0056	*				SB
10/04/2007	517	105	2007	0057	*				SB
10/04/2007	517	86	2007	0058	*				SB
10/09/2007	507	101	2007	0059	*				SB
10/09/2007	507	98	2007	0060	*				SB
10/10/2007	705	95	2007	0063	*				SR
10/10/2007	705	92	2007	0064	*				SR
10/10/2007	705	111	2007	0065	*				SR
10/10/2007	705	98	2007	0066	*				SR
10/10/2007	705	82	2007	0067					SR
10/10/2007	705	81	2007	0068					SR
10/10/2007	705	78	2007	0069					SR
10/10/2007	705	90	2007	0070					SR
10/10/2007	705	103	2007	0071					SR
10/10/2007	703	108	2007	0073					SR
10/10/2007	703	104	2007	0074					SR

Table 7a. Juvenile striped bass collected in the Mid-Water Trawl Survey.

Table 8a: 2007 POD Samples									
Sample ID	**Fork Length (mm)**	**Station**	**Date**	**Time**	**Histology**	**Otolith**	**Viral**	**Biochem**	**Comments**
1		801	070508	12:30				*	Mangled got caught for too long due to snag
2		801	070508	12:45			*		
3		801	070508	12:45			*		
4		801	070508	12:45			*		
5	10	606	070523	10:10					1 fish
6	10	520	070523	12:10					2 fish
7	10	520	070523	12:25					4 fish
8	10	520	070523	12:40					1 fish
9	10	804	070523	13:10					3 fish
10	10	804	070523	13:25					3 fish
11	10	804	070523	13:40					5 fish
12	24	519	070702	10:31	*				1 fish
13	28	519	070702	10:47	*				1 fish
14	16	910	070717	13:00				*	1 fish
15	180	815	070816	10:45				*	EtOH bottle ruined
16	168	706	070817	14:17				*	1 year old
17	98	706	070817	14:44	*				1 fish
18	200	BS863	070828	8:08	*	*		*	1 year old
19	168	BS863	070828	8:08	*	1		*	1 year old
20	170	BS863	070828	8:08	*	1		*	1 year old Chipped
21	175	BS863	070828	8:08	*	2		*	1 year old
22	155	BS863	070828	8:08	*	2		*	1 year old
23	80	BS863	070828	8:08	*	Missing			1 fish
24	110	BS863	070828	8:08	*	2			1 fish
25	120	BS863	070828	8:08	*	1			1 fish
26	105	BS863	070828	8:08	*	1			1 fish
27	115	BS863	070828	8:08			*		1 fish
28	125	BS863	070828	8:08			*		1 fish
29	130	BS863	070828	8:08			*		1 fish
30	115	BS863	070828	8:08			*		1 fish
31	125	BS863	070828	8:08				*	1 fish
32	115	BS863	070828	8:08				*	1 fish
33	110	BS863	070828	8:08				*	1 fish
34	100	BS863	070828	8:08				*	1 fish
35	110	BS864	070828	11:30	*	1			1 fish
36	90	BS864	070828	11:30	*	1			1 fish
37	100	BS864	070828	11:30	*	1			1 fish
38	105	BS864	070828	11:30	*	1			1 fish
39	95	BS864	070828	11:30			*		1 fish
40	110	BS864	070828	11:30			*		1 fish
41	90	BS864	070828	11:30				*	1 fish
42	110	BS864	070828	11:30				*	1 fish

Table 8a: 2007 POD Samples									
Sample ID	**Fork Length (mm)**	**Station**	**Date**	**Time**	**Histology**	**Otolith**	**Viral**	**Biochem**	**Comments**
43	90	BS864	070828	11:30				*	1 fish
44	90	BS864	070828	11:30				*	1 fish
45	100	BS864	070828	11:30				*	1 fish
46	182	BS752	070829	7:41	*	1		*	1 year old
47	73	BS752	070829	8:16				*	1 fish
48	57	BS752	070829	8:16	*	1			1 fish
49	54	BS752	070829	8:16	*	Missing			1 fish
50	70	BS752	070829	8:16		*	*		1 fish
51	64	BS752	070829	8:16				*	1 fish
52	85	BS752	070829	8:16				*	1 fish
53	86	BS752	070829	8:16				*	1 fish
54	72	BS752	070829	8:16				*	1 fish
55	63	BS760	070829	9:21	*	Missing			1 fish
56	77	BS760	070829	9:21				*	1 fish
57	68	BS762	070829	11:06				*	1 fish
58	65	BS762	070829	11:37	*	1			1 fish
59	68	BS762	070829	11:37				*	1 fish
60	222	807	070921	8:25	*			*	1 year old
61	119	812	070921	9:35	*	2			1 fish
62	143	812	070921	9:35	*	1			1 fish
63	100	812	070921	9:35	*	Missing			1 fish
64	100	812	070921	9:35	*	Missing			1 fish
65	115	812	070921	9:35	*	Missing			1 fish
66	80	812	070921	9:35	*	2			1 fish
67	133	812	070921	9:35				*	1 fish
68	122	812	070921	9:35				*	1 fish
69	145	812	070921	9:35				*	1 fish
70	136	812	070921	9:35				*	1 fish, no muscle
71	141	812	070921	9:35				*	1 fish
72	126	812	070921	9:35				*	1 fish
73	115	812	070921	9:35				*	1 fish
74	125	812	070921	9:35				*	1 fish
75	120	812	070921	9:35			*		1 fish
76	108	812	070921	9:35			*		1 fish
77	115	812	070921	9:35			*		1 fish
78	110	812	070921	9:35			*		1 fish
79	122	812	070921	9:35			*		1 fish
80	111	812	070921	9:35			*		1 fish
81	123	812	070921	9:35			*		1 fish
82	117	812	070921	9:35			*		1 fish
83	90	812	070921	9:35	*	Missing			1 fish
84	100	812	070921	9:35	*	Missing			1 fish
85	128	812	070921	9:35	*	Missing			1 fish
86	125	912	070921	11:36				*	
87	94	703	070924	9:45	*	1		*	1 year old
88	168	903	070924	9:45	*	1		*	1 year old Chipped
89	78	707	070924	9:45				*	

Table 8a: 2007 POD Samples									
Sample ID	Fork Length (mm)	Station	Date	Time	Histology	Otolith	Viral	Biochem	Comments
90	80	707	070924	9:45				*	
91	53	707	070924	9:45	*	1			
92	90	707	070924	9:45	*	2			
93	84	707	070924	9:45	*	1			
94	108	707	070924	9:45				*	
95	73	707	070924	9:45				*	
96	94	707	070924	9:45				*	
97	93	707	070924	9:45				*	
98	93	707	070924	9:45				*	
99	70	707	070924	9:45	*	1			
100	77	707	070924	9:45			*		
101	78	707	070924	9:45			*		
102	69	710	070924	11:08	*	1			
103	87	710	070924	11:08	*	1			
104	213	601	070925	9:04	*	1		*	1 year old
105	112	601	070925	9:04	*	1		*	
106	114	601	070925	9:04	*	1		*	
107	103	601	070925	9:04	*	1		*	
108	111	601	070925	10:01	*	1			
109	109	601	070925	10:01	*	Missing			
110	135	601	070925	10:01	*	1			
111	105	601	070925	10:01	*	1			
112	103	601	070925	10:01	*	1			
113	122	601	070925	10:01			*		
114	123	601	070925	10:01			*		
115	115	601	070925	10:01			*		
116	122	601	070925	10:01			*		
117	125	601	070925	10:01				*	
118	107	601	070925	10:01				*	
119	103	601	070925	10:01				*	
120	112	601	070925	10:01				*	
121	98	601	070925	10:01				*	
122	118	601	070925	10:01				*	
123	115	601	070925	10:01				*	
124	105	602	070925	11:55	*	1		*	
125	127	602	070925	11:55	*	1		*	
126	108	602	070925	11:55	*	Missing		*	
127	118	602	070925	11:55	*	Missing		*	
128	102	602	070925	11:55	*	1		*	
129	82	BS863	071030	8:07	*	1			
130	108	BS863	071030	8:07	*	1			
131	118	BS863	071030	8:07	*	1			
132	107	BS863	071030	8:07	*	1			
133	131	BS863	071030	8:07	*	1			
134	126	BS863	071030	8:07				*	
135	120	BS863	071030	8:07				*	
136	115	BS863	071030	8:07				*	
137	114	BS863	071030	8:07				*	
138	110	BS863	071030	8:07				*	

Table 8a: 2007 POD Samples									
Sample ID	Fork Length (mm)	Station	Date	Time	Histology	Otolith	Viral	Biochem	Comments
139	107	BS863	071030	8:07				*	
140	107	BS863	071030	8:07			*		
141	93	BS863	071030	8:07			*		
142	109	BS863	071030	8:07			*		
143	106	BS865	071030	8:52	*	1			
144	130	BS865	071030	8:52	*	1			
145	103	BS865	071030	8:52	*	2			
146	104	BS865	071030	8:52	*	1			
147	117	BS865	071030	8:52				*	
148	123	BS865	071030	8:52				*	
149	125	BS865	071030	8:52				*	
150	103	BS865	071030	8:52				*	
151	105	BS865	071030	8:52				*	
152	124	BS865	071030	8:52			*		
153	124	BS865	071030	8:52			*		
154	94	BS865	071030	8:52			*		
155	89	BS752	071030	10:20	*	1			Chipped
156	83	BS752	071030	10:20	*	1			
157	106	BS752	071030	10:20	*	2			
158	93	BS752	071030	10:20				*	
159	98	BS752	071030	10:20				*	
160	102	BS752	071030	10:20				*	
161	88	BS752	071030	10:20				*	
162	76	BS752	071030	10:20				*	
163	74	BS752	071030	10:20				*	
164	89	BS752	071030	10:20			*		
165	68	BS752	071030	10:20			*		
166	72	BS750	071030	11:43	*	1		*	
167	87	BS750	071030	11:43	*	1		*	
168	92	BS750	071030	11:43	*	1		*	
200	103	507	071030	8:12	*	1		*	
201	93	508	071030	8:12	*	1		*	
202	99	508	071030	8:12	*	1		*	
203	111	508	071030	8:12	*	1		*	
204	131	508	071030	8:12	*	1		*	
205	109	508	071030	8:12	*	1		*	
206	108	508	071030	8:12	*	1		*	
207	114	508	071030	8:12		1		*	
208	115	508	071030	8:12		1		*	
209	104	508	071030	8:12		*		*	
210	91	508	071030	8:12		2		*	
211	98	508	071030	8:12		2		*	
212	87	508	071030	8:12		2		*	Popped Gall Bladder
213	90	508	071030	8:12		2		*	
214	92	508	071030	8:12		2		*	
215	79	508	071030	8:12		2		*	
216	96	602	071119	8:13	*	1			

Table 8a: 2007 POD Samples									
Sample ID	**Fork Length (mm)**	**Station**	**Date**	**Time**	**Histology**	**Otolith**	**Viral**	**Biochem**	**Comments**
217	110	602	071119	8:13	*	*			
218	88	602	071119	8:13	*	1			
219	111	602	071119	8:13				*	
220	101	602	071119	8:13				*	
221	91	602	071119	8:13		*		*	Otolith
222	128	602	071119	8:13				*	
223	94	602	071119	8:13				*	
224	121	602	071119	8:13	*	*			Face Lesion
225	99	602	071119	8:13				*	
226	104	602	071119	8:13				*	
227	70	602	071119	8:13				*	No Spleen or Liver
228	85	602	071119	8:13				*	
229	91	519	071119	9:37	*	1			
230	127	519	071119	9:37	*	*			
231	93	519	071119	9:37	*	2			
232	102	519	071119	9:37	*	2			
233	114	519	071119	9:37				*	
234	87	519	071119	9:37				*	
235	120	519	071119	9:37				*	
236	100	519	071119	9:37				*	Broke Gall Bladder
237	110	519	071119	9:37				*	
238	127	519	071119	9:37				*	
239	78	802	071119	11:01	*	*			
240	114	802	071119	11:01	*	*		*	No Brain
241	132	TNS 816	071120	9:17	*	*			
242	98	TNS 816	071120	9:17	*	*			
243	107	TNS 816	071120	9:17	*	*		*	
244	141	TNS 816	071120	9:17	*	*		*	
245	123	TNS 816	071120	9:17				*	
246	93	BS751	071120	10:08	*	*		*	
247	112	BS863	071120	11:14	*	*			
248	131	BS863	071120	11:14	*	2			
249	113	BS863	071120	11:14	*	1			
250	93	BS863	071120	11:14	*	*			
251	116	BS863	071120	11:14				*	
252	138	BS863	071120	11:14				*	
253	118	BS863	071120	11:14		*		*	
254	128	BS863	071120	11:14				*	
255	110	BS863	071120	11:14				*	
256	117	BS863	071120	11:14				*	Broke Gall Bladder
257	82	FMWT602	071127	10:30	*	1			
258	89	FMWT602	071127	10:30	*	*			
259	92	FMWT602	071127	10:30		*		*	
260	78	FMWT602	071127	10:30		*		*	
261	78	FMWT602	071127	10:30		*		*	

Table 8a: 2007 POD Samples									
Sample ID	Fork Length (mm)	Station	Date	Time	Histology	Otolith	Viral	Biochem	Comments
262	93	FMWT602	071127	10:30				*	
263	99	FMWT602	071127	10:30				*	
264	93	FMWT509	071127	11:13	*	1			
265	102	FMWT509	071127	11:13	*	1			
266	104	FMWT509	071127	11:13	*	*			
267	106	FMWT509	071127	11:13	*	1			
268	121	FMWT509	071127	11:13				*	
269	118	FMWT509	071127	11:13				*	
270	92	FMWT509	071127	11:13				*	No Kidney
271	136	FMWT509	071127	11:13				*	No Liver
272	88	FMWT509	071127	11:13				*	
273	96	FMWT509	071127	11:13				*	
274	109	FMWT519	071127	11:43	*	1			
275	123	FMWT519	071127	11:43				*	
276	112	FMWT519	071127	11:43				*	
277	116	20mm705	071128	9:25	*	1			
278	117	20mm705	071128	9:25	*	2			
279	101	20mm705	071128	9:25	*	2			
280	100	20mm705	071128	9:25	*	2			
281	116	20mm705	071128	9:25		*		*	
282	124	20mm705	071128	9:25		1		*	
283	101	20mm705	071128	9:25				*	
284	125	20mm705	071128	9:25				*	
285	103	20mm705	071128	9:25				*	
286	117	20mm705	071128	9:25				*	
287	122	BS863	071128	12:32	*	1			
288	106	BS863	071128	12:32				*	
289	123	BS863	071128	12:32		1		*	
290	95	FMWT 602	071219	8:07	*	1			
291	137	FMWT 602	071219	8:07	*	1			Chipped
292	117	FMWT 602	071219	8:07	*	1			
293	102	FMWT 602	071219	8:07	*	1			
294	110	FMWT 602	071219	8:07	*	2			
295	94	FMWT 602	071219	8:07	*	1			
296	104	FMWT 602	071219	8:07	*	Missing			
297	89	FMWT 602	071219	8:07	*	1			
298	138	FMWT 602	071219	8:07				*	
299	133	FMWT 602	071219	8:07				*	
300	147	FMWT 602	071219	8:07		1		*	
301	142	FMWT 602	071219	8:07				*	
302	138	FMWT 602	071219	8:17		1		*	
303	128	FMWT 602	071219	8:17				*	
304	133	FMWT 602	071219	8:17				*	
305	124	FMWT 602	071219	8:17				*	
306	122	FMWT 602	071219	8:17				*	
307	134	FMWT 602	071219	8:17				*	
308	121	FMWT 602	071219	8:17		2		*	
309	117	FMWT 602	071219	8:17				*	
310	122	FMWT 602	071219	8:17				*	

Table 8a: 2007 POD Samples									
Sample ID	**Fork Length (mm)**	**Station**	**Date**	**Time**	**Histology**	**Otolith**	**Viral**	**Biochem**	**Comments**
311	118	FMWT 602	071219	8:17				*	
312	69	FMWT 509	071219	8:55	*	1			USFG gave these
313	113	FMWT 509	071219	8:55				*	to us
314	104	FMWT 519	071219	8:58	*	1			
315	65	FMWT 519	071219	8:58	*	1			
316	73	FMWT 519	071219	8:58	*	1			
317	101	FMWT 519	071219	8:58	*	Missing			
318	98	FMWT 519	071219	8:58	*	1			
319	95	FMWT 519	071219	8:58	*	1			
320	128	FMWT 519	071219	8:58	*	*			
321	112	FMWT 519	071219	8:58	*	1			
322	103	FMWT 519	071219	8:58				*	
323	104	FMWT 519	071219	8:58				*	
324	114	FMWT 519	071219	8:58				*	
325	128	FMWT 519	071219	8:58				*	
326	100	FMWT 519	071219	8:58				*	
327	123	FMWT 519	071219	8:58				*	
328	108	FMWT 519	071219	8:58				*	
329	100	FMWT 519	071219	8:58				*	
330	103	FMWT 519	071219	8:58				*	
331	105	FMWT 519	071219	8:58				*	
332	110	20mm705	071220	9:47	*	1			
333	116	20mm705	071220	9:47	*	1		*	
334	109	20mm705	071220	9:47	*	1		*	
335	86	BS863	071220	11:49	*	1			
336	84	BS863	071220	11:49	*	1		*	Broke gall bladder
337	117	BS863	071220	11:49	*	1		*	
338	123	602	080129	8:29				*	
339	225	602	080129	8:45	*	*			
340	80	602	080129	9:05		*		*	No kidney
341	98	602	080129	9:05	*	*			
342	124	602	080129	9:27	*	*			
343	120	602	080129	9:27	*	*			
344	168	602	080129	9:27	*	*			
345	79	602	080129	9:27	*	*			
346	108	602	080129	9:27				*	
347	108	602	080129	9:27		*		*	
348	108	602	080129	9:27	*			*	
349	100	519	080129	10:20	*				
350	103	519	080129	10:20	*				
351	129	519	080129	10:20				*	
352	104	519	080129	10:20				*	
353	110	519	080129	10:45	*				
354	121	519	080129	10:45	*				
355	127	519	080129	10:45				*	
356	131	519	080129	10:45				*	No muscle
357	124	806	080129	12:30				*	

Table 8a: 2007 POD Samples									
Sample ID	**Fork Length (mm)**	**Station**	**Date**	**Time**	**Histology**	**Otolith**	**Viral**	**Biochem**	**Comments**
358	130	806	080129	12:30					
359	181	806	080129	1:00					
360	123	20mm705	080130	10:07	*				
361	120	20mm705	080130	10:07				*	
362	73	20mm705	080130	10:07	*				
363	124	20mm705	080130	10:07	*				
364	129	20mm705	080130	10:07	*				
365	94	20mm705	080130	10:07	*				
366	99	20mm705	080130	10:07				*	
367	101	20mm705	080130	10:07				*	
368	142	20mm705	080130	10:07	*				Parasite in liver
369	95	BS863	080130	12:13	*				
370	125	BS863	080130	12:13	*				
371	126	BS863	080130	12:13	*				
372	90	BS863	080130	12:13	*				
373	112	BS863	080130	12:13	*				
374	148	BS863	080130	12:13	*				
375	89	BS863	080130	12:13	*				
376	141	BS863	080130	12:13	*				
377	163	BS863	080130	12:13				*	
378	126	BS863	080130	12:13				*	
379	139	BS863	080130	12:13				*	
380	92	BS863	080130	12:13				*	
381	131	BS863	080130	12:13				*	
382	120	BS863	080130	12:13				*	
383	104	BS863	080130	12:13				*	

Table 8a. Juvenile striped bass collected during POD special sampling events.

Table 9a. Summary of EROD activities from S9 Liver Fractions of Fish taken from the 2007 POD Special Survey			
Month	**Site**	**Number of Induced Fish**	**Total Fish From Site**
August	BS 752	5	6
	BS 762	0	1
	BS 863	4	7
	BS 864	3	5
	TNS 706	0	1
	TNS 815	1	1
September	FMWT 601	9	11
	FMWT 602	3	4
	FMWT 703	0	1
	FMWT 707	5	7
	FMWT 812	6	8
	FMWT 912	0	1
October	BS 750	0	3
	BS 752	0	6
	BS 863	4	6
	BS 865	0	5
	FMWT 507	0	1
	FMWT 508	12	15
November	20mm 705	4	6
	BS 751	1	1
	BS 863	5	6
	FMWT 509	0	6
	FMWT 519	4	7
	FMWT 602	9	13
	TNS 816	3	3
December	20mm 705	1	2
	BS 863	0	1
	FMWT 519	8	10
	FMWT 602	10	14
January	20mm 705	3	3
	BS 863	6	7
	FMWT 519	4	4
	FMWT 602	3	5
	FMWT 806	1	1

Table 9a. Summary of EROD activities from S9 Liver Fractions of Fish taken from the 2007 POD Special Survey

Table 10a.

Fish ID	Capture Date	Capture Location	Sex	Age (years)	Fork Length (mm)	Mass (Kg)
9R99	99.05.05	Knights Landing/Colusa	F	7	800	8.2
11R99	99.05.05	Knights Landing/Colusa	F	7	850	11.4
13R99	99.05.05	Knights Landing/Colusa	F	7	740	6.8
14R99	99.05.05	Knights Landing/Colusa	F	7	770	8.2
15R99	99.05.11	Knights Landing/Colusa	F	7	730	7.3
16R99	99.05.11	Knights Landing/Colusa	F	6	830	9.1
17R99	99.05.11	Knights Landing/Colusa	F	9	710	5.5
18R99	99.05.11	Knights Landing/Colusa	F	9	940	13.6
19R99	99.05.25	Knights Landing/Colusa	F	9	910	13.6
20R99	99.05.25	Knights Landing/Colusa	F	9	980	15.9
25R99	99.06.09	Knights Landing/Colusa	F	10	1010	13.6
28R99	99.06.09	Knights Landing/Colusa	F	5	600	3.2
1R01	08.05.01	Knights Landing/Colusa	F	7	740	6.8
3R01	08.05.01	Knights Landing/Colusa	F	7	790	9.1
4R01	08.05.01	Knights Landing/Colusa	F	7	760	6.8
5R01	08.05.01	Knights Landing/Colusa	F	10	1000	15.9
7R01	08.05.01	Knights Landing/Colusa	F	9	940	13.6
11R01	08.05.08	Knights Landing/Colusa	F	11	790	5.5
13R01	08.05.08	Knights Landing/Colusa	F	10	930	11.4
14R01	08.05.08	Knights Landing/Colusa	F	6	580	2.3

Table 10a.

15R01	08.05.15	Knights Landing/Colusa	F	10	940	15.9
SJ060516-1	06.05.16	San Joaquin	F	6	810	4.5
MOK060516-2	06.05.16	Mokelumne	F	7	720	3.4
MOK060516-3	06.05.16	Mokelumne	F	6	680	3.2
MOK060523-4	06.05.23	Mokelumne	F	8	725	4.1
MOK060523-5	06.05.23	Mokelumne	F	6	730	3.9
MOK060523-6	06.05.23	Mokelumne	F	5	680	2.7
MOK060523-7	06.05.23	Mokelumne	F	7	650	2.7
MOK060523-8	06.05.23	Mokelumne	F	4	590	2
MOK060523-9	06.05.23	Mokelumne	F	4	570	2
MOK650SL	06.05.23	Mokelumne	M	5		
MOK620MM	06.05.23	Mokelumne	M	7	620	
2R 06	06.05.31	Knights Landing/Colusa	F	16	1050	15.9
3R 06	06.05.31	Knights Landing/Colusa	F	7	809	4.5
4R 06	06.05.31	Knights Landing/Colusa	F	5		3.6
5R 06	06.05.31	Knights Landing/Colusa	F	7	819	4.5
6R 06	06.05.31	Knights Landing/Colusa	F	10	610	3.6
7R 06	06.06.08	Knights Landing/Colusa	F	15	1170	15.9
8R 06	06.06.08	Knights Landing/Colusa	F			
9R 06	06.06.08	Knights Landing/Colusa	F	5	630	2.9
10R 06	06.06.08	Knights Landing/Colusa	F	8	640	2.9
11R 06	06.06.08	Knights Landing/Colusa	F	5		1.8
12R 06	06.06.08	Knights Landing/Colusa	F			13.6
13R 06	06.06.08	Knights Landing/Colusa	F	16	1080	15.9

Table 10a.

14R 06	06.06.08	Knights Landing/Colusa	F	4	610	2.3
2Oc06	06.07.16	Ocean	F	6	762	4.5
3Oc06	06.08.13	Ocean	F	8	914	8.2
4Oc06	06.08.13	Ocean	F	14	1066	12.2
5Oc06	06.08.13	Ocean	F	10	889	7.3
6Oc06	06.07.23	Ocean	F	9	889	6.4
31R07	07.05.15	Knights Landing	F	10	850	9.1
32R07	07.05.15	Knights Landing	F	9	785	9.1
33R07	07.05.15	Knights Landing	F	8	800	9.1
35R07	07.05.15	Knights Landing	F	6	725	5.9
36R07	07.05.15	Knights Landing	F	9	821	4.75
37R07	07.05.30	Knights Landing	F	9	832	5
38R07	07.05.30	Knights Landing	F	10	890	5
39R07	07.05.30	Knights Landing	F	7	815	9.1
40R07	07.05.30	Knights Landing	F	6	722	3.5
Wild AZ DOA	07.05.23		M	6		
WM A2	07.06.04		M	4		
1RM07	07.06.05	Knights Landing	M	4		
2RMO7	07.06.05	Knights Landing	M	5		
3RM07	07.06.05	Knights Landing	M	5		
4RM07	07.06.05	Knights Landing	M	3		
5RM07	07.06.05	Knights Landing	M	6		
6RM07	07.06.05	Knights Landing	M	4		
RMD07	07.06.05	Knights Landing	M	5		
SB1	07.07.22	Stinson Beach		14		
SB2	07.07.22	Stinson Beach		7		

Table 10a.

SB3	07.07.22	Stinson Beach		6		
SB4	07.07.25	Stinson Beach		6		
SB5	07.07.25	Stinson Beach		7		
SB6	07.07.25	Stinson Beach		7		
SB7	07.07.25	Stinson Beach		7		
SB8	07.07.25	Stinson Beach		8		
SB9	07.07.25	Stinson Beach		6		
SB10	07.07.25	Stinson Beach		13		
SB11	07.07.25	Stinson Beach		11		
CC6176	06.12.06	Clifton Court	U	5	446	1.542
CC6177	06.12.06	Clifton Court	U	4	463	1.406
CC6178	06.12.06	Clifton Court	F	7	691	4.1
CC6179	06.12.06	Clifton Court	F	7	669	4.2
CC6180	06.12.06	Clifton Court	M	4	443	1.179
CC6181	06.12.06	Clifton Court	F	5	601	2.313
CC6182	06.12.06	Clifton Court	F	6	659	3.628
CC6183	06.12.06	Clifton Court	M	5	483	1.27
CC6184	06.12.06	Clifton Court	F	5	686	1.63
CC6185	06.12.06	Clifton Court	F	7	678	4.535
CC6226	07.03.22	Clifton Court	M	9	790	7.3
CC6227	07.03.22	Clifton Court	F	22	1080	15
CC6228	07.03.22	Clifton Court	F	4	478	1.3
CC6229	07.03.22	Clifton Court	M	5	483	1.2
CC6230	07.03.22	Clifton Court	F	5	519	1.6
CC6231	07.03.22	Clifton Court	F	6	591	2.8
CC6232	07.03.22	Clifton Court	M	5	497	1
CC6233	07.03.22	Clifton Court	M	6	570	2.3
CC6234	07.03.22	Clifton Court	F	5	503	0.9
CC6235	07.03.22	Clifton Court	M	5	485	1.3
CR4617	06.10.12	Cosumnes River	F	5	565	2.2
KL6169	06.12.05	Knights Landing	M	4	443	1
KL6170	06.12.05	Knights Landing	M	4	447	1.2
KL6171	06.12.05	Knights Landing	M	5	544	2.4
KL6172	06.12.05	Knights Landing	M	4	434	1
KL6173	06.12.05	Knights Landing	M	4	459	1.3
KL6174	06.12.05	Knights Landing	M	4	471	1.6
KL6175	06.12.05	Knights Landing	F	9	798	5.7
KL6216	07.03.21	Knights Landing	F	5	561	2.2
KL6217	07.03.21	Knights Landing	M	5	493	1.8
KL6218	07.03.21	Knights Landing	M	5	438	1.2
KL6219	07.03.21	Knights Landing	M	8	673	4.4

Table 10a.

KL6221	07.03.21	Knights Landing	M	4	494	1.4
KL6222	07.03.21	Knights Landing	M	7	622	2.9
KL6223	07.03.21	Knights Landing	M	6	600	2.7
KL6224	07.03.21	Knights Landing	F	4	470	1.4
KL6225	07.03.21	Knights Landing	M	4	435	1.1
LI6151	06.11.08	Liberty Island	M	5	515	1.7
LI6152	06.11.08	Liberty Island	F	5	510	1.6
LI6153	06.11.08	Liberty Island	F	4	443	1.1
LI6154	06.11.08	Liberty Island	M	4	456	1.3
LI6160	06.11.09	Liberty Island	F	4	495	1.5
LI6161	06.11.09	Liberty Island	F	5	540	1.8
LI6162	06.11.09	Liberty Island	F	4	450	1.1
LI6163	06.11.09	Liberty Island	M	4	433	0.9
LI6164	06.11.09	Liberty Island	F	4	427	0.9
LI6165	06.11.09	Liberty Island	M	4	435	1.1
LI6166	06.11.09	Liberty Island	F	4	498	1.4
LI6167	06.11.09	Liberty Island	F	4	413	0.8
LI6168	06.11.09	Liberty Island	M	5	698	4.2
LI6237	07.03.22	Liberty Island	M	4	499	1.9
LI6238	07.03.22	Liberty Island	M	7	633	3.4
LI6239	07.03.22	Liberty Island	M	9	686	3.8
LI6240	07.03.22	Liberty Island	M	4	426	1.1
LI6241	07.03.22	Liberty Island	M	4	471	1.3
LI6242	07.03.22	Liberty Island	F	4	478	1.2
LI6243	07.03.22	Liberty Island	F	6	637	3.5
LI6244	07.03.22	Liberty Island	M	5	498	1.2
MS6135	06.11.01	Miner Slough	F	6	616	2.8
RV4658	06.10.13	Rio Vista Fish Derby	M	5	555	1.9
RV4659	06.10.13	Rio Vista Fish Derby	M	8	705	4.1
CS6159	06.11.08	Cache Slough	F	5	555	2
TD4669	06.10.13	Toe Drain	M	5	525	1.9
TD4690	06.10.13	Toe Drain	M	5	540	2.3
TD4691	06.10.13	Toe Drain	M	7	640	2.8
IO35501		San Pablo Bay	U	7		
IO35502		San Pablo Bay	U	5		
IO35503		San Pablo Bay	U	6		
IO35504		San Pablo Bay	U	5		
IO35505		San Pablo Bay	U	4		
IO35506		San Pablo Bay	U	6		
IO35507		San Pablo Bay	U	5		
IO35508		San Pablo Bay	U	4		
IO35509		San Pablo Bay	U	6		
IO61301	06.06.19	South Bay	U	5		

Table 10a.

IO65301	06.05.22	San Pablo Bay	U	5		
IO65302	06.05.22	San Pablo Bay	U	5		
IO65303	06.05.22	San Pablo Bay	U	5		
IO65304	06.05.22	San Pablo Bay	U	4		
IO65305	06.05.22	San Pablo Bay	U	5		
IO65306	06.05.24	San Pablo Bay	U	9		
IO65307	06.05.24	San Pablo Bay	U	5		
IO65308	06.08.17	San Pablo Bay	U	5		
IO65309	06.08.17	San Pablo Bay	U	5		
IO65310	06.09.19	San Pablo Bay	U	8		
IO65311	06.09.20	San Pablo Bay	U	5		
IO65312	06.09.20	San Pablo Bay	U	11		
IO65313	06.09.21	San Pablo Bay	U	4		
IO65314	06.09.21	San Pablo Bay	U	5		
IO66301	06.07.05	Central Bay	U	4		

Table 10a. Adult striped bass subjected to otolith microgeochemical habitat use analysis.

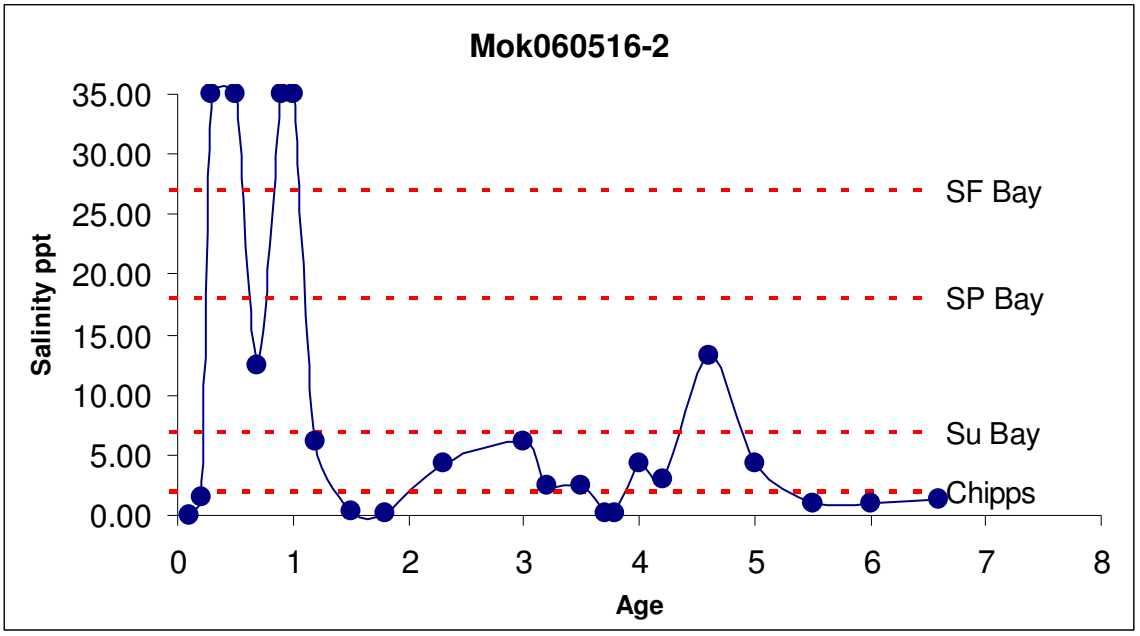


Figure 1a:
Mokelumne River 060516-2 Female, 3.4 kg, 7 years

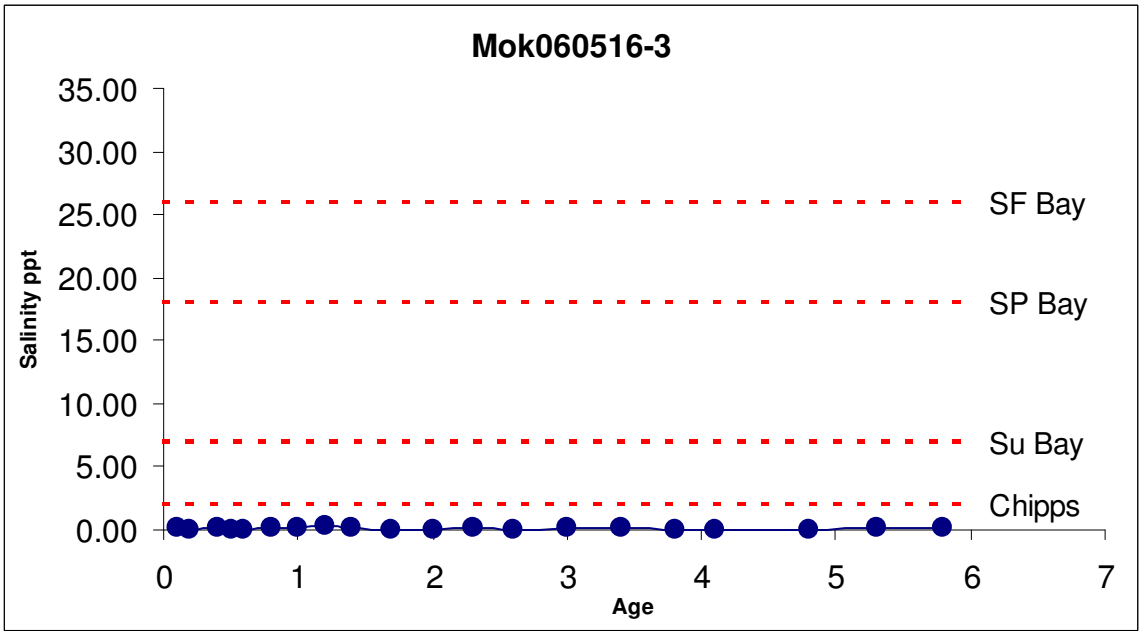


Figure 2a:
Mokelumne River 060516-3 Female, 3.2 kg, 6 years

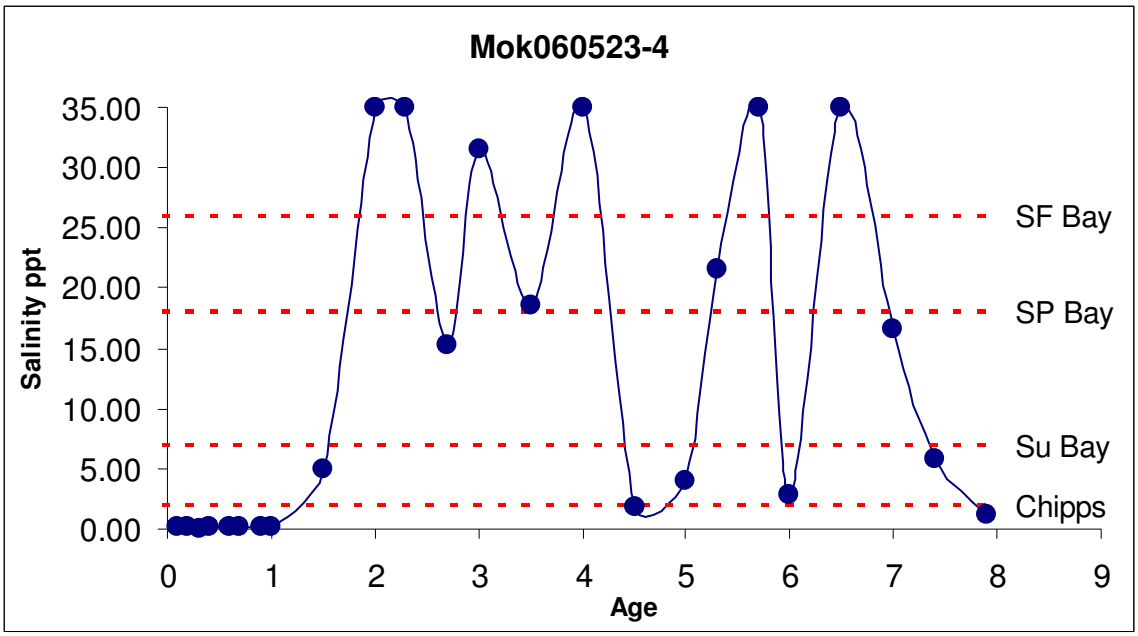


Figure 3a:
Mokelumne River 060523-4 Female, 4.1 kg, 8 years

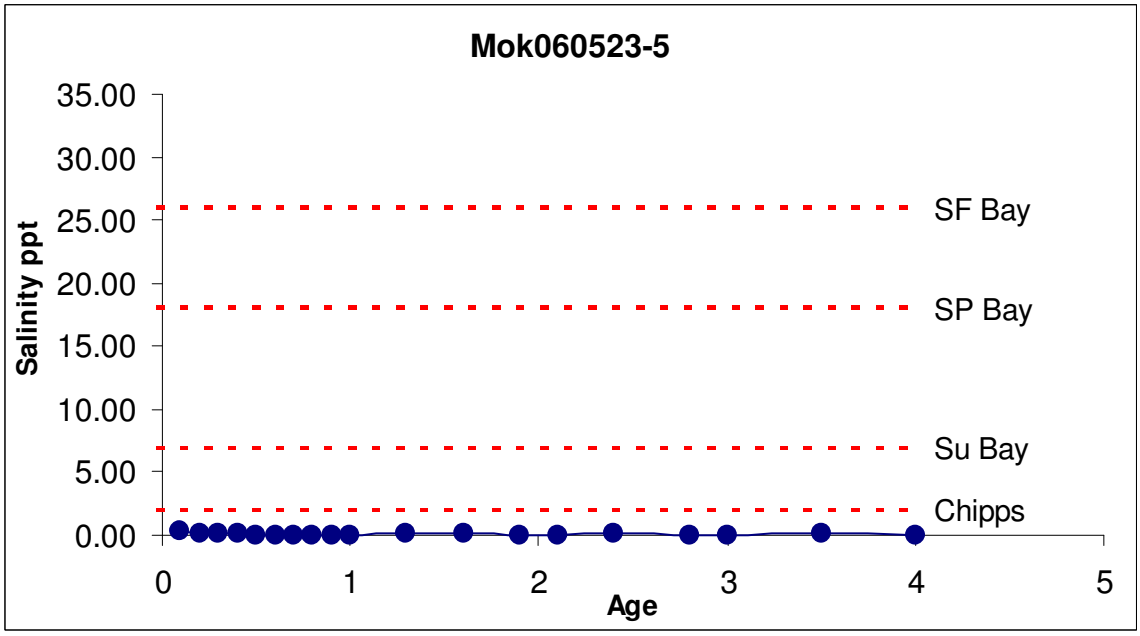


Figure 4a:
Mokelumne River 060523-5 Female, 3.9 kg, 4 years

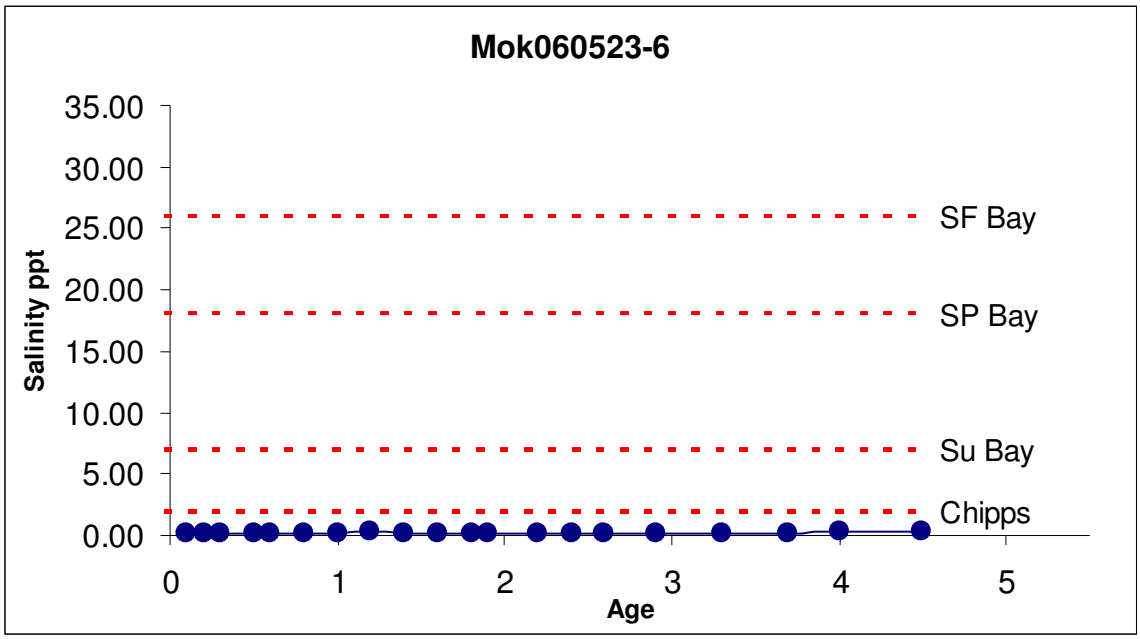


Figure 5a:
Mokelumne River 060523-6 Female, 2.7 kg, 5 years

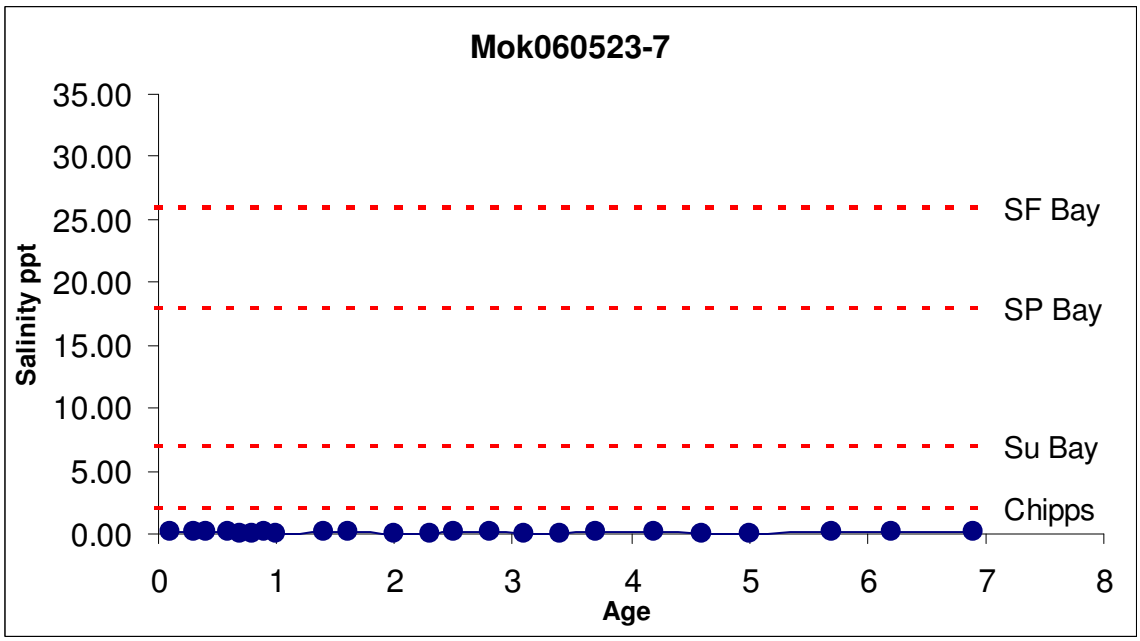


Figure 6a:
Mokelumne River 060523-7 Female, 2.7 kg, 7 years

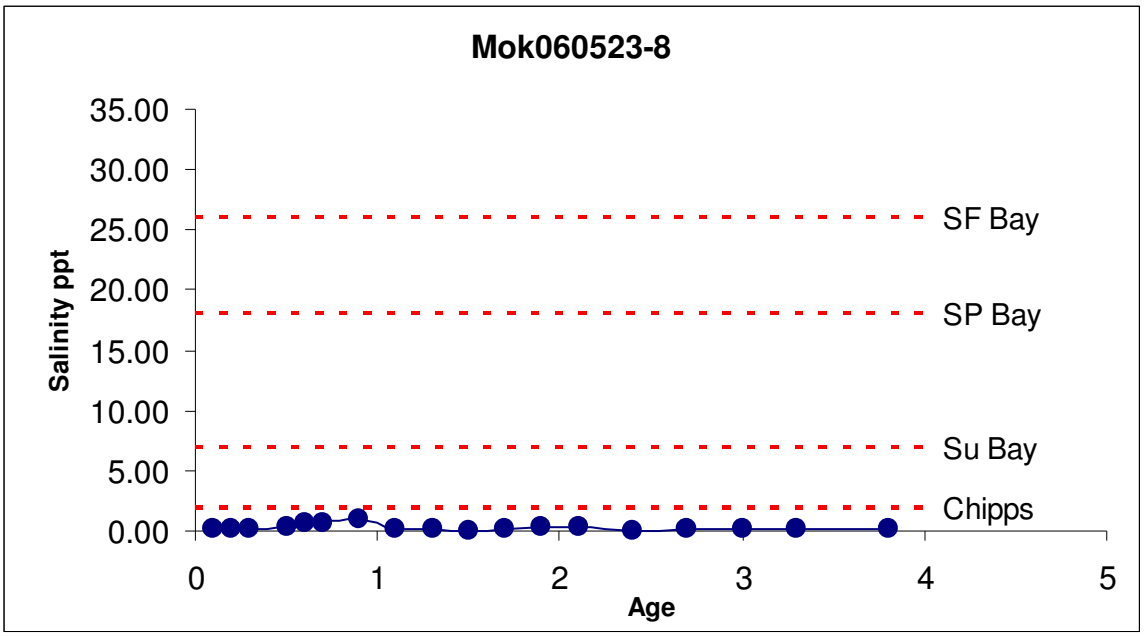


Figure 7a:
Mokelumne River 060523-8 Female, 2 kg, 4 years

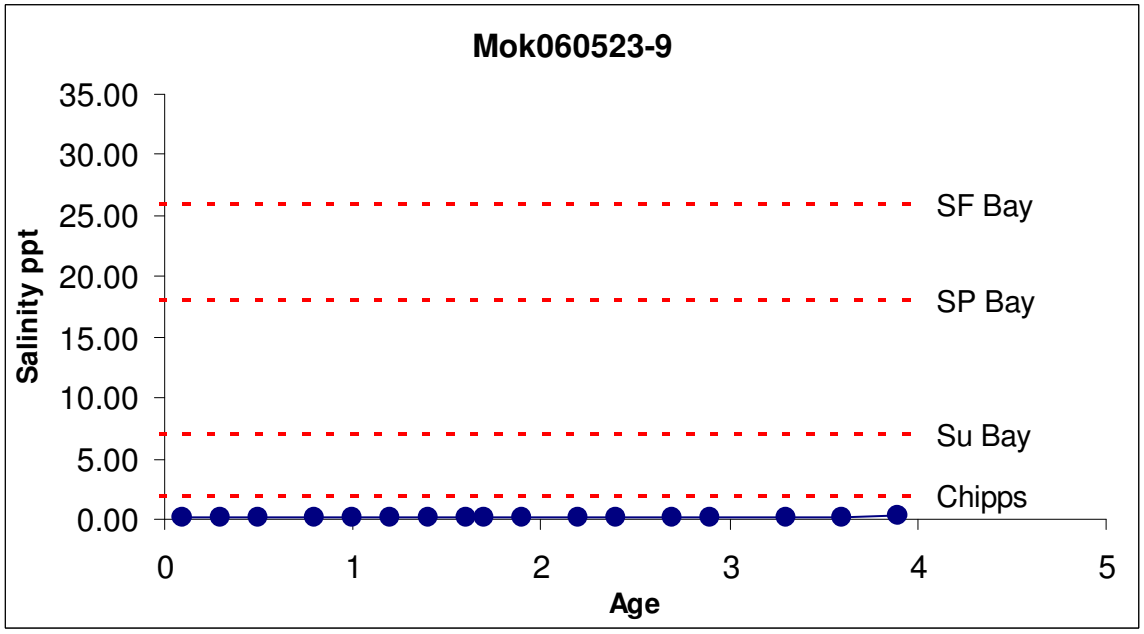


Figure 8a:
Mokelumne River 060523-9 Female, 2 kg, 4 years

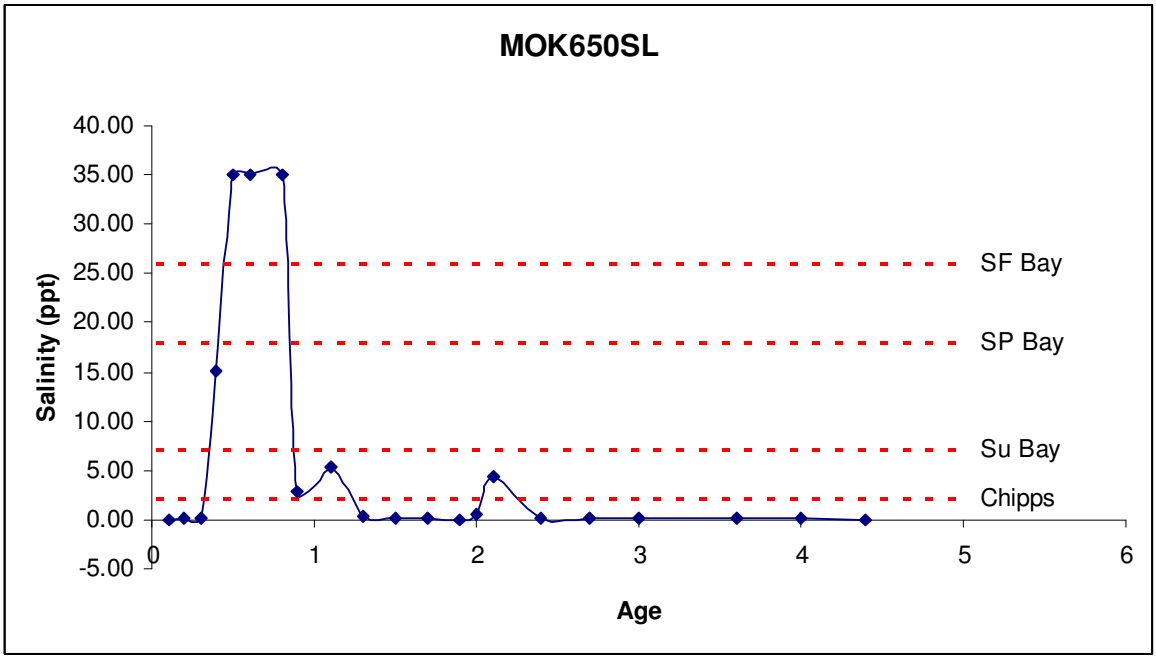


Figure 9a:
Mokelumne River 650SL Male, 5 years

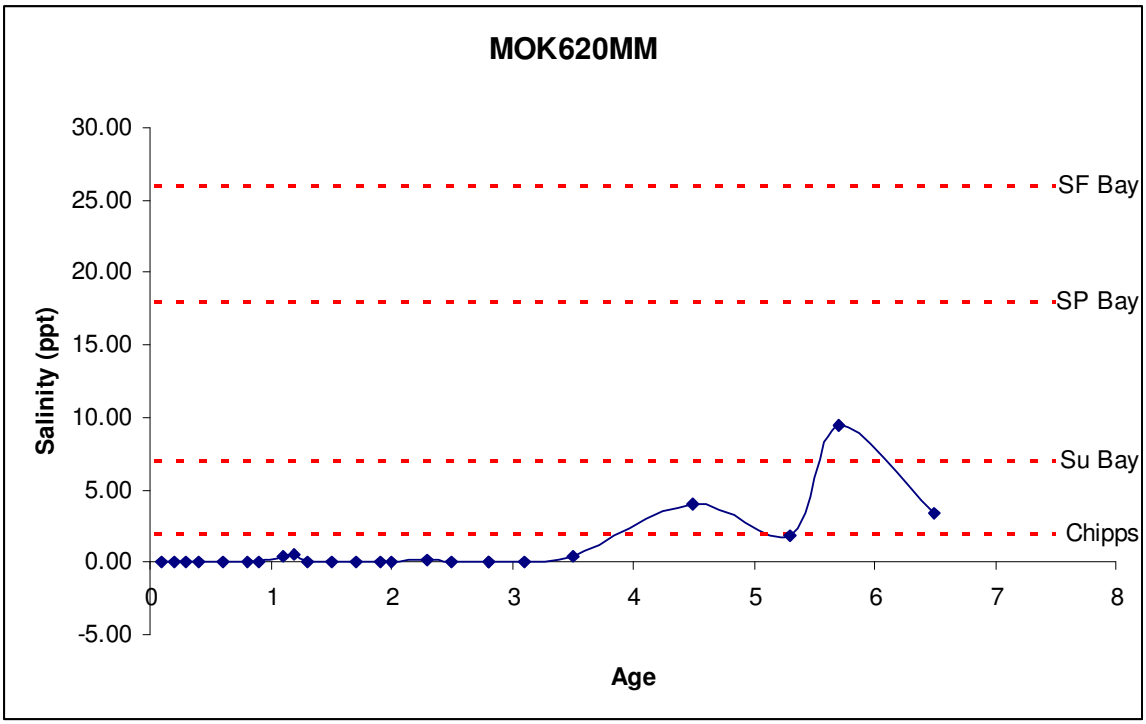


Figure 10a:
Mokelumne River 620MM Male, 7 years

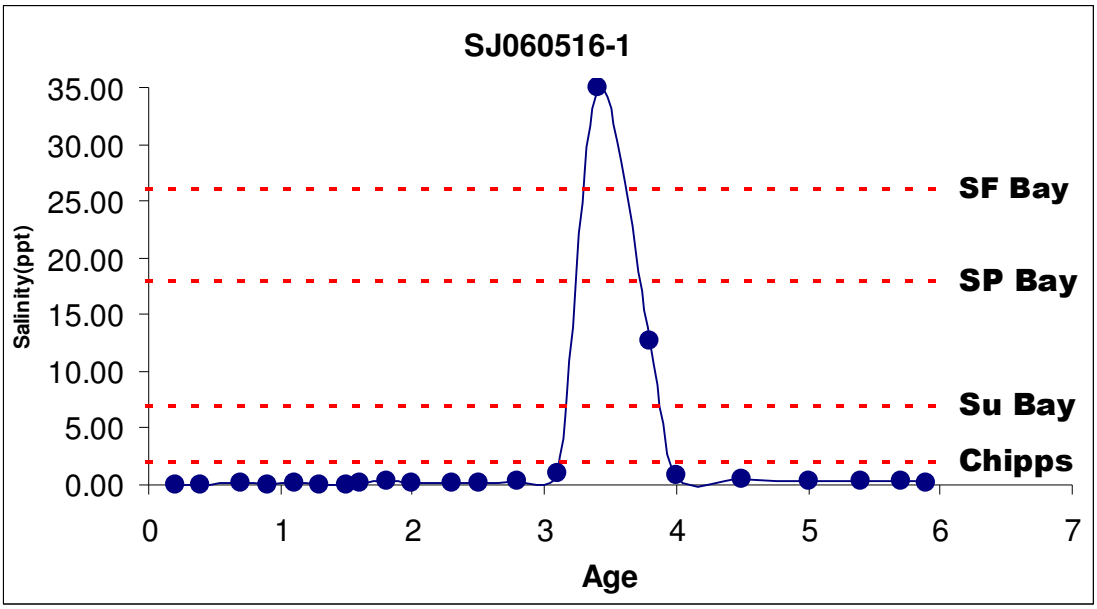


Figure 11a:
San Joaquin 060516-1 Female, 4.5 kg, 6 years

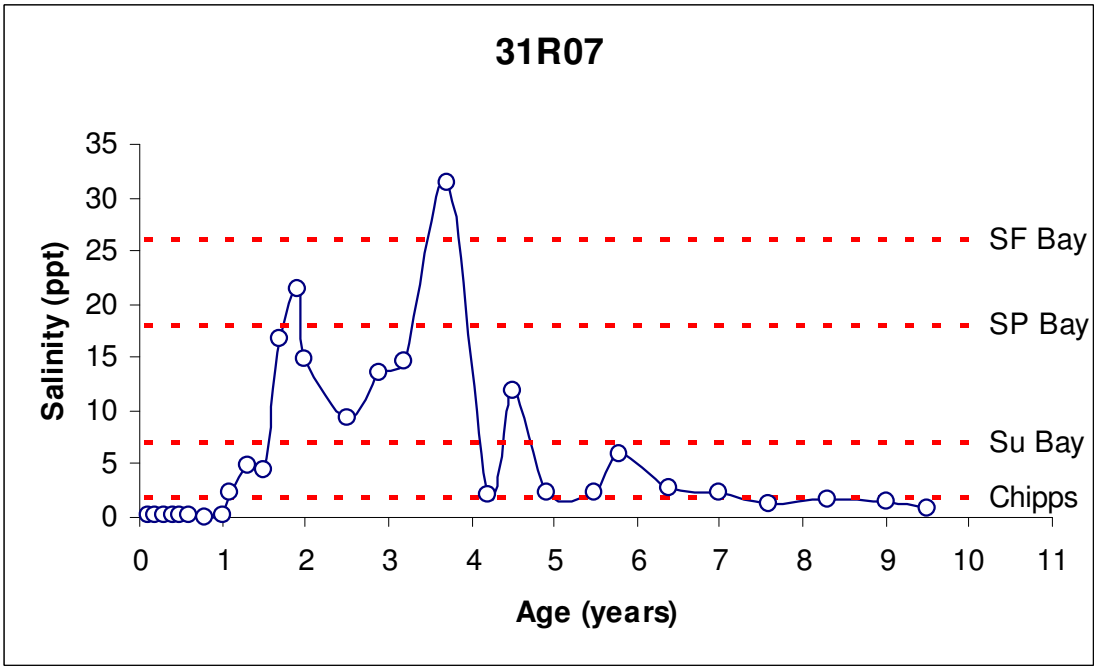


Figure 12a:
Knights Landing 31R07 Female, 9.1 kg, 10 years

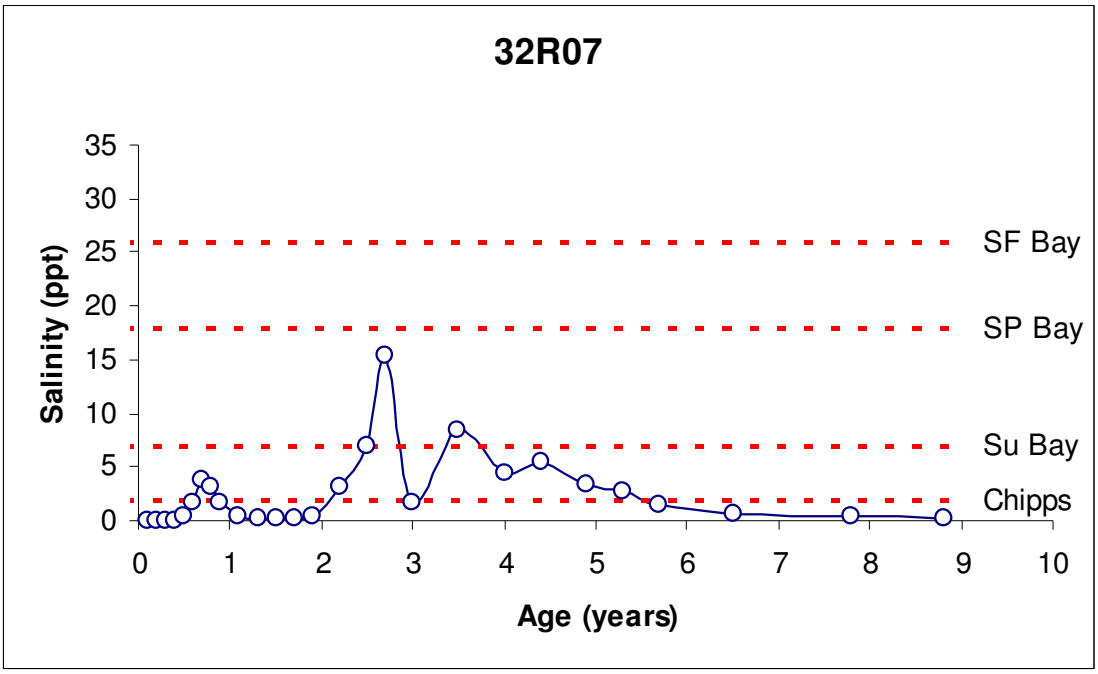


Figure 13a:
Knights Landing 32R07 Female, 9.1 kg, 9 years

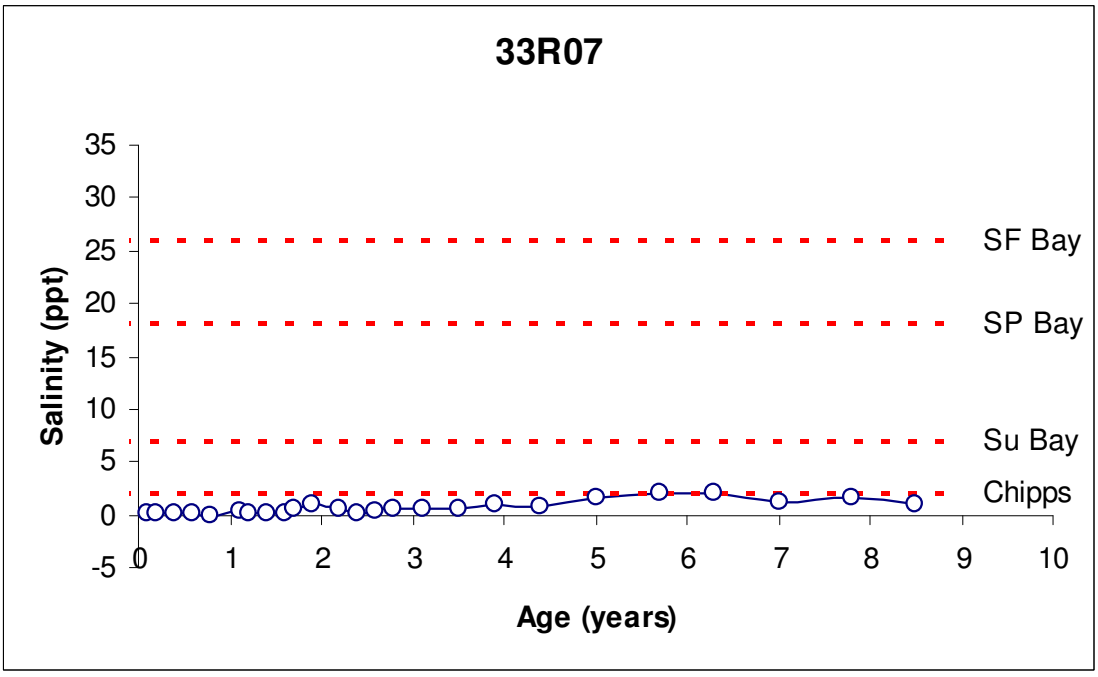


Figure 14a:
Knights Landing 33R07 Female, 9.1 kg, 9 years

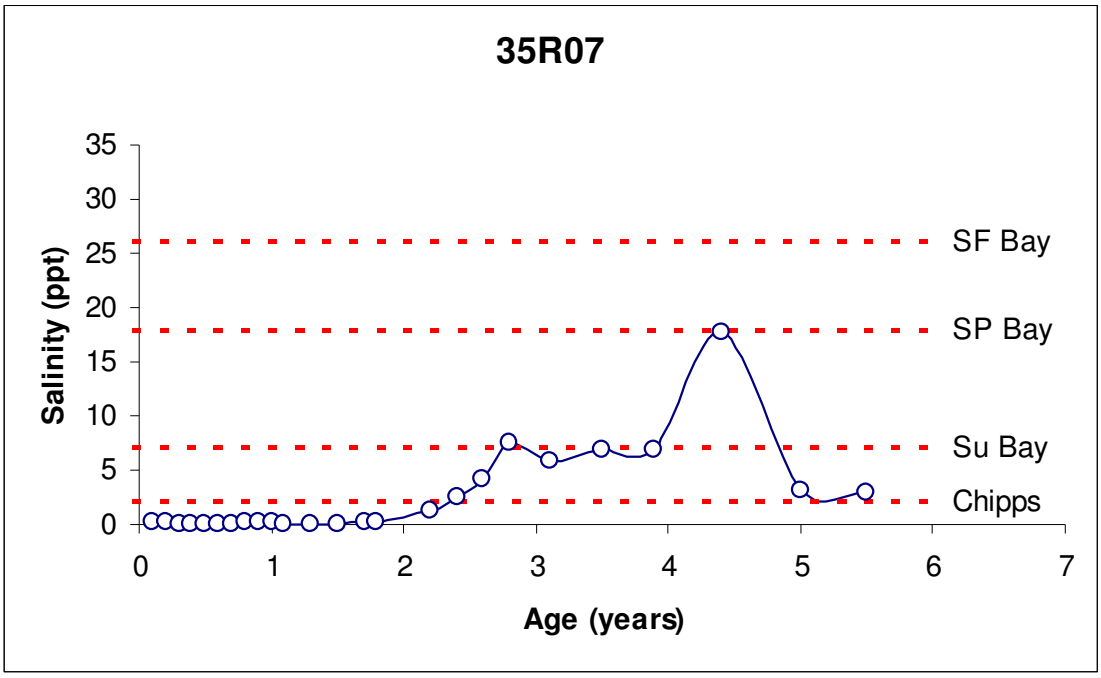


Figure 15a:
Knights Landing 35R07 Female, 5.9 kg, 6 years

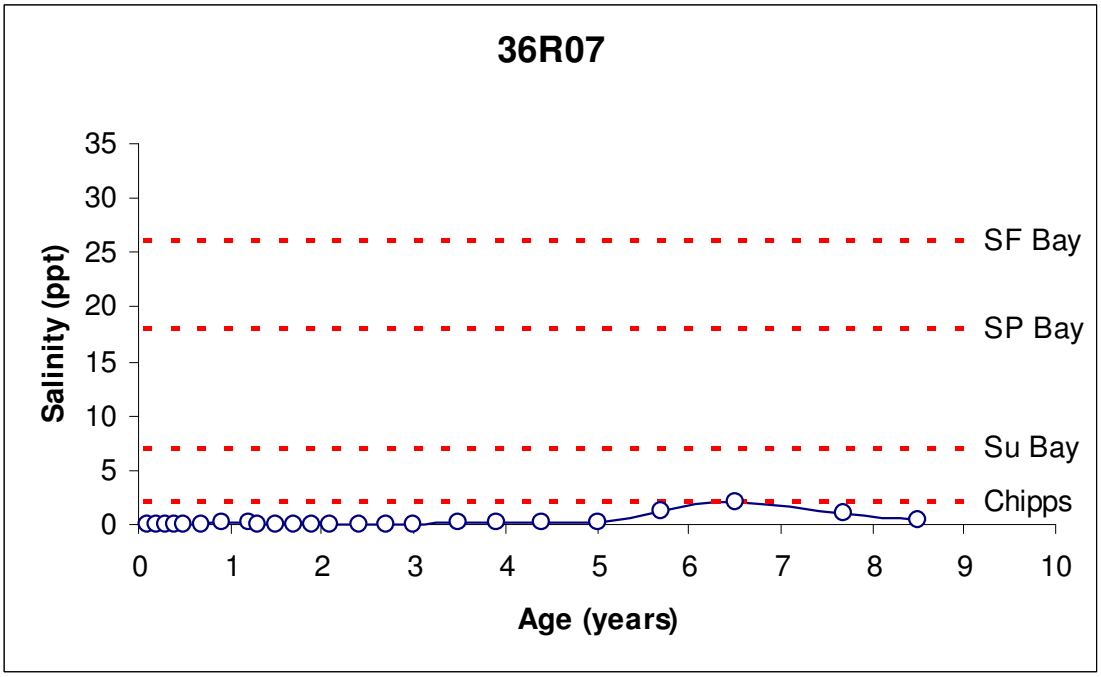


Figure 16a:
Knights Landing 36R07 Female, 9 years

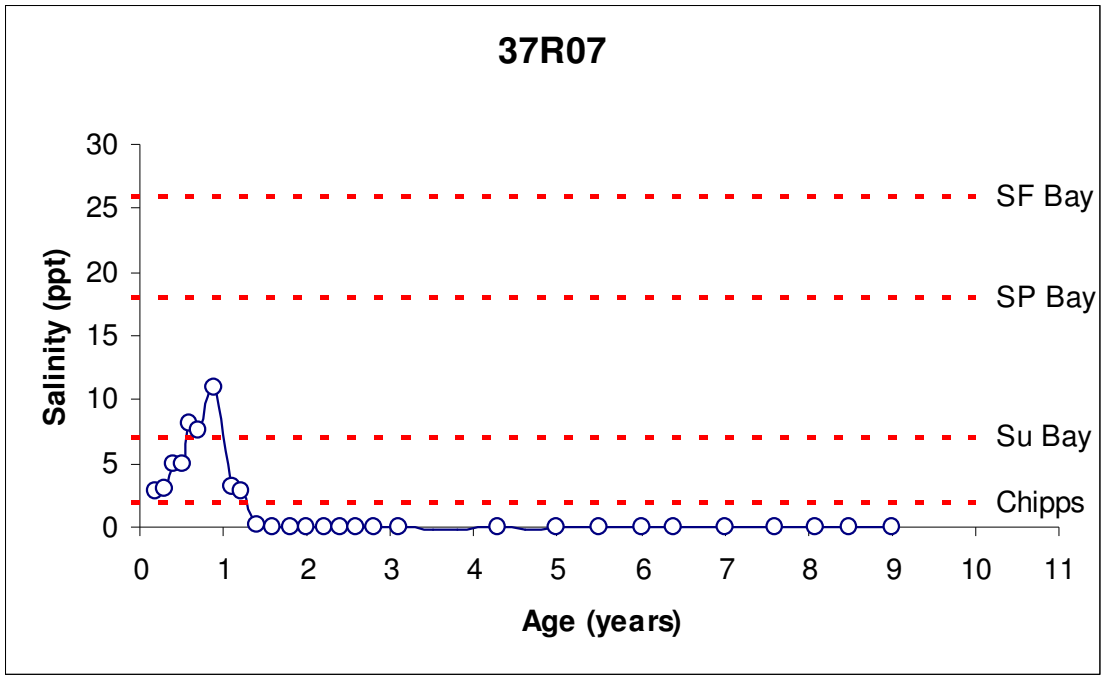


Figure 17a:
Knights Landing 37R07 Female, 9 years

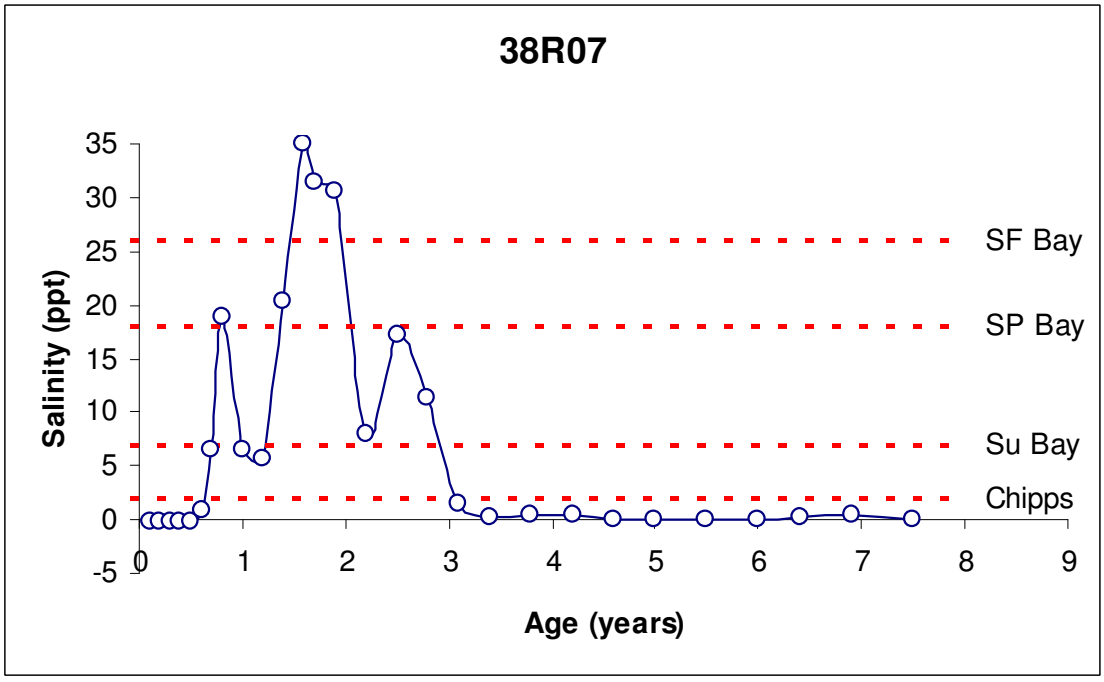


Figure 18a:
Knights Landing 38R07 Female, 8 years

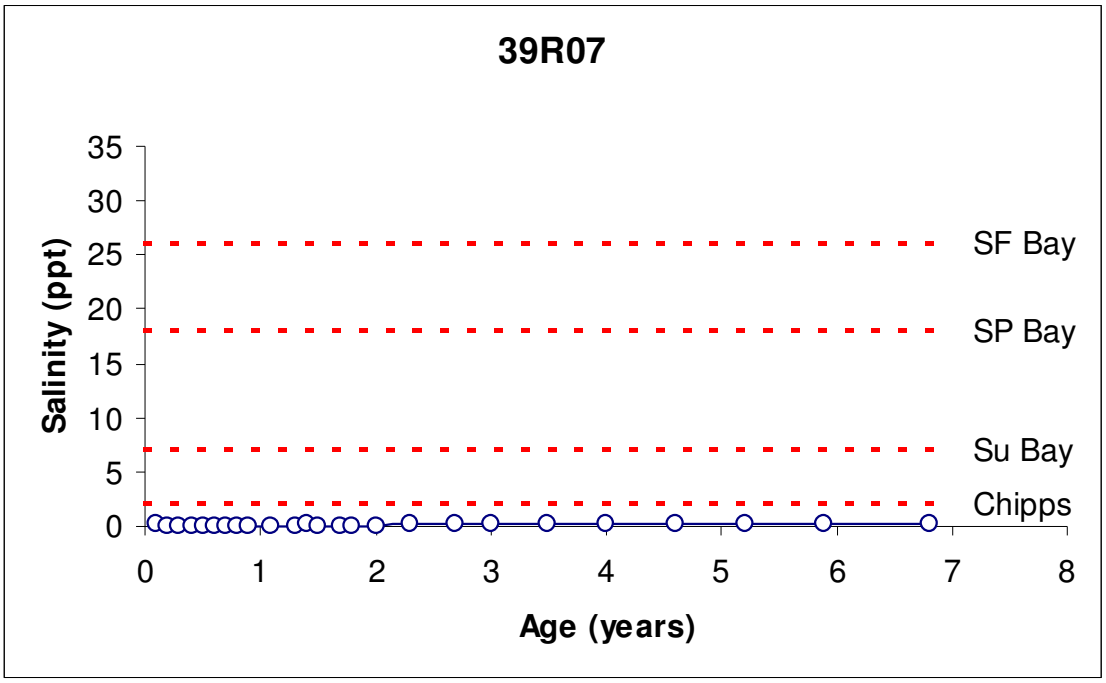


Figure 19a:
Knights Landing 39R07 Female, 9.1 kg, 7 years

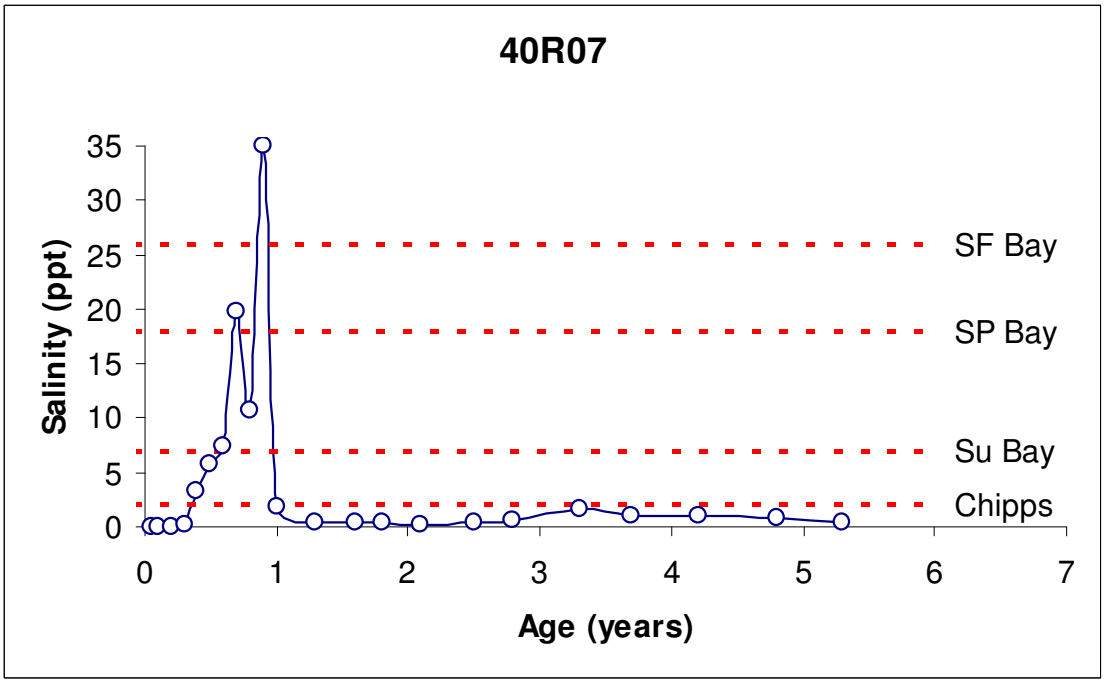


Figure 20a:
Knights Landing 40R07 Female, 6 years

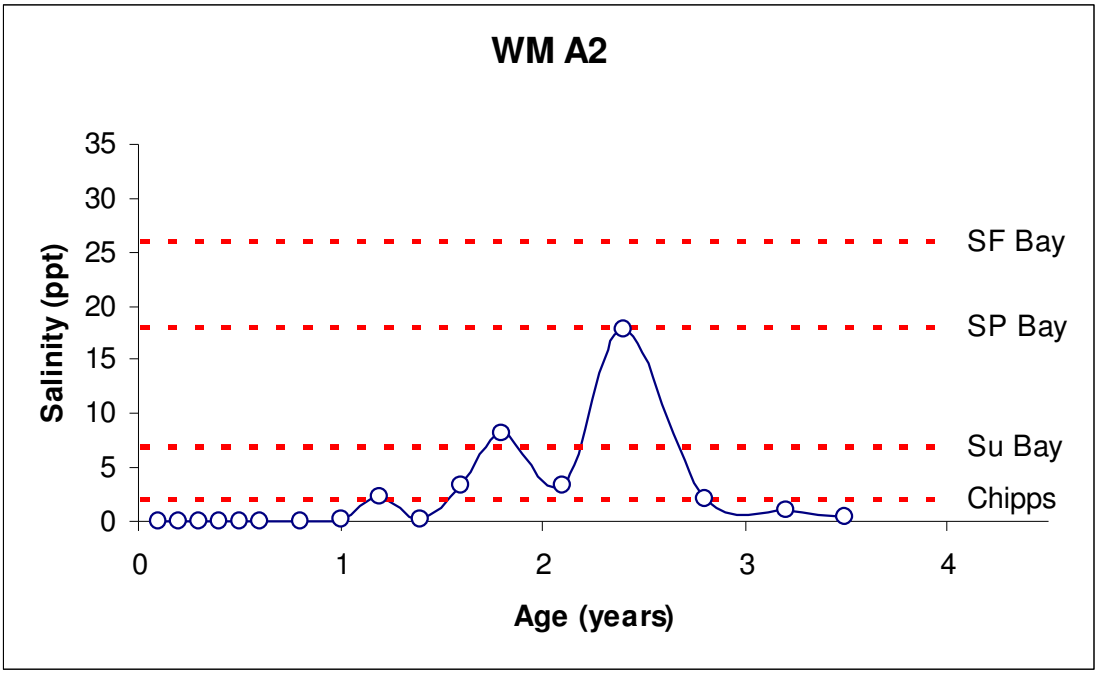


Figure 21a:
WM A2 Male, 4 years

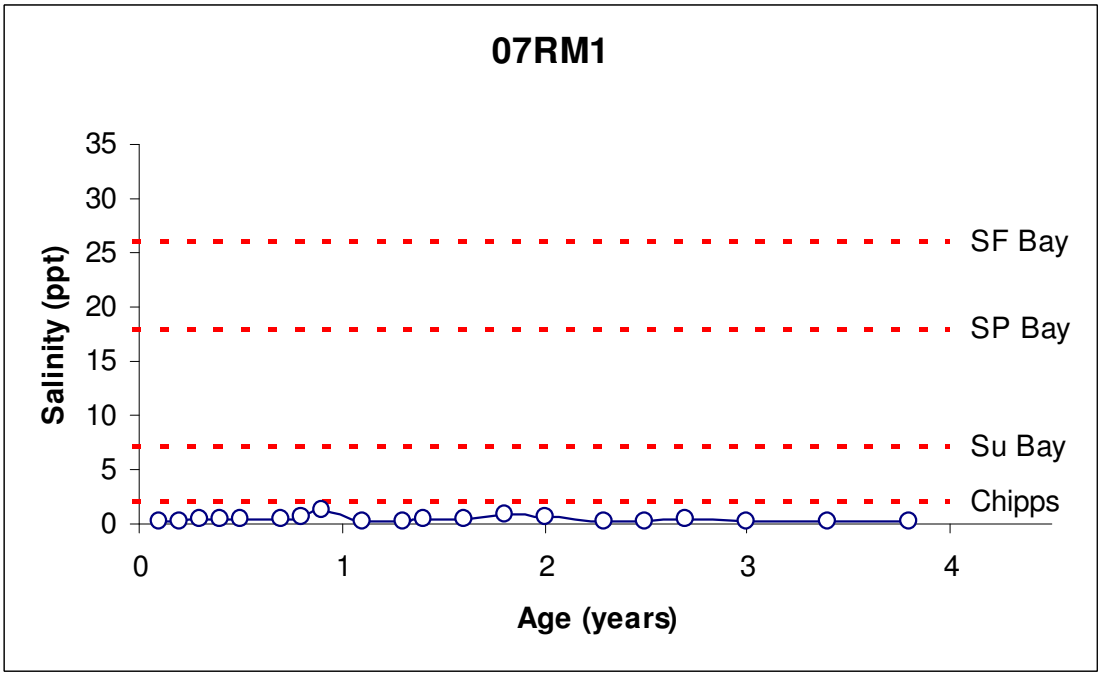


Figure 22a:
Knights Landing/Colusa 07RM1 Male, 4 years

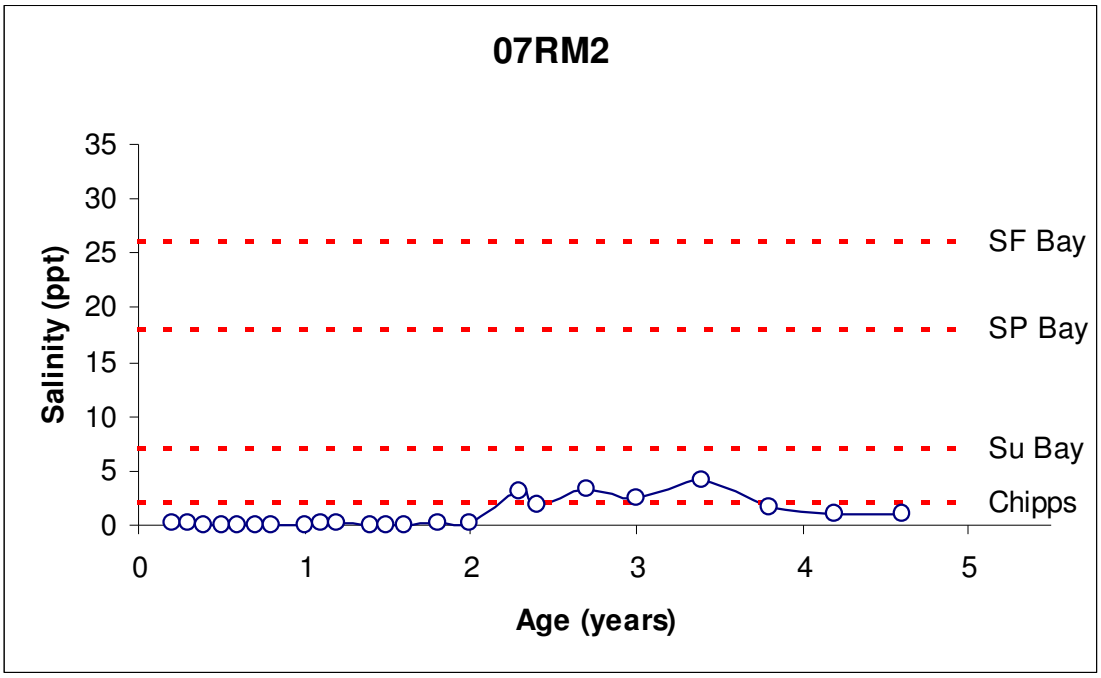


Figure 23a:
Knights Landing/Colusa 07RM2 Male, 5 years

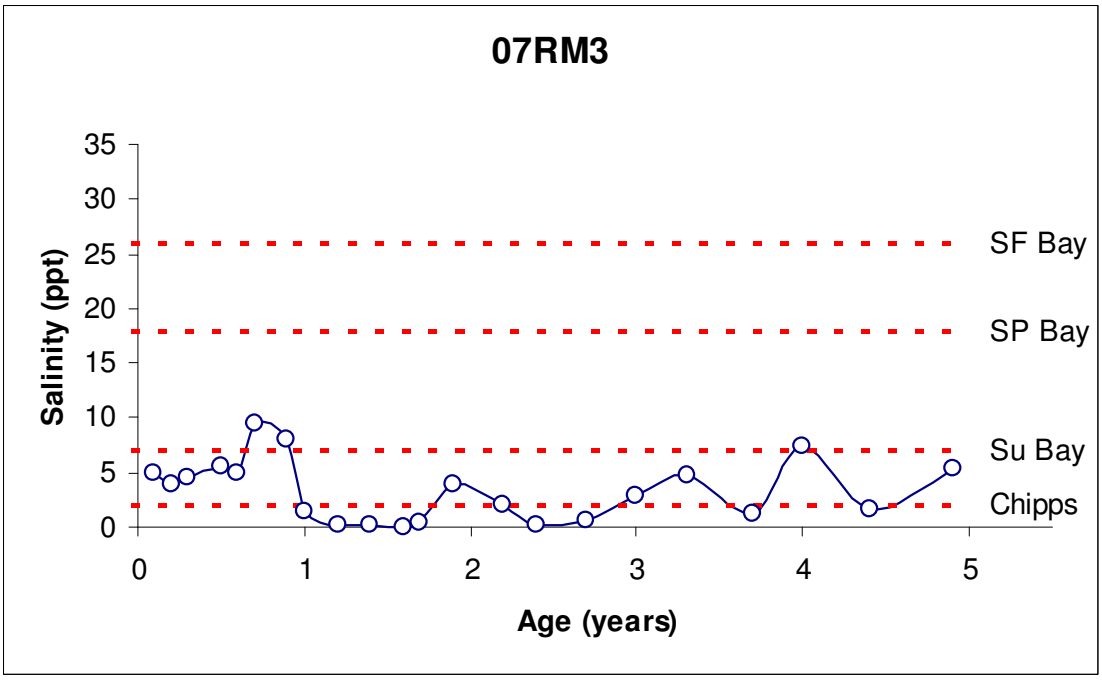


Figure 24a:
Knights Landing/Colusa 07RM3 Male, 5 years

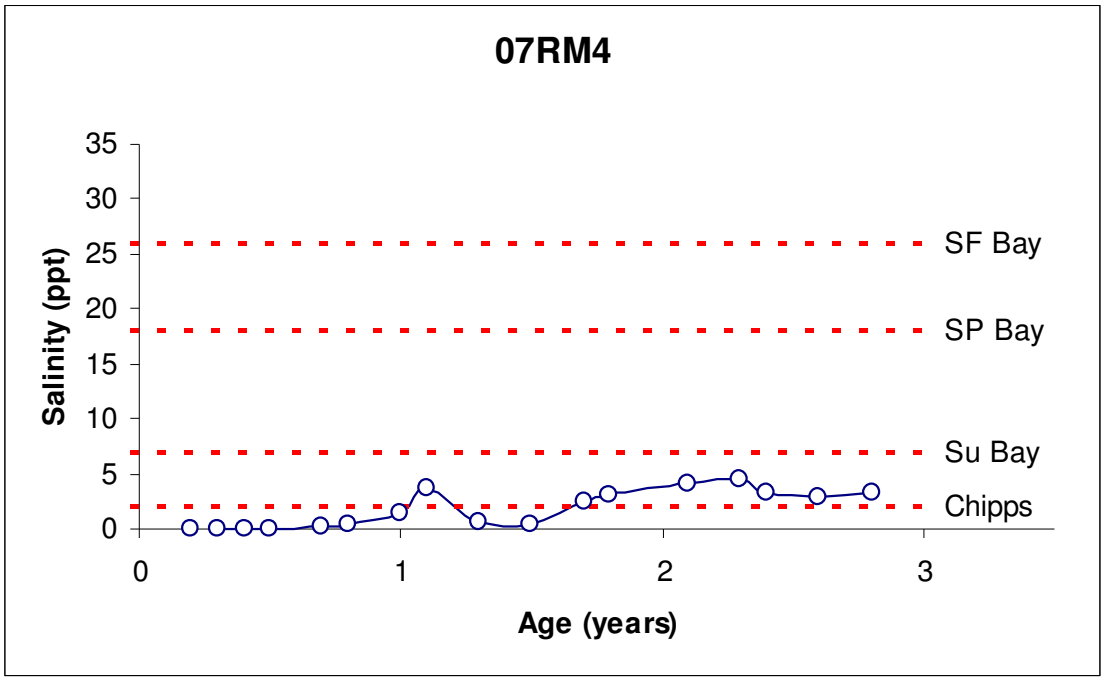


Figure 25a:
Knights Landing/Colusa 07RM4 Male, 3 years

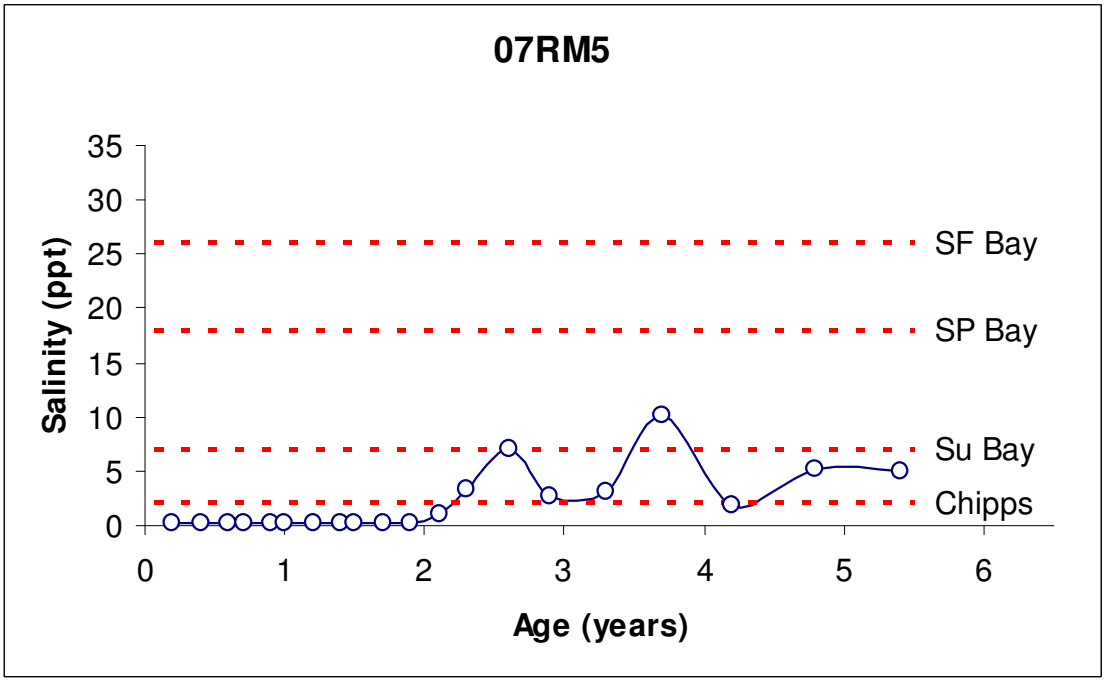


Figure 26a:
Knights Landing/Colusa 07RM5 Male, 6 years

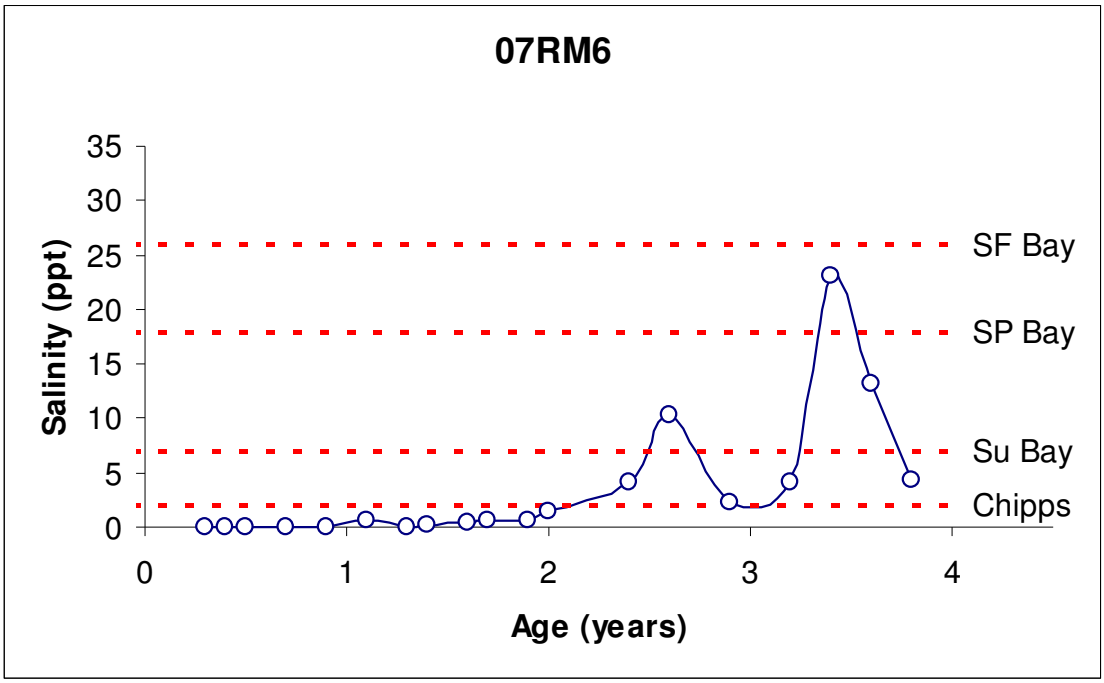


Figure 27a:
Knights Landing/Colusa 07RM6 Male, 4 years

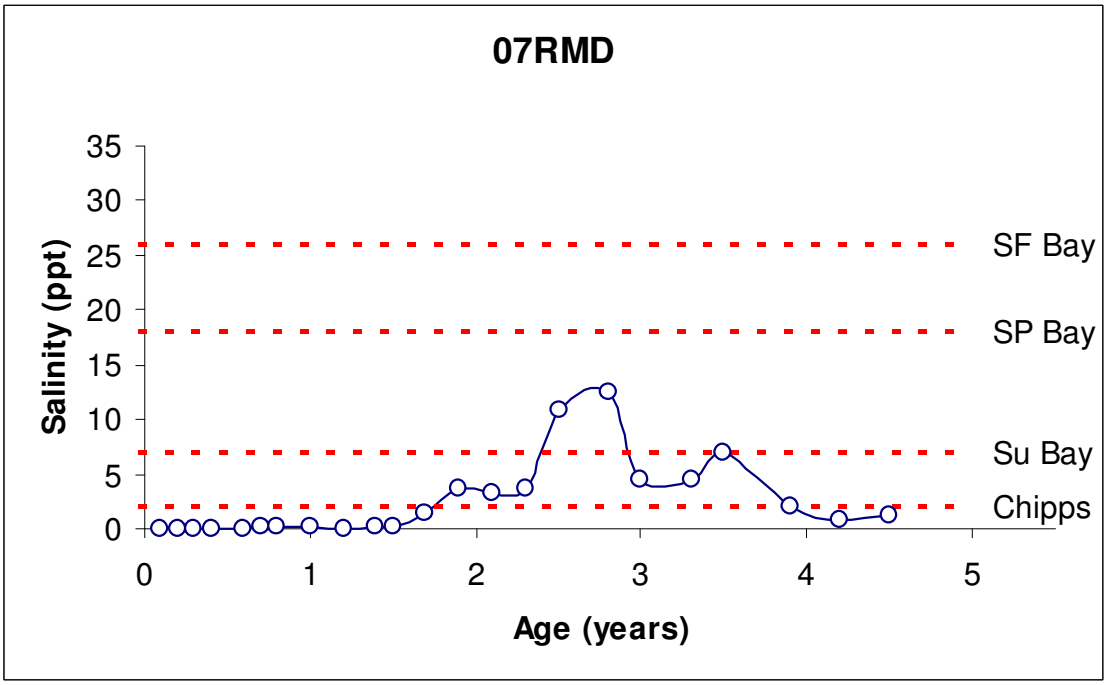


Figure 28a:
Knights Landing/Colusa 07RMD Male, 5 years

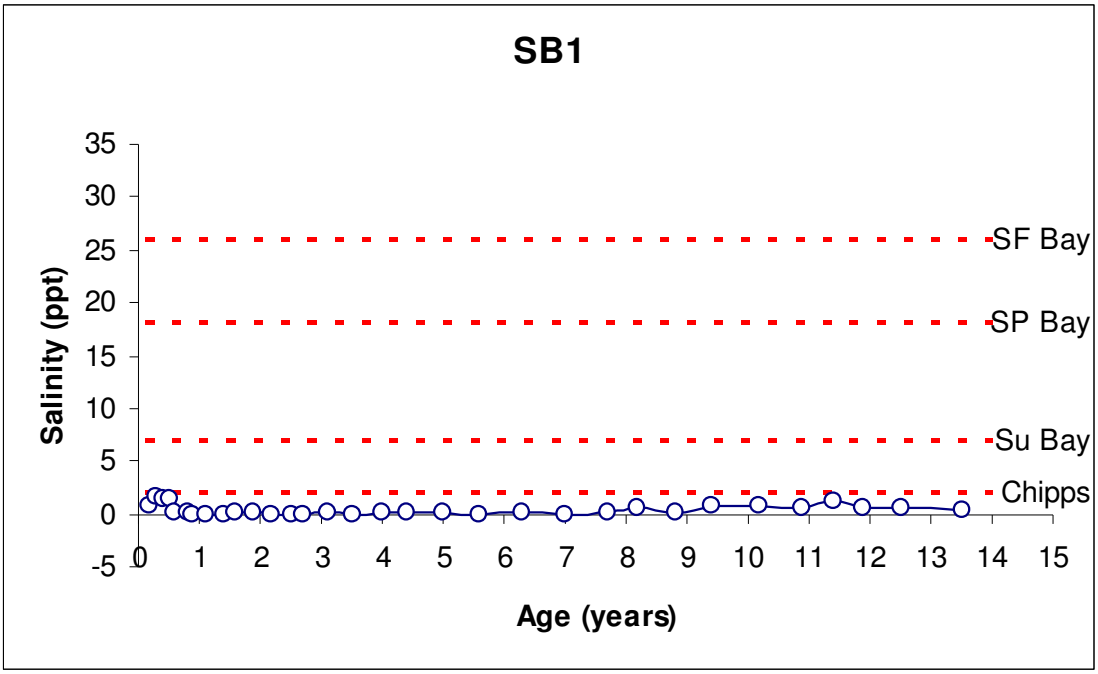


Figure 29a:
Stinson Beach 1, 14 years

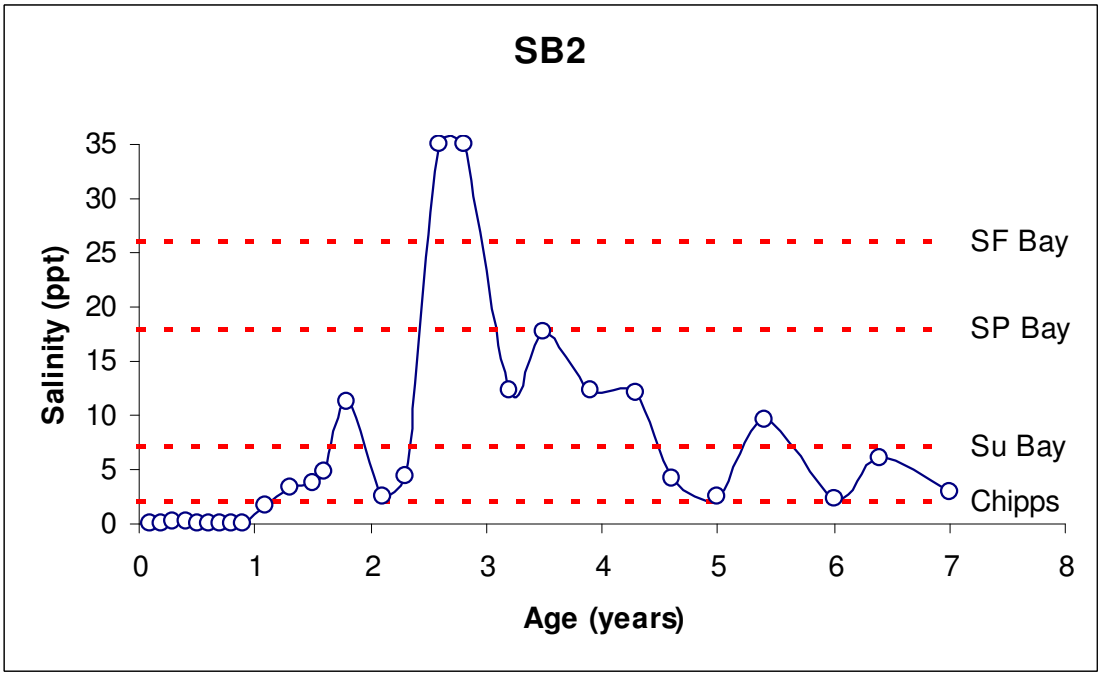


Figure 30a:
Stinson Beach 2, 7 years

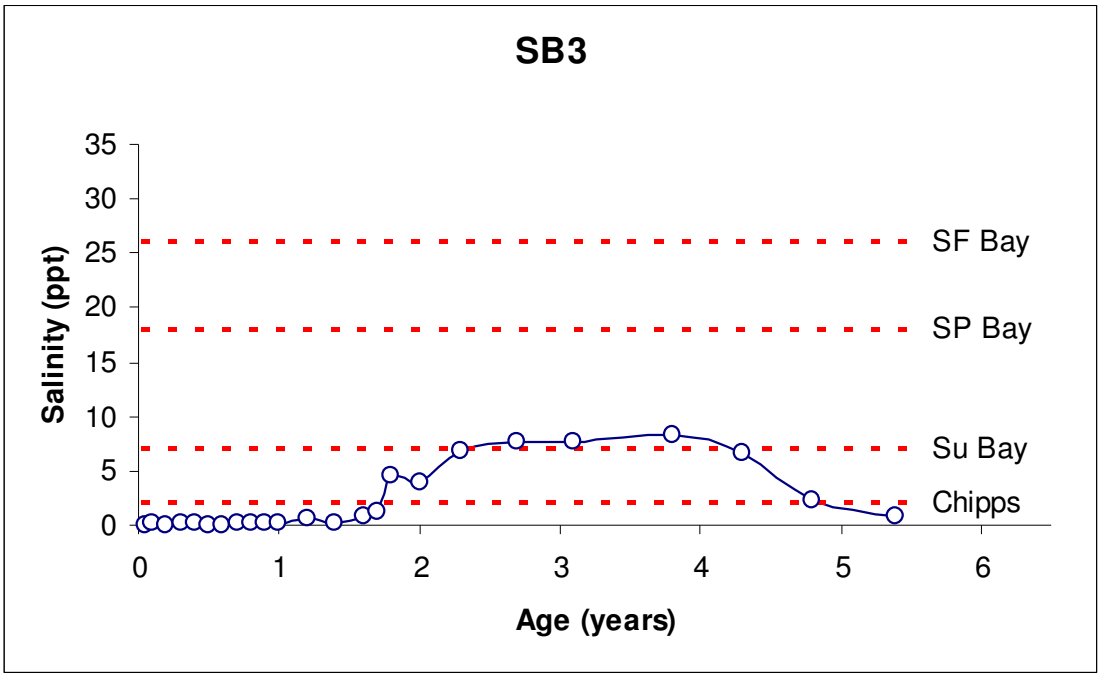


Figure 31a:
Stinson Beach 3, 6 years

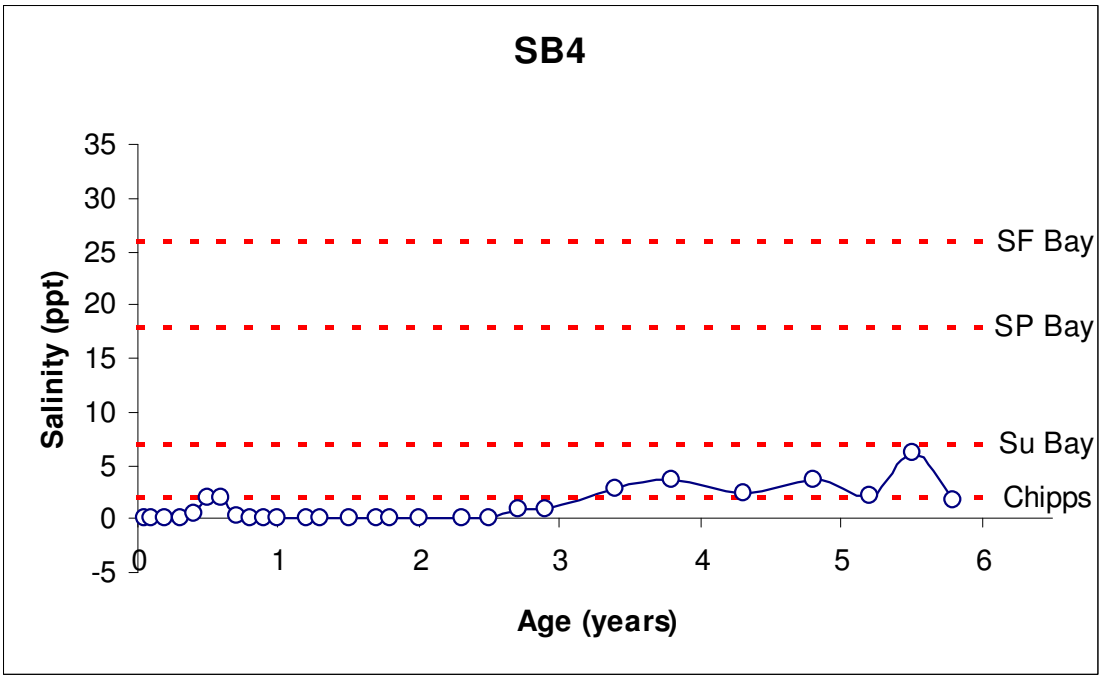


Figure 32a:
Stinson Beach 4, 6 years

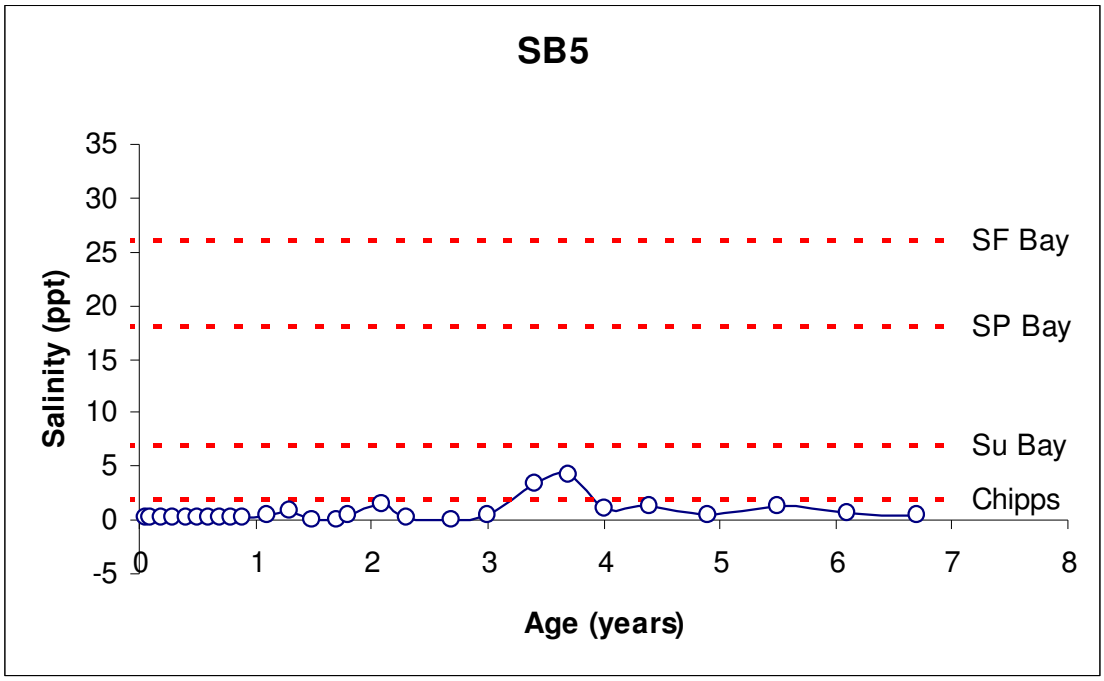


Figure 33a:
Stinson Beach 5, 7 years

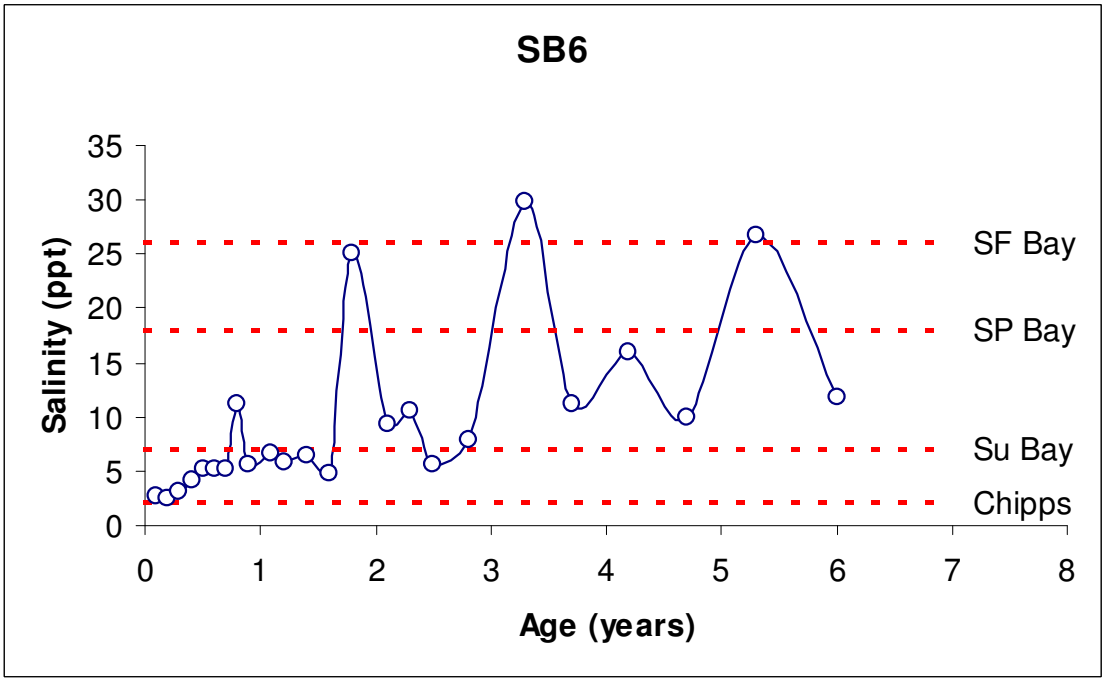


Figure 34a:
Stinson Beach 6, 7 years

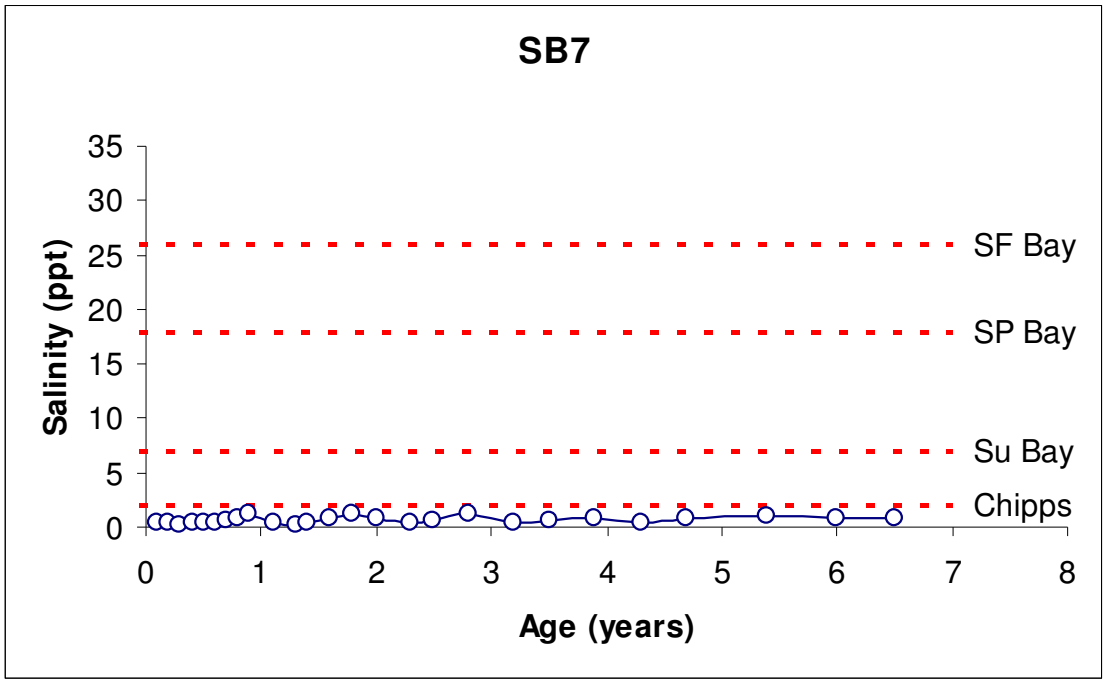


Figure 35a:
Stinson Beach 7, 7 years

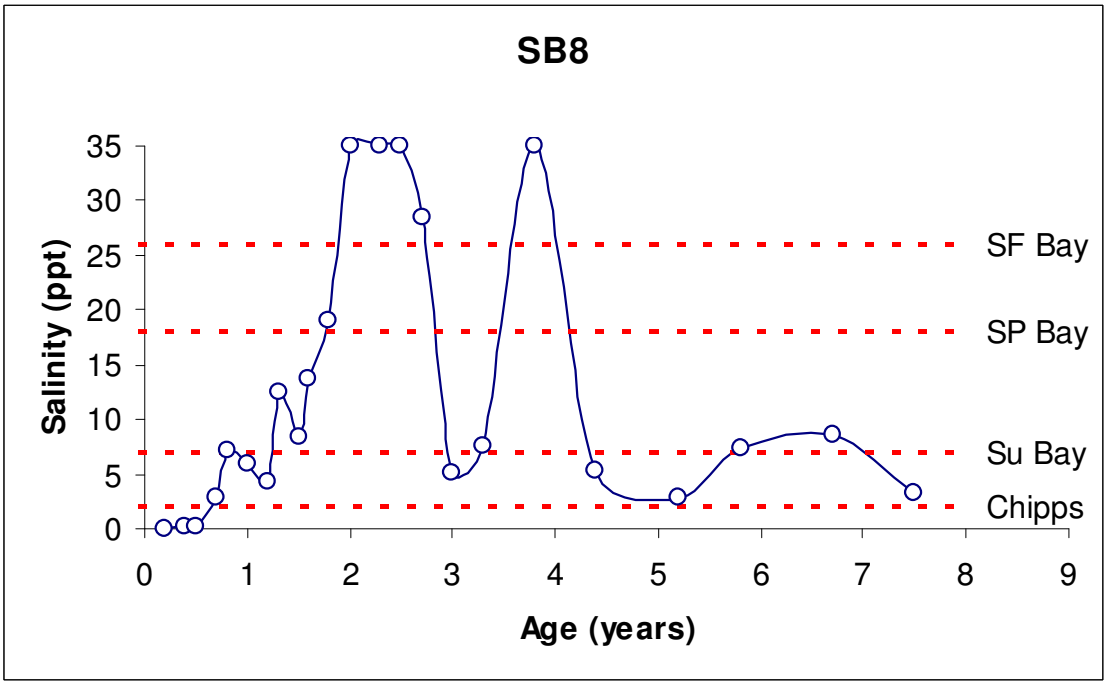


Figure 36a:
Stinson Beach 8, 8 years

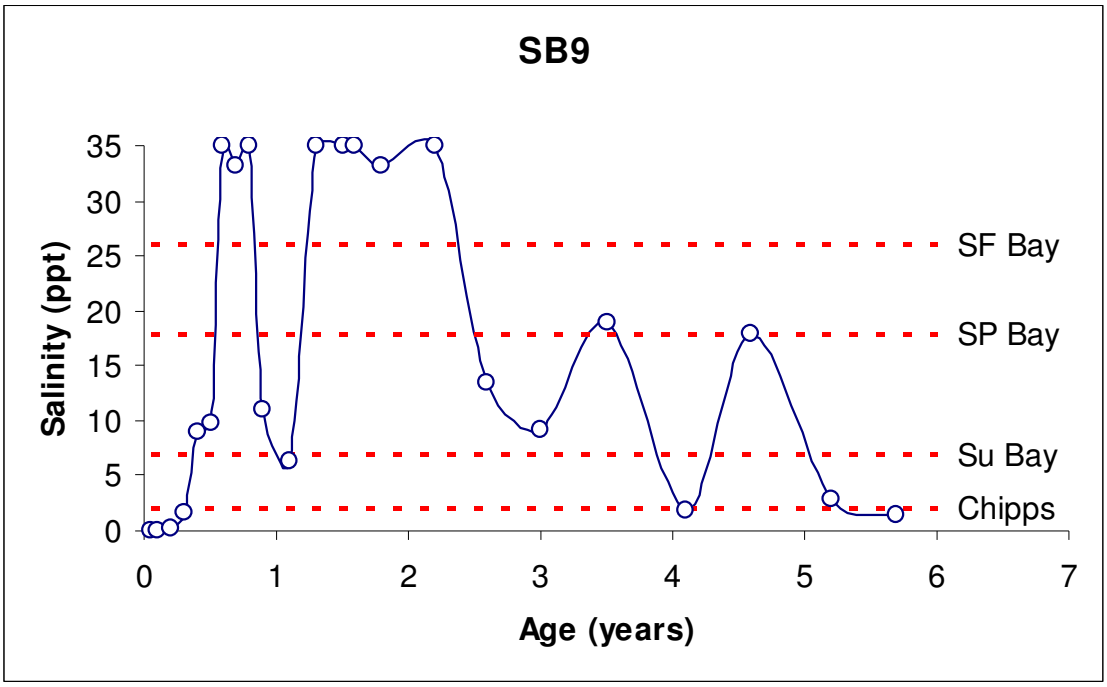


Figure 37a:
Stinson Beach 9, 6 years

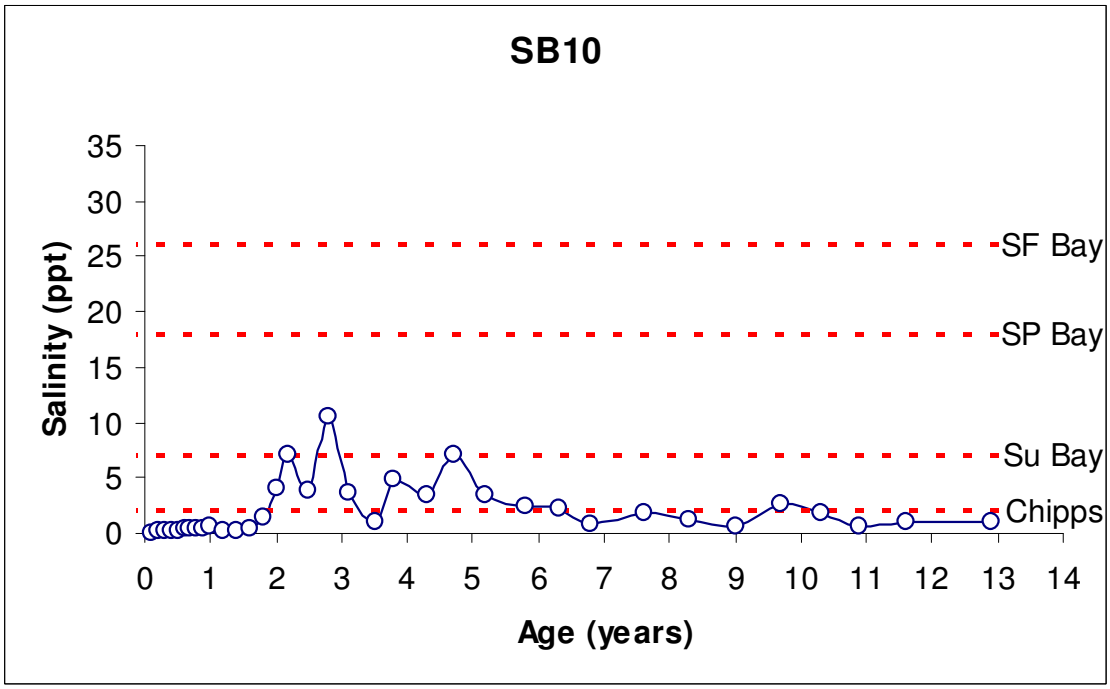


Figure 38a:
Stinson Beach 10, 13 years

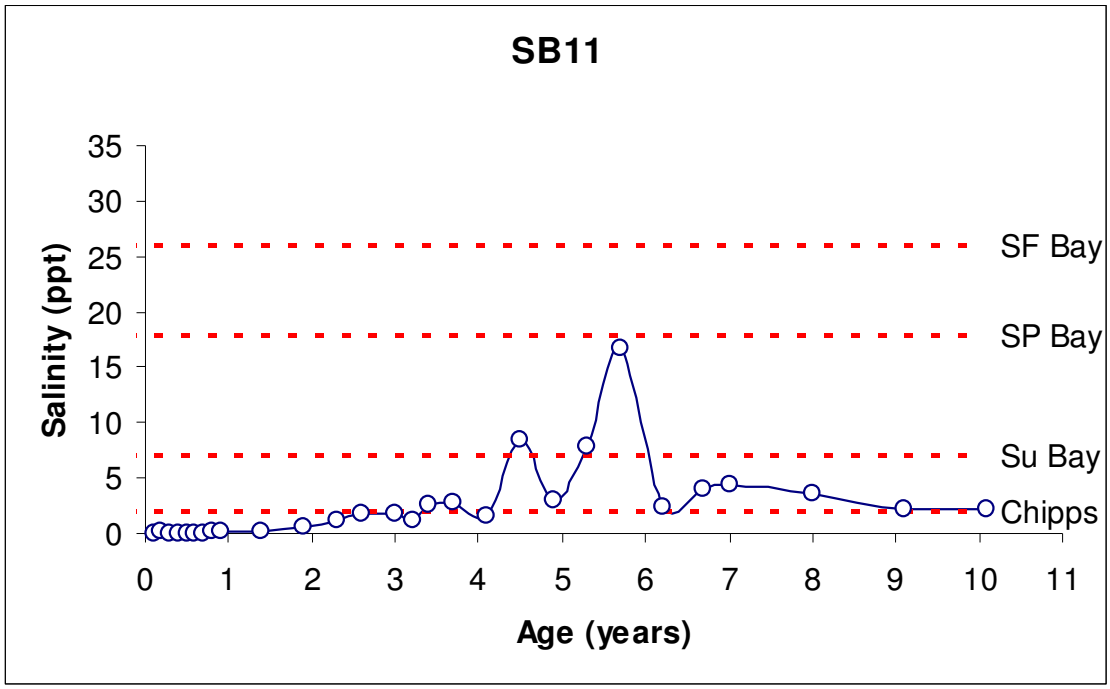


Figure 39a:
Stinson Beach 11, 11 years

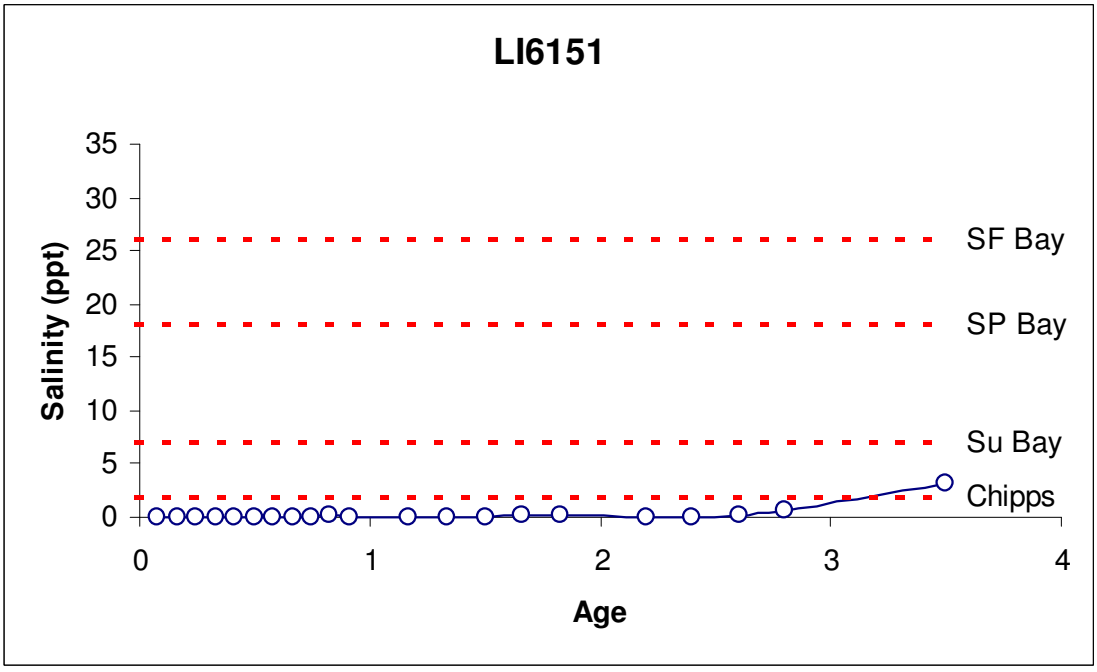


Figure 40a:
Liberty Island 6151 Male, 51.5 cm, 1.7 kg, 4 years

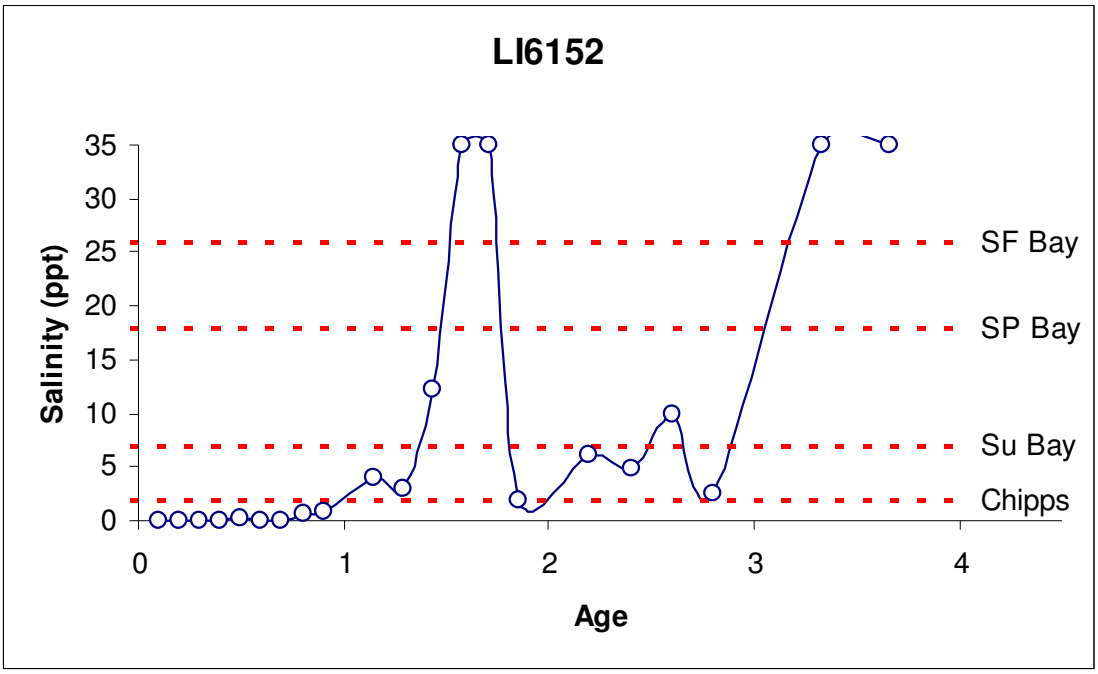


Figure 41a:
Liberty Island 6152 Female, 51.0 cm, 1.6 kg, 4 years

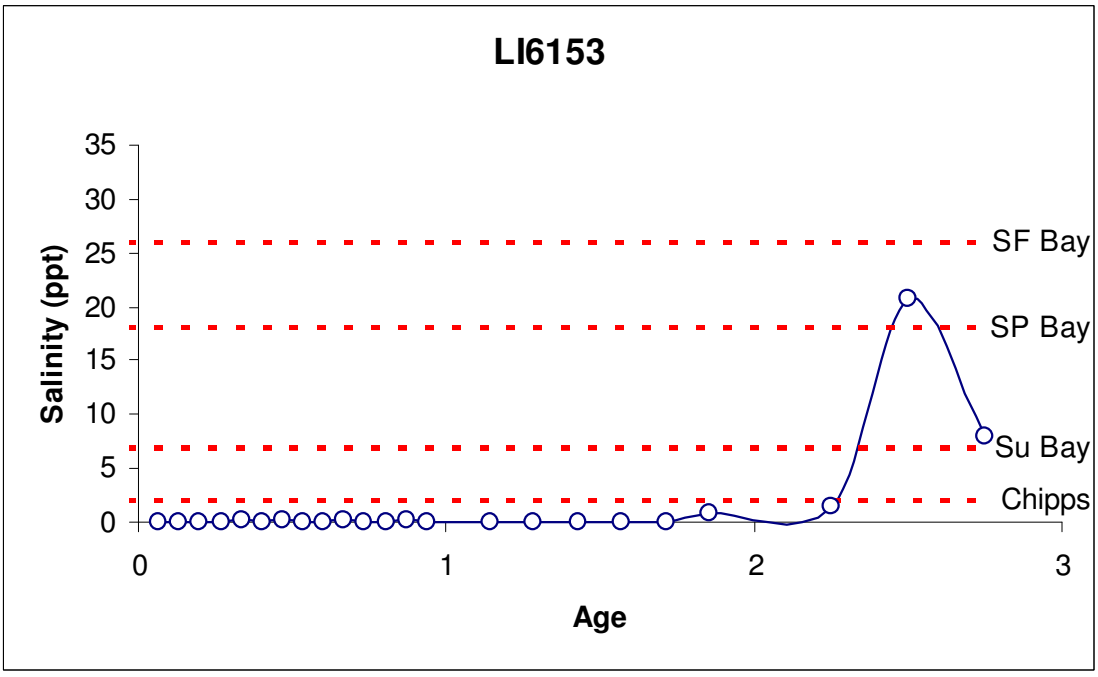


Figure 42a:
Liberty Island 6153 Female, 44.3 cm, 1.1 kg, 3 years

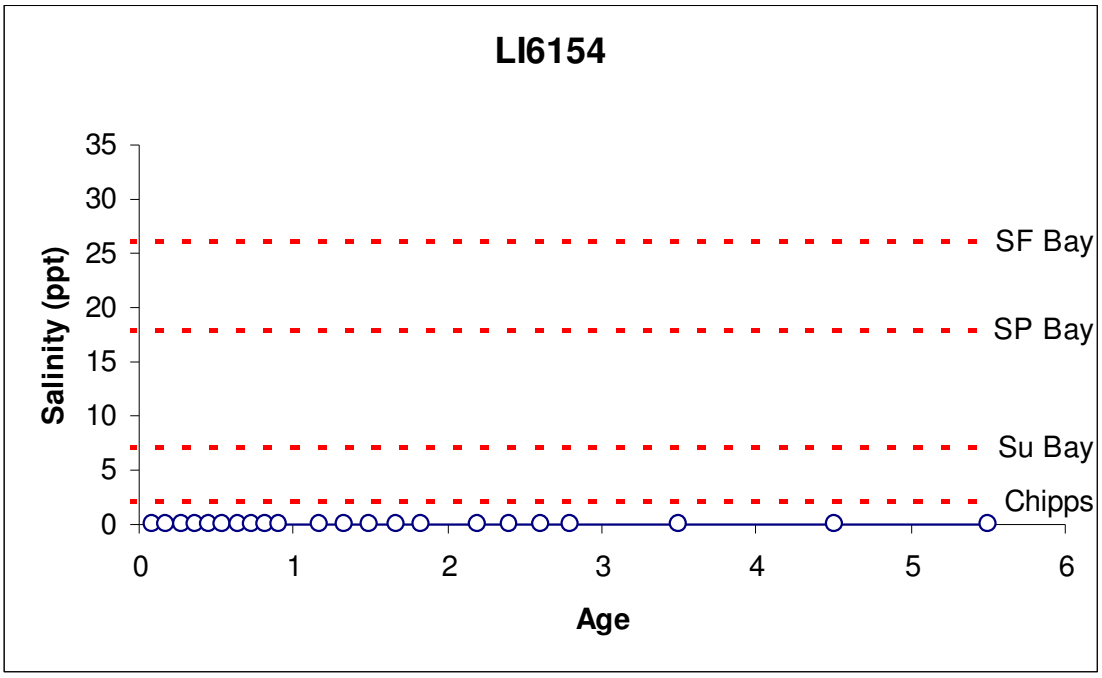


Figure 43a:
Liberty Island 6154 Male, 45.6 cm, 1.3 kg, 6 years

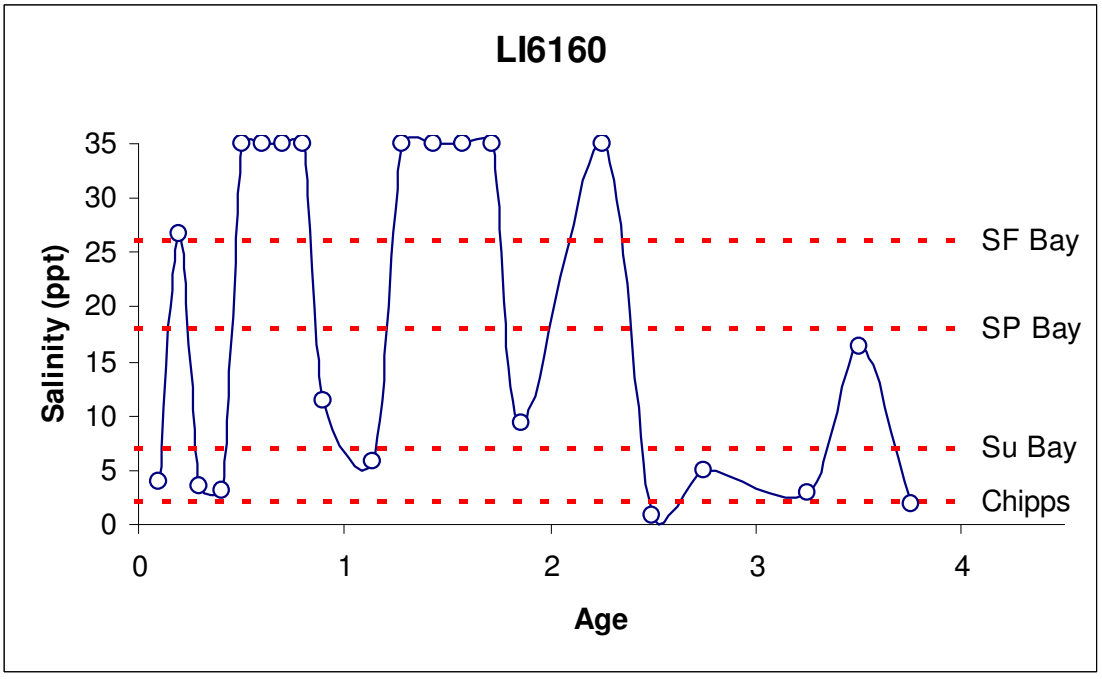


Figure 44a:
Liberty Island 6160 Female, 49.5 cm, 1.5 kg, 4 years

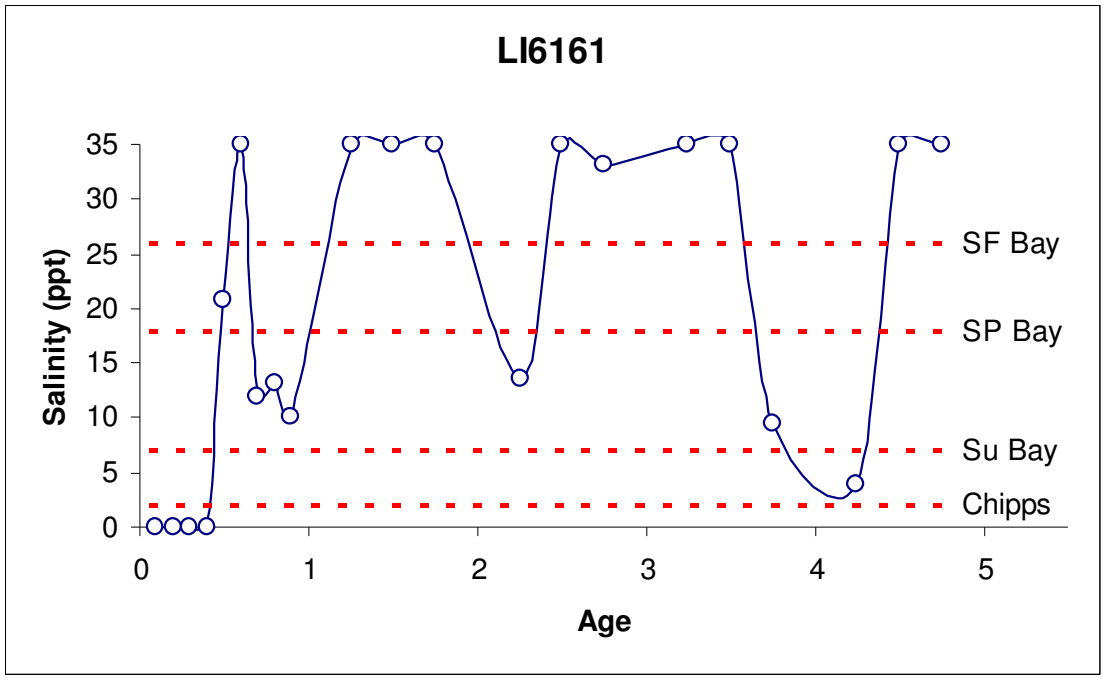


Figure 45a:
Liberty Island 6161 Female, 54.0 cm, 1.8 kg, 5 years

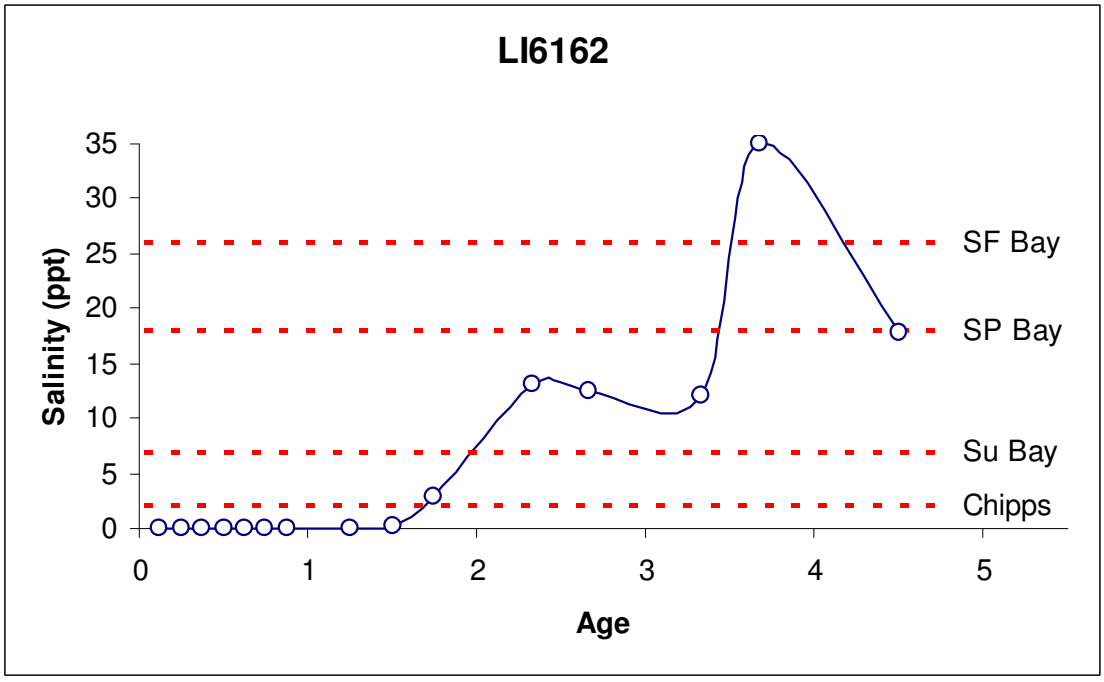


Figure 46a:
Liberty Island 6162 Female, 45.0 cm, 1.1 kg, 5 years

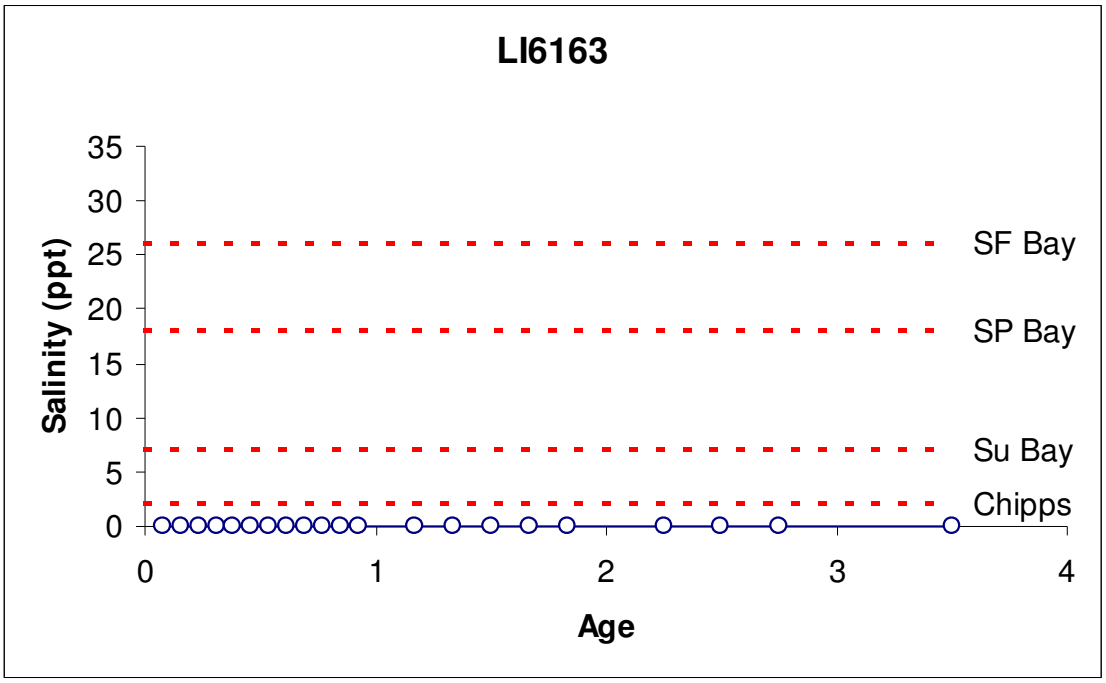


Figure 47a:
Liberty Island 6163 Male, 43.3 cm, 0.9 kg, 4 years

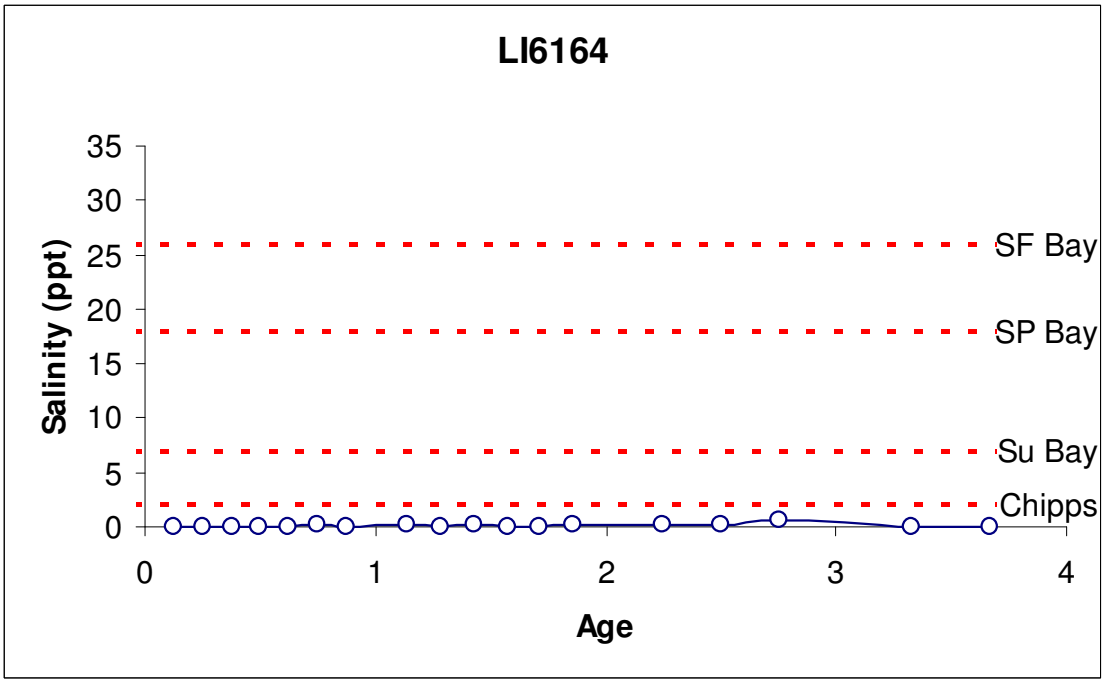


Figure 48a:
Liberty Island 6164 Female, 42.7 cm, 0.9 kg, 4 years

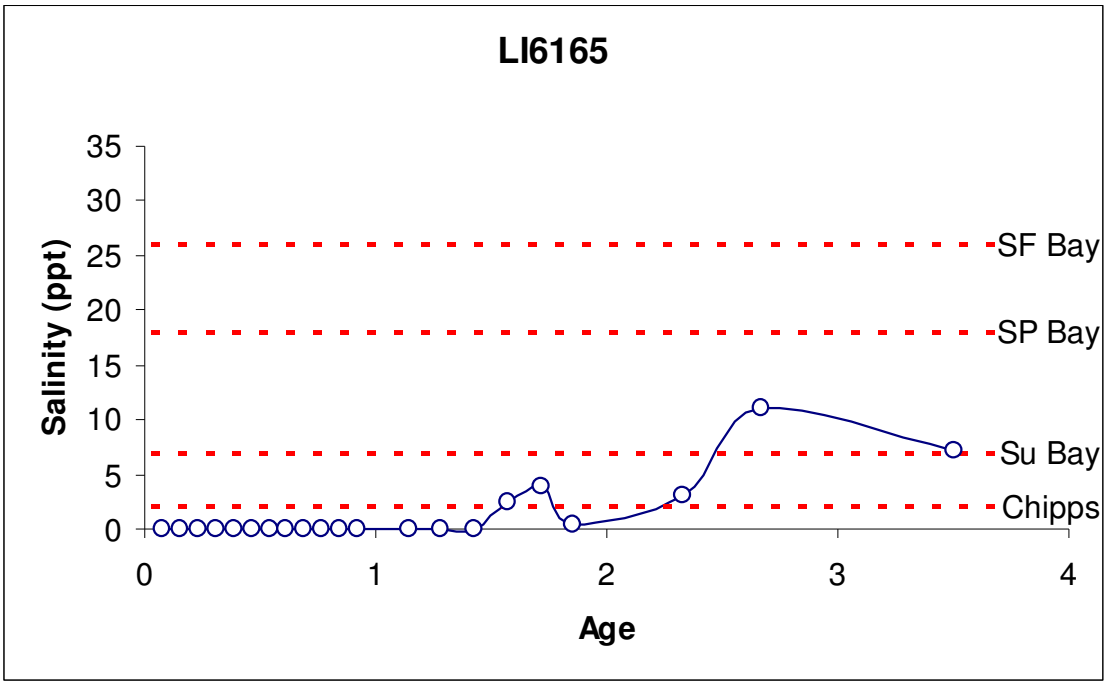


Figure 49a:
Liberty Island 6165 Male, 43.5 cm, 1.1 kg, 4 years

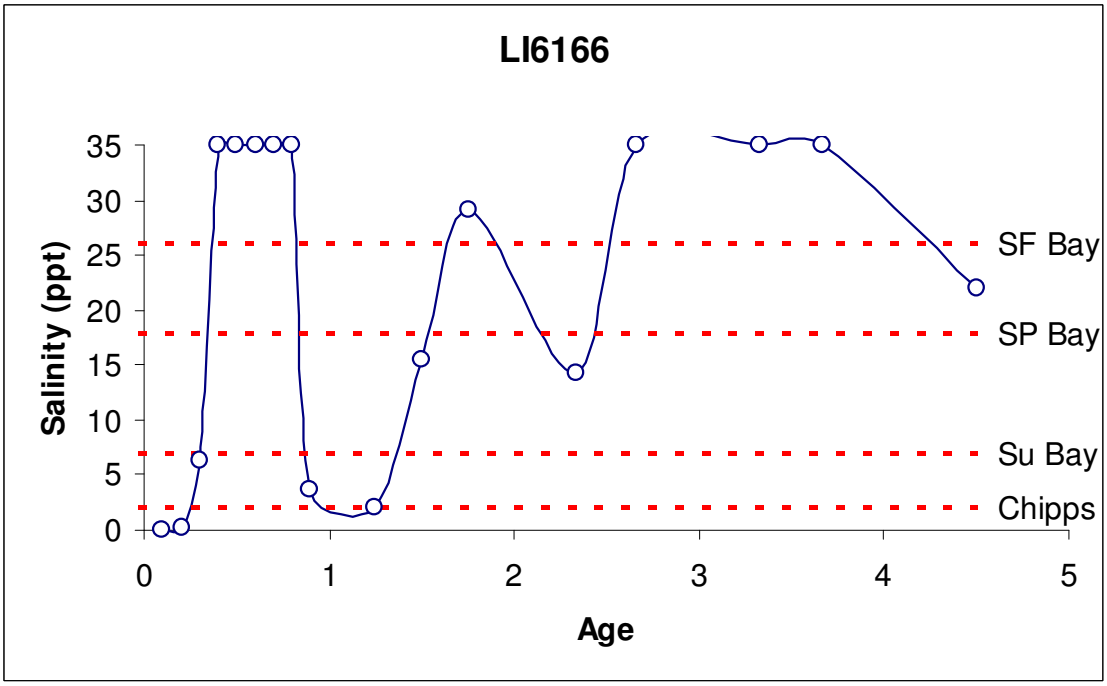


Figure 50a:
Liberty Island 6166 Female, 49.8 cm, 1.4 kg, 5 years

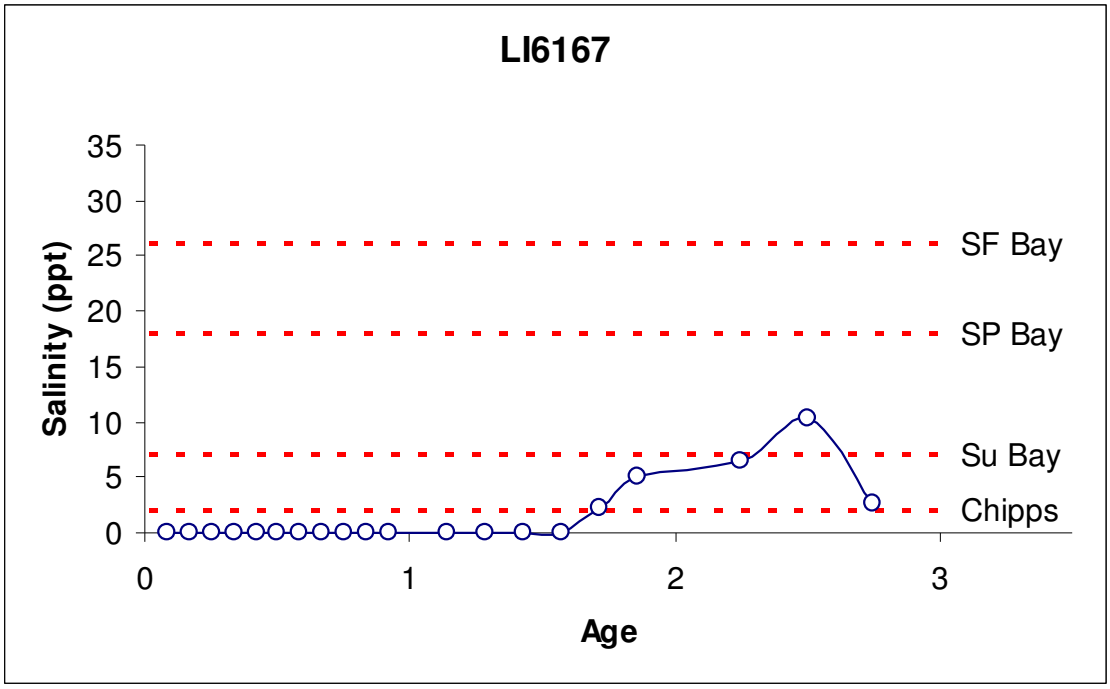


Figure 51a:
Liberty Island 6167 Female, 41.3 cm, 0.8 kg, 3 years

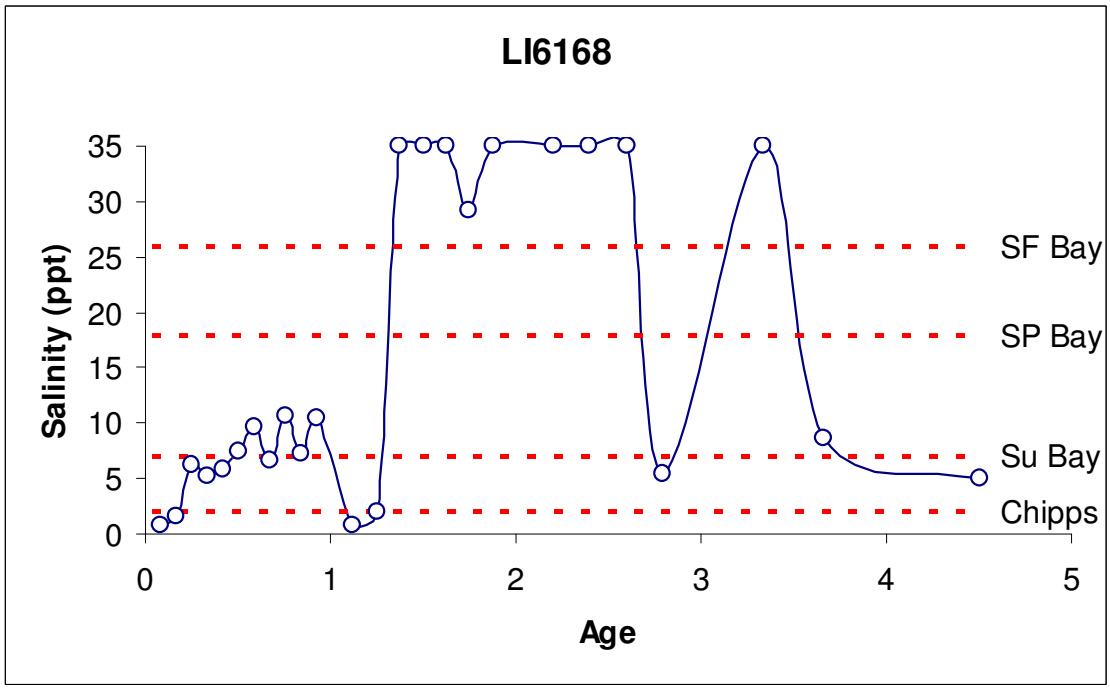


Figure 52a:
Liberty Island 6168 Male, 69.8 cm, 4.2 kg, 5 years

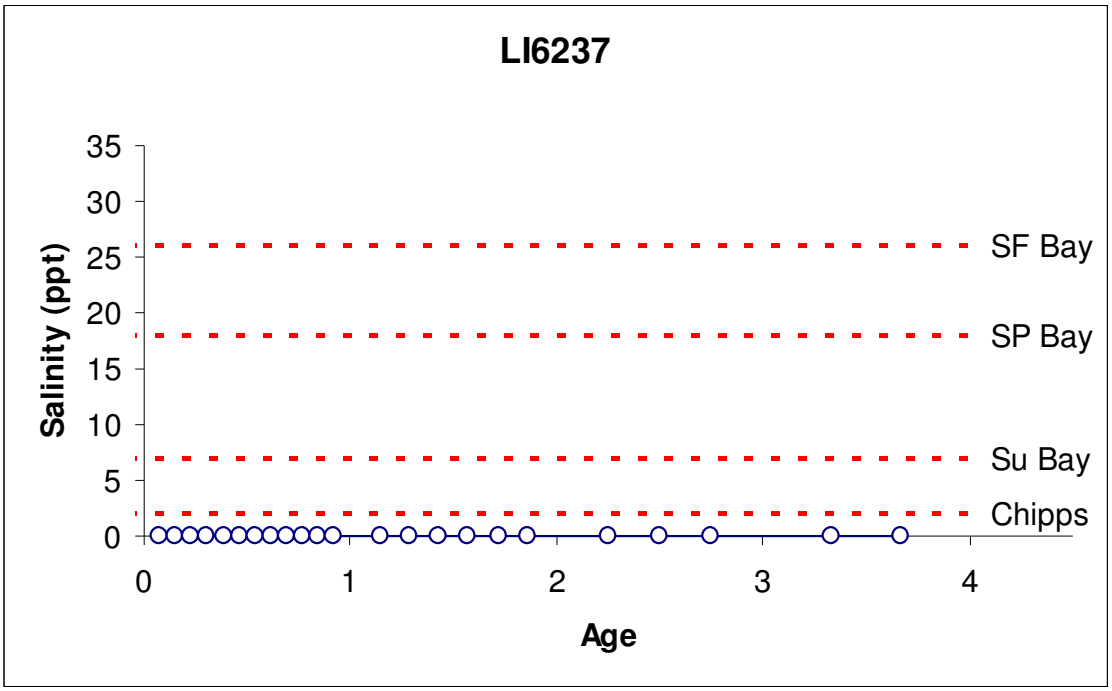


Figure 53a:
Liberty Island 6237 Male, 49.9 cm, 1.9 kg, 4 years

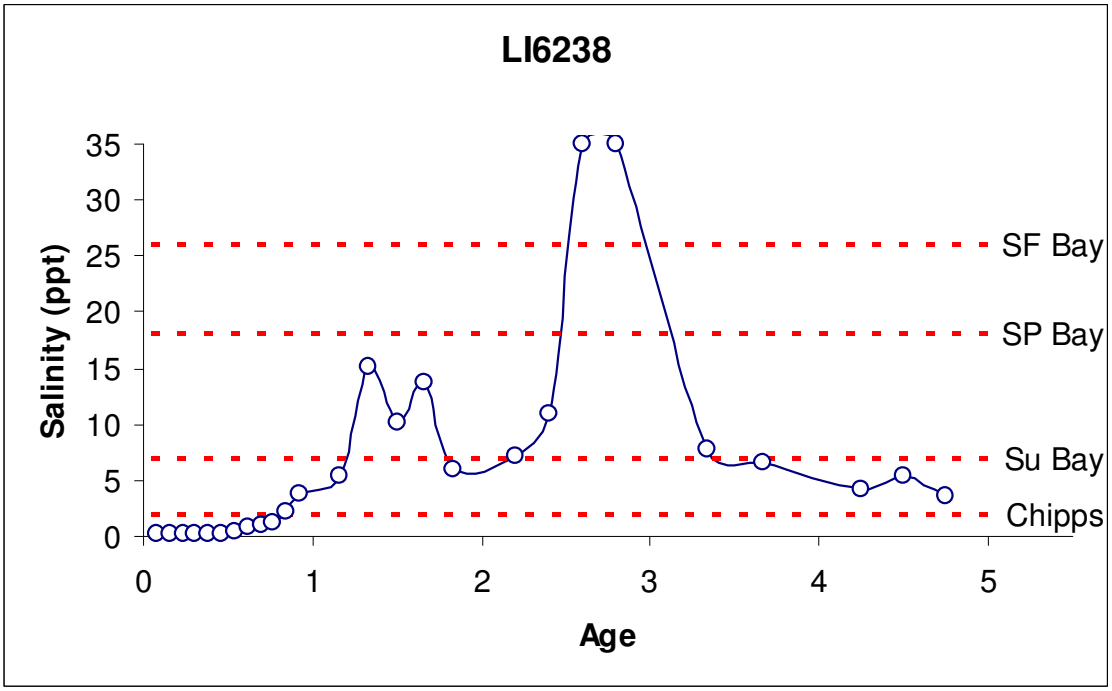


Figure 54a:
Liberty Island 6238 Male, 63.3 cm, 3.4 kg, 5 years

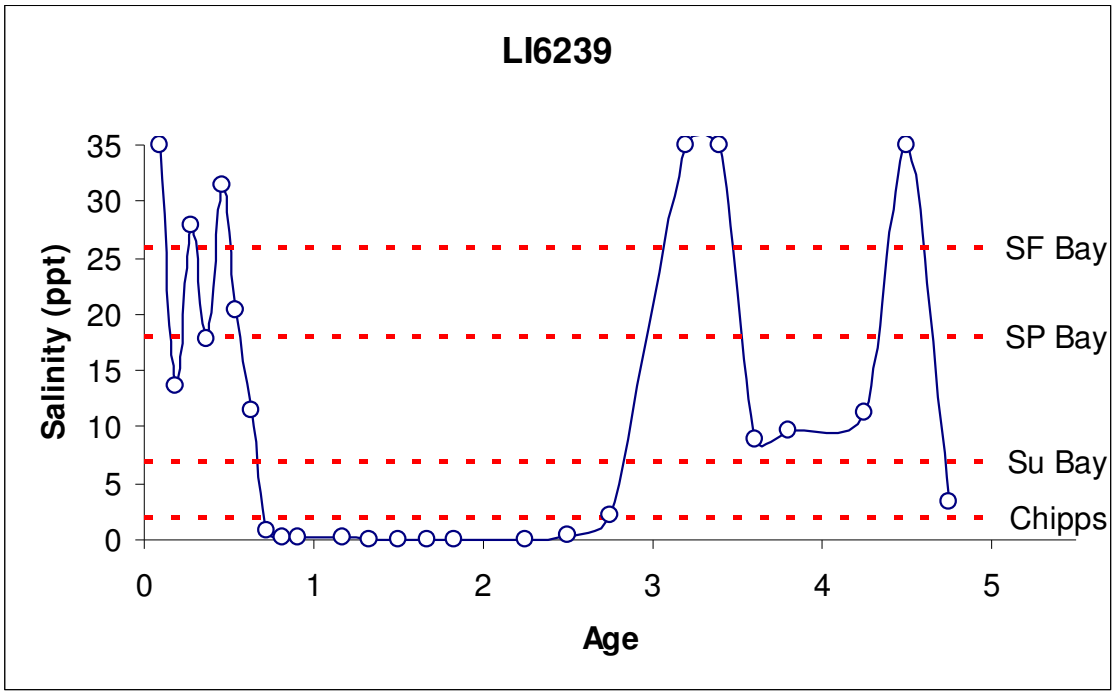


Figure 55a:
Liberty Island 6239 Male, 68.6 cm, 3.8 kg, 5 years

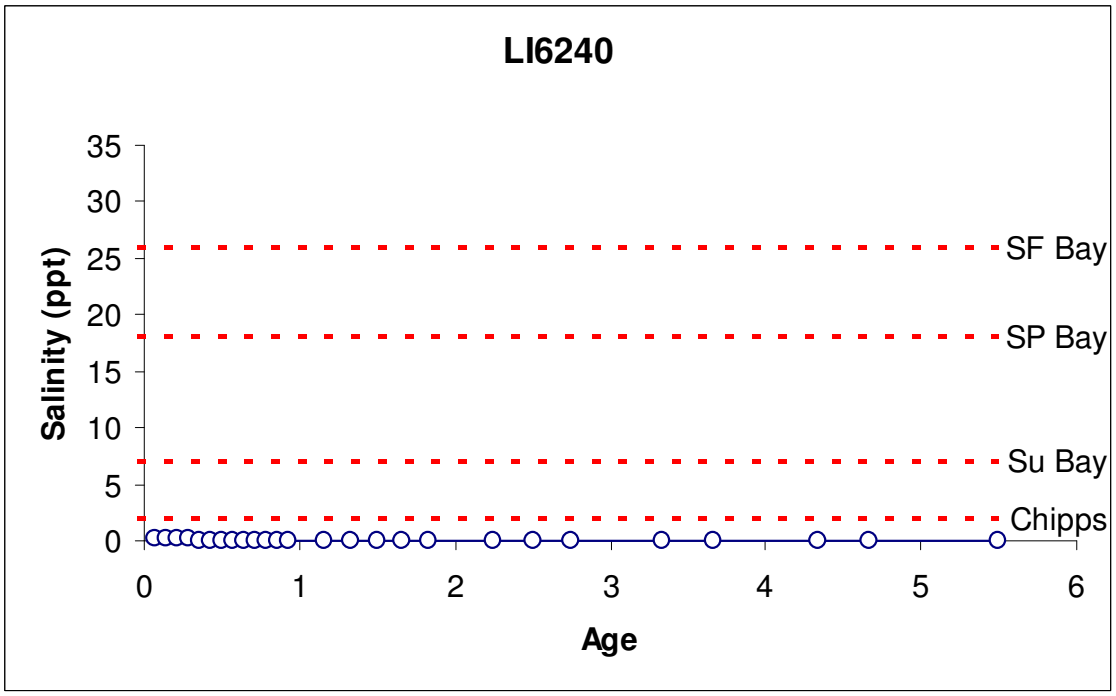


Figure 56a:
Liberty Island 6240 Male, 42.6 cm, 1.1 kg, 6 years

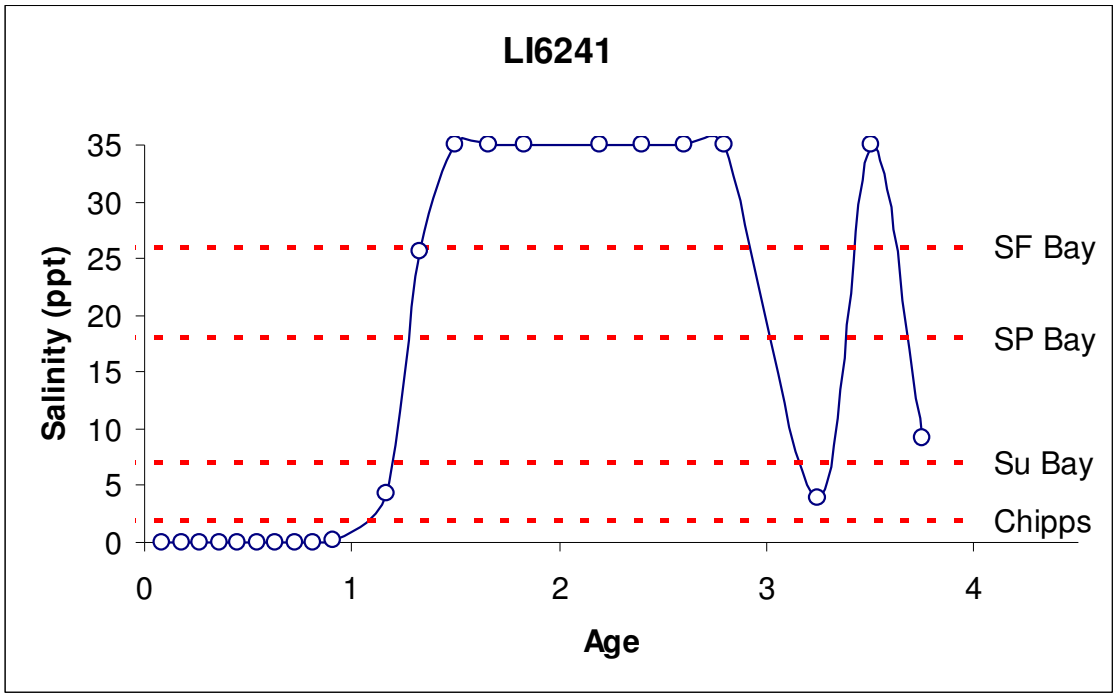


Figure 57a:
Liberty Island 6241 Male, 47.1 cm, 1.3 kg, 4 years

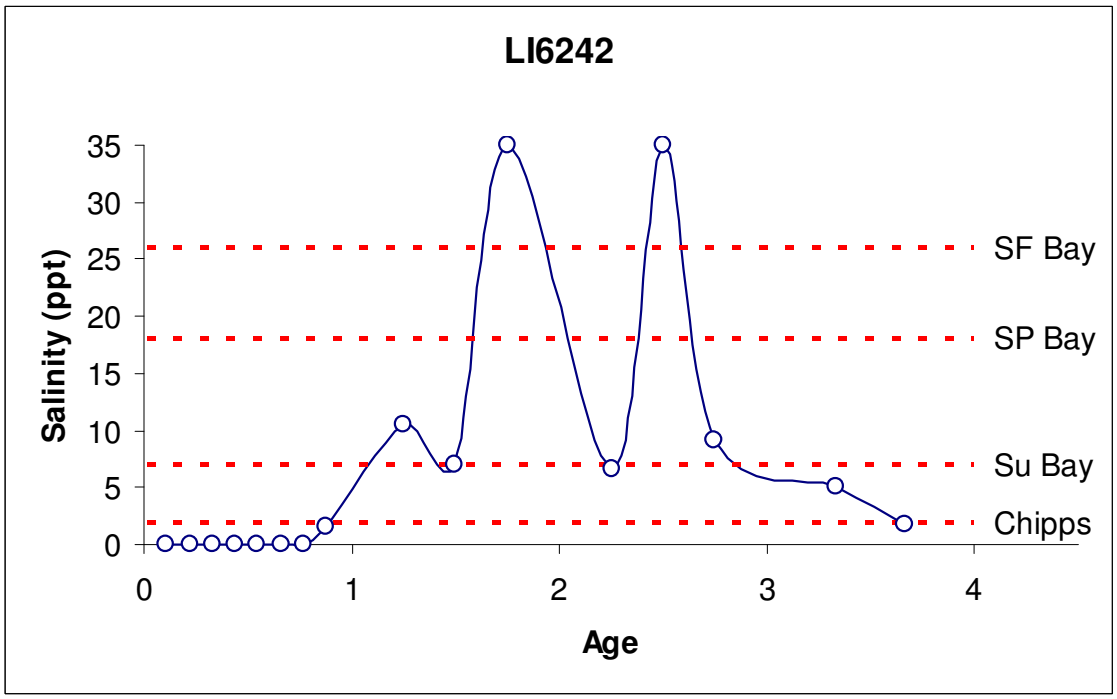


Figure 58a:
Liberty Island 6242 Female, 47.8 cm, 1.2 kg, 4 years

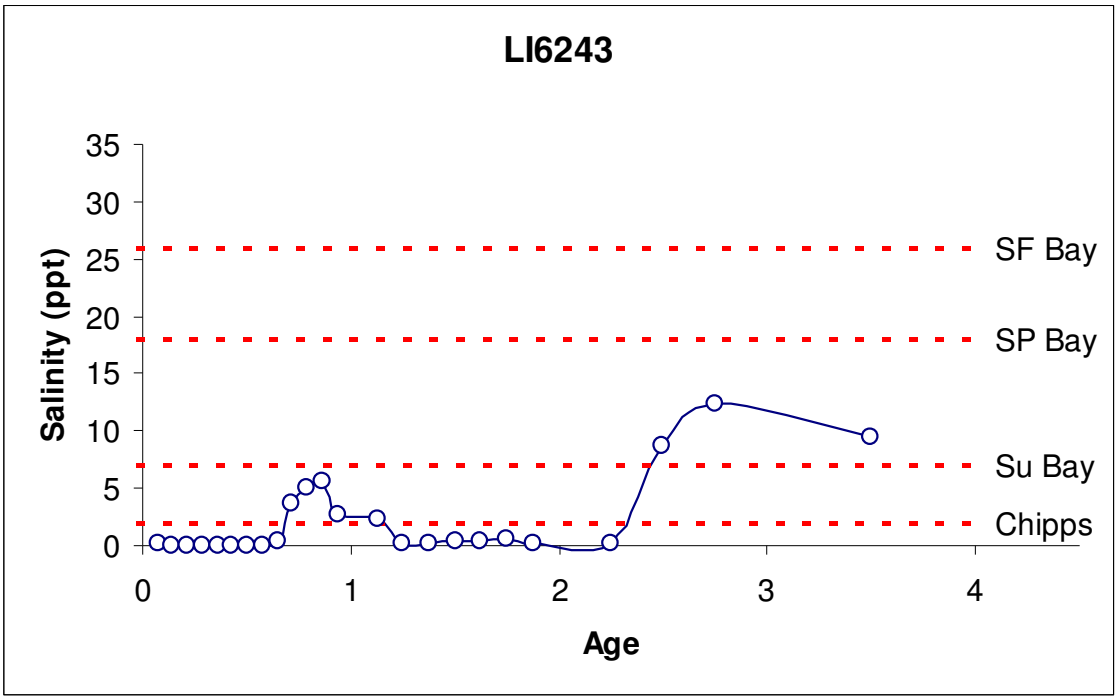


Figure 59a:
Liberty Island 6243 Female, 63.7 cm, 3.5 kg, 4 years

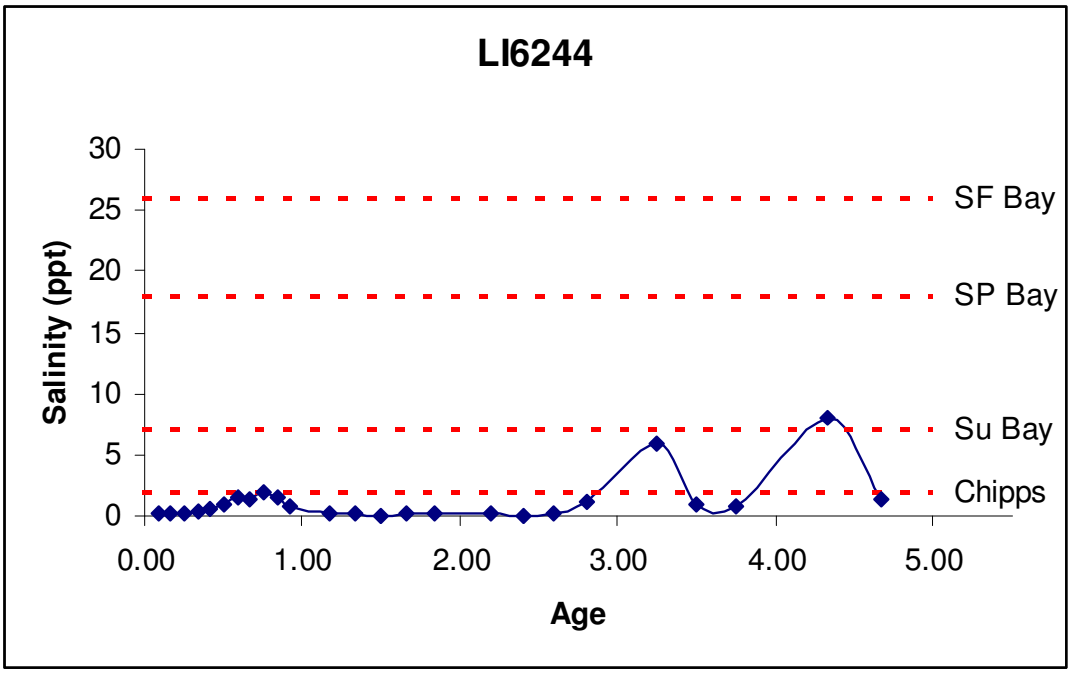


Figure 60a:
Liberty Island 6244 Male, 49.8 cm, 1.2 kg, 5 years

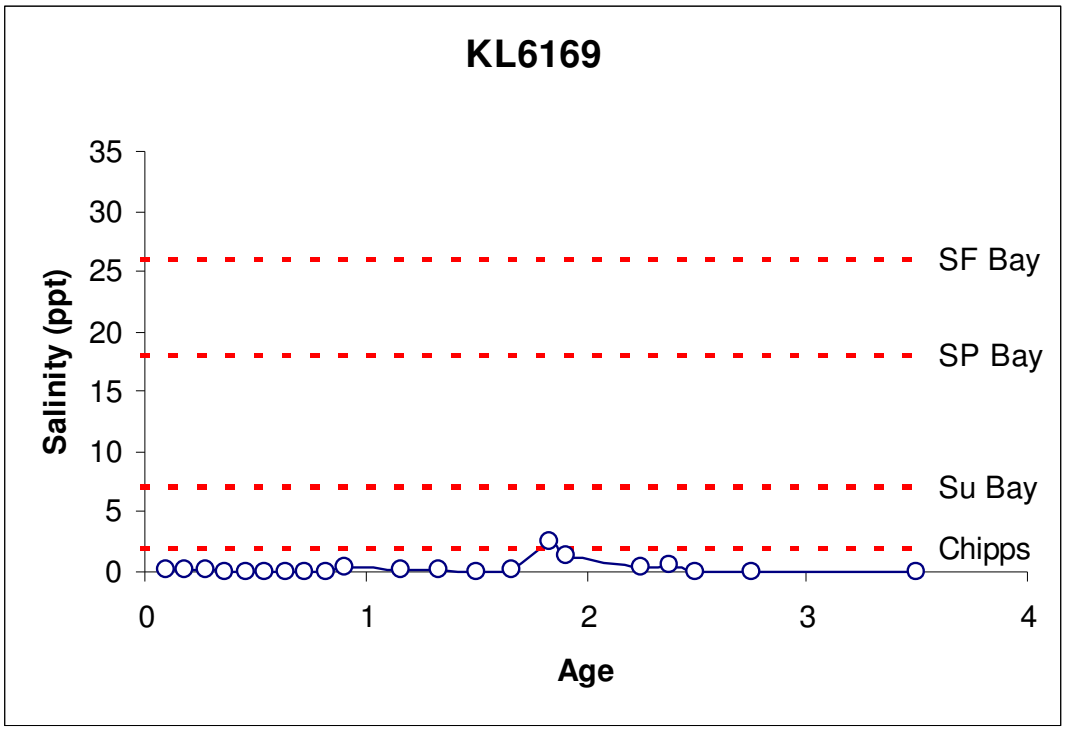


Figure 61a:
Knights Landing 6169 Male, 44.3 cm, 1.0 kg, 4 years

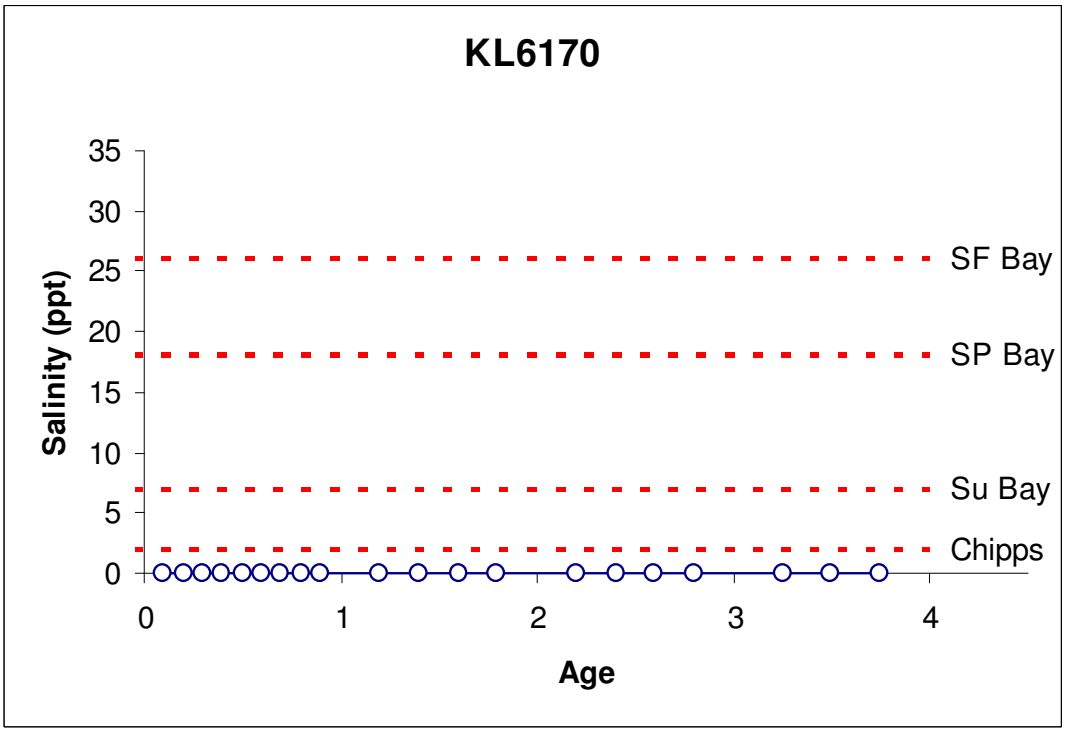


Figure 62a:
Knights Landing 6170 Male, 44.7 cm, 1.2 kg, 4 years

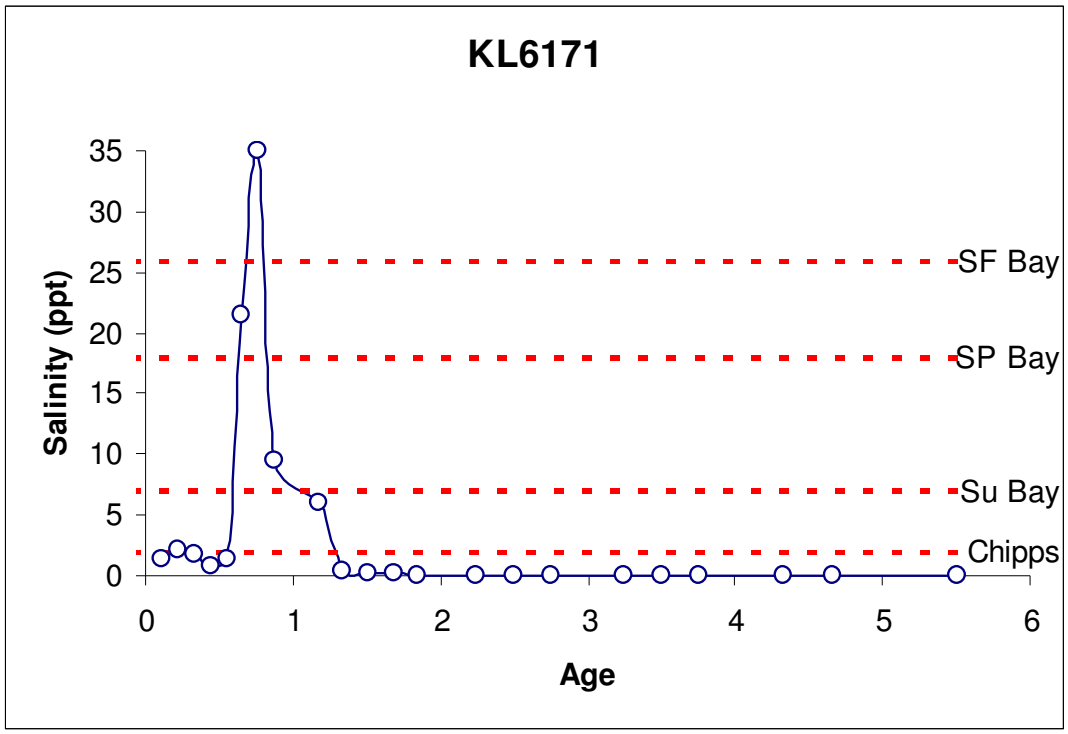


Figure 63a:
Knights Landing 6171 Male, 54.4 cm, 2.4 kg, 6 years

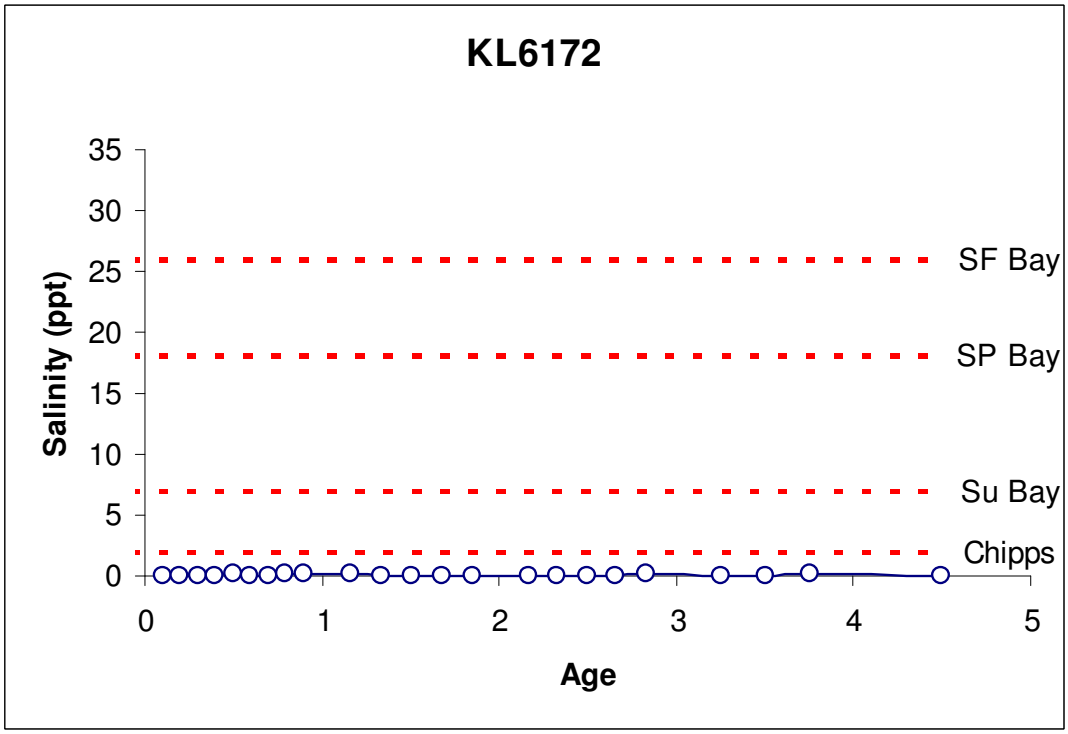


Figure 64a:
Knights Landing 6172 Male, 43.4 cm, 1.0 kg, 5 years

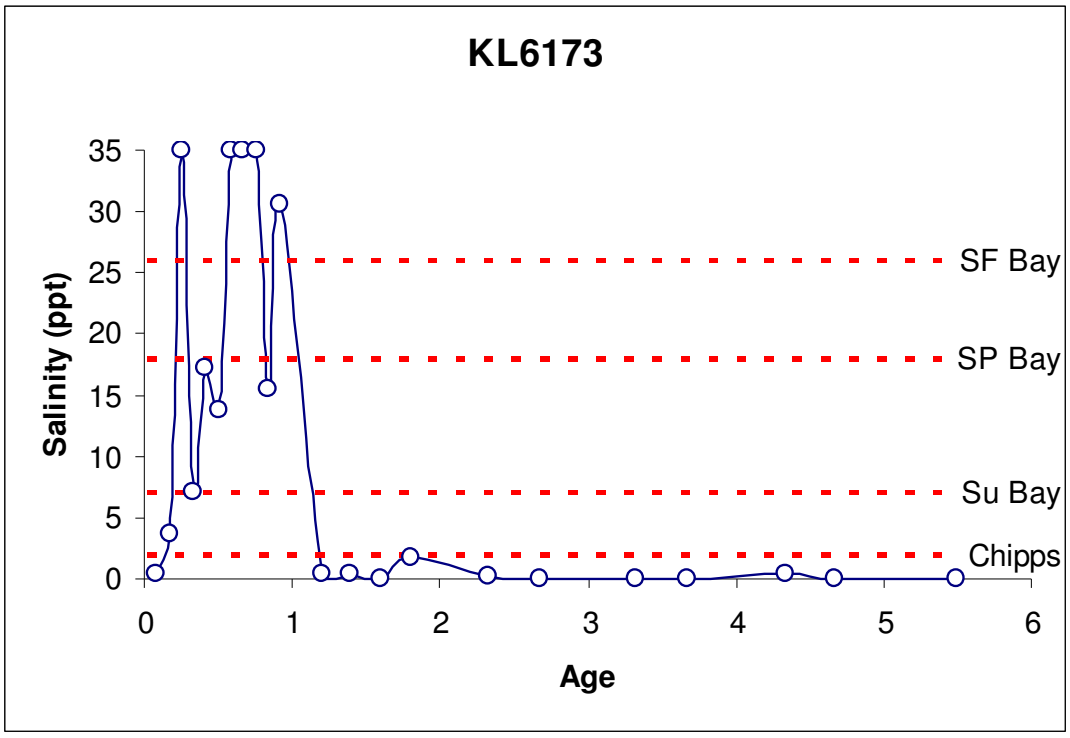


Figure 65a:
Knights Landing 6173 Male, 45.9 cm, 1.3 kg, 6 years

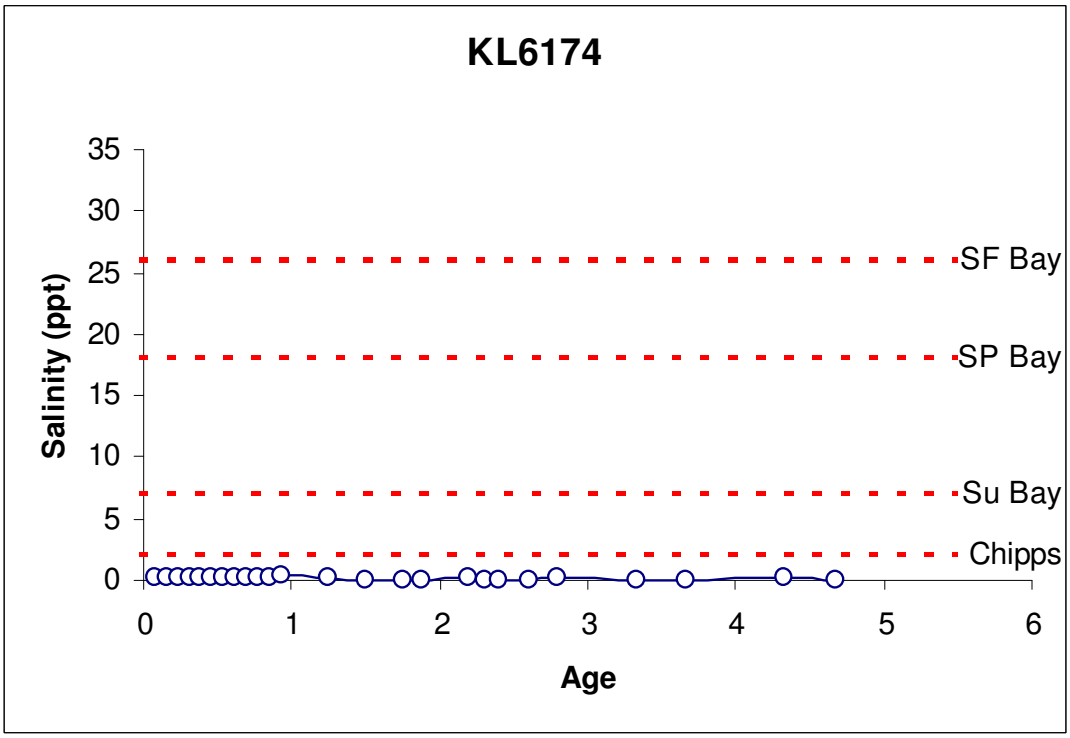


Figure 66a:
Knights Landing 6174 Male, 47.1 cm, 1.6 kg, 5 years

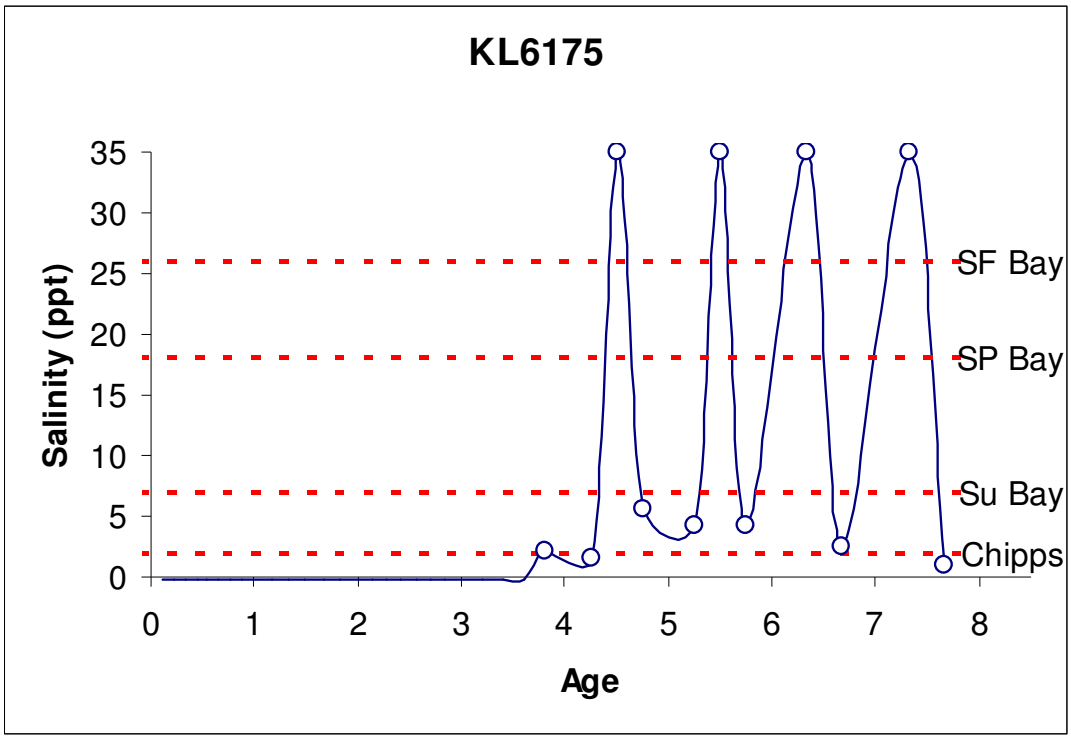


Figure 67a:
Knights Landing 6175 Female, 79.8 cm, 5.7 kg, 8 years

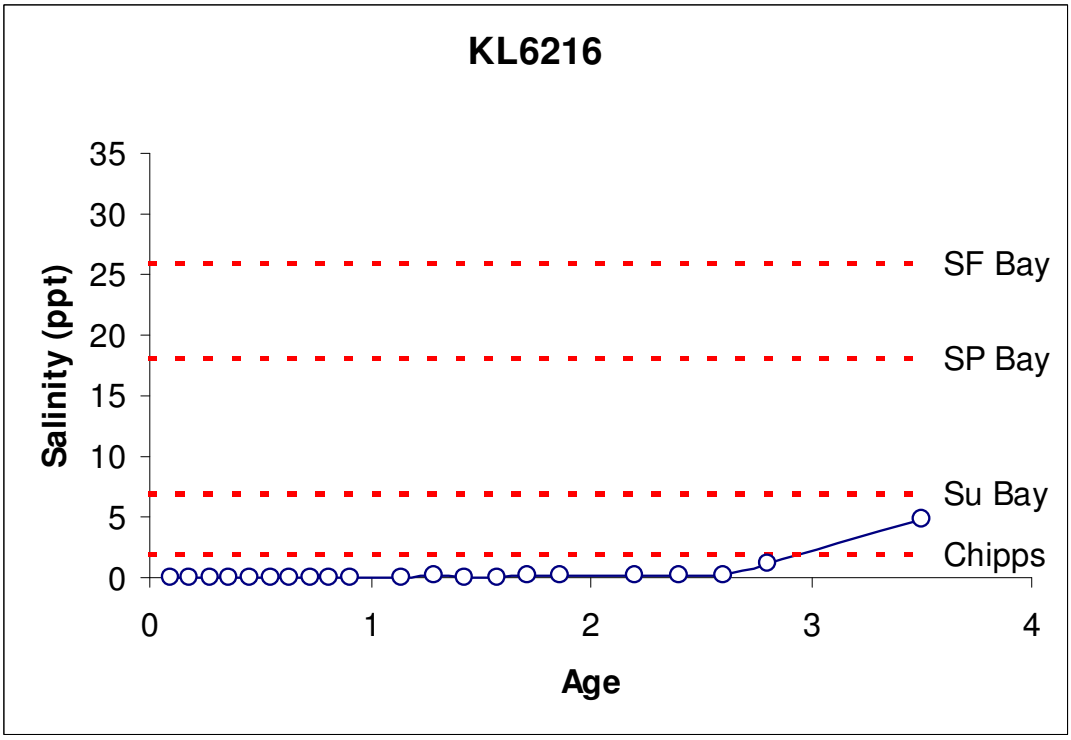


Figure 68a:
Knights Landing 6216 Female, 56.1 cm, 2.2 kg, 4 years

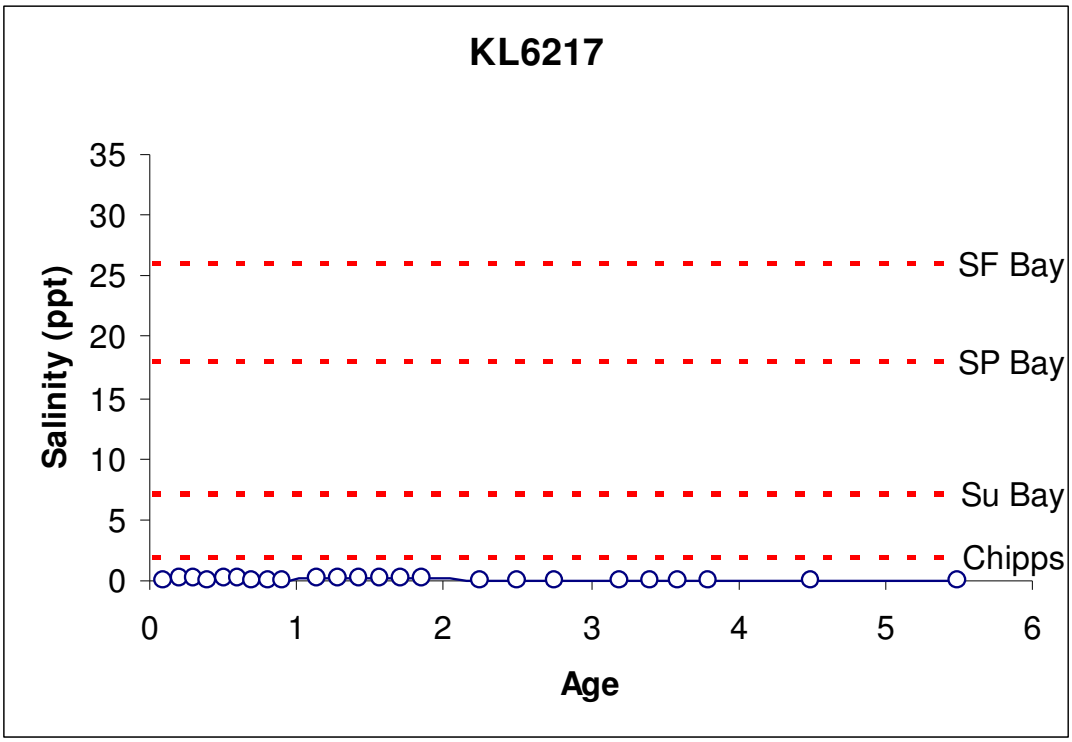


Figure 69a:
Knights Landing 6217 Male, 49.3 cm, 1.8 kg, 6 years

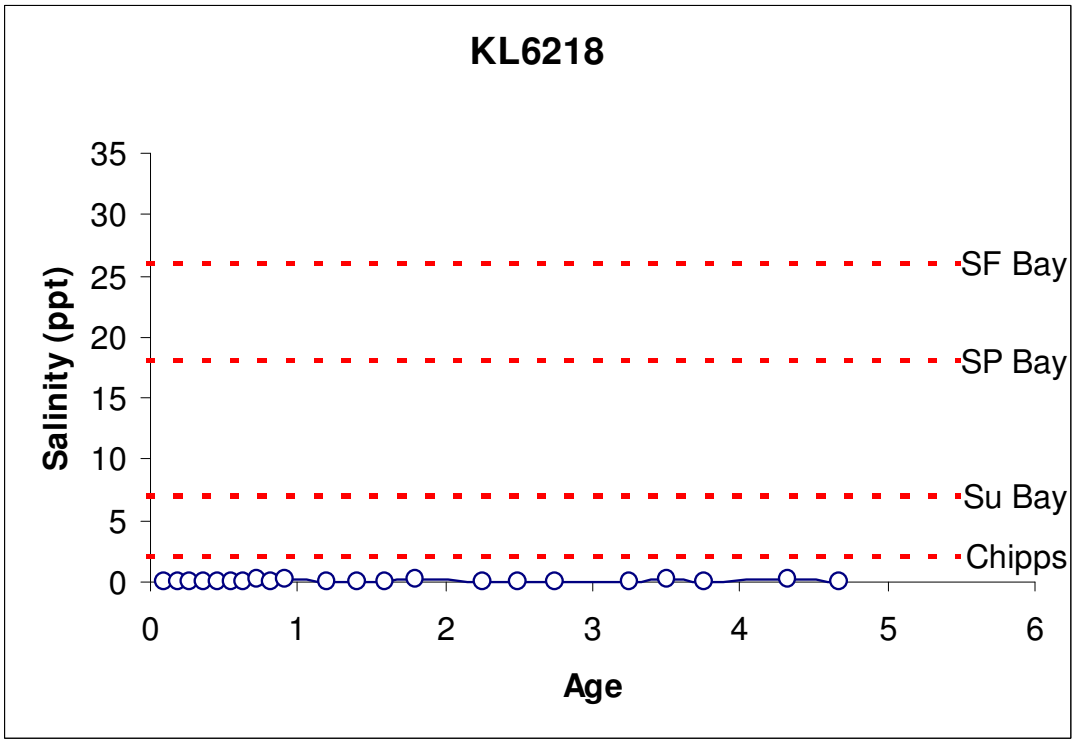


Figure 70a:
Knights Landing 6218 Male, 43.8 cm, 1.2 kg, 5 years

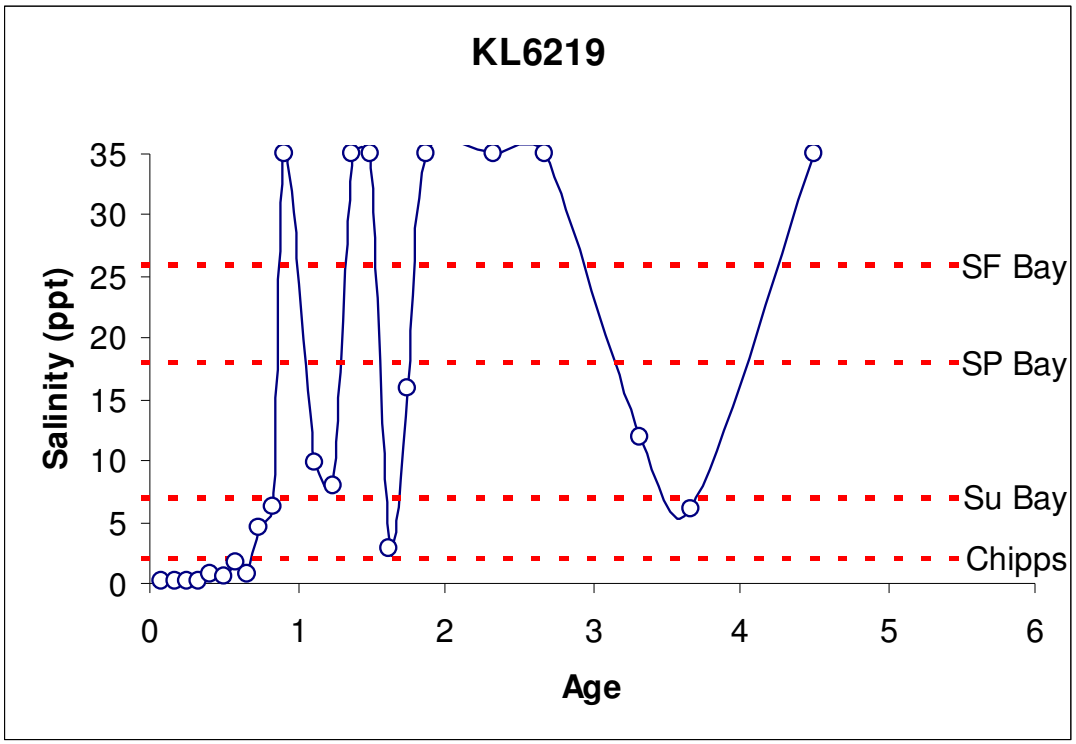


Figure 71a:
Knights Landing 6219 Male, 67.3 cm, 4.4 kg, 5 years

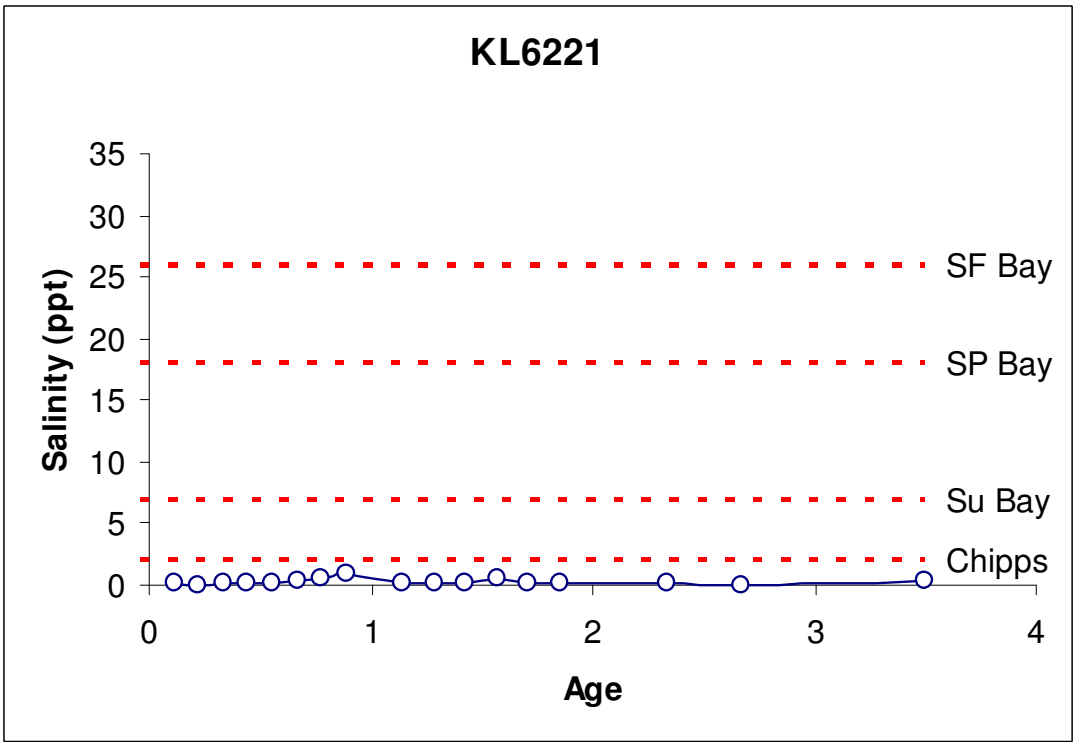


Figure 72a:
Knights Landing 6221 Male, 49.4 cm, 1.4 kg, 4 years

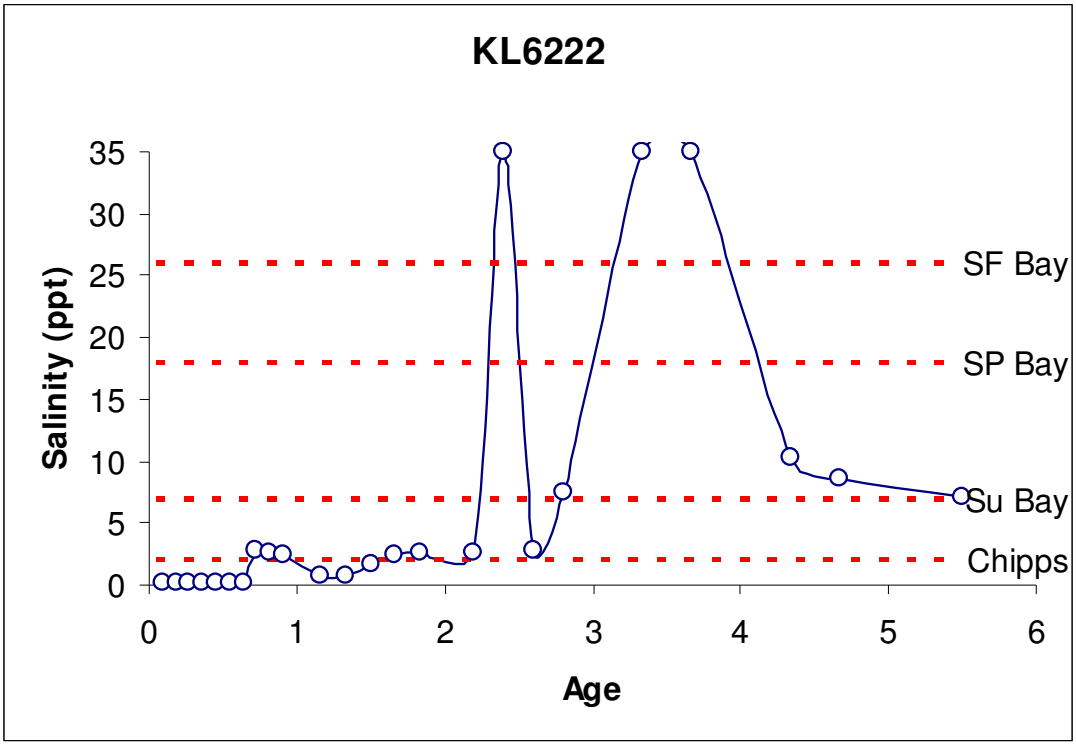


Figure 73a:
Knights Landing 6222 Male, 62.2 cm, 2.9 kg, 6 years

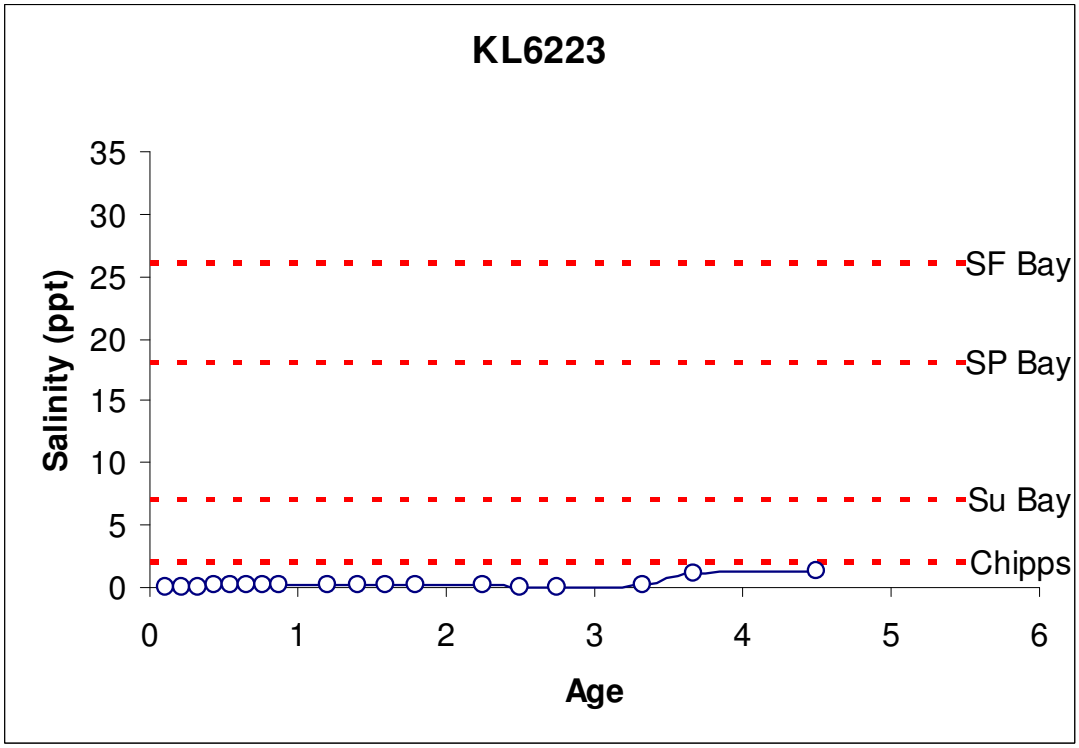


Figure 74a:
Knights Landing 6223 Male, 60.0 cm, 2.7 kg, 5 years

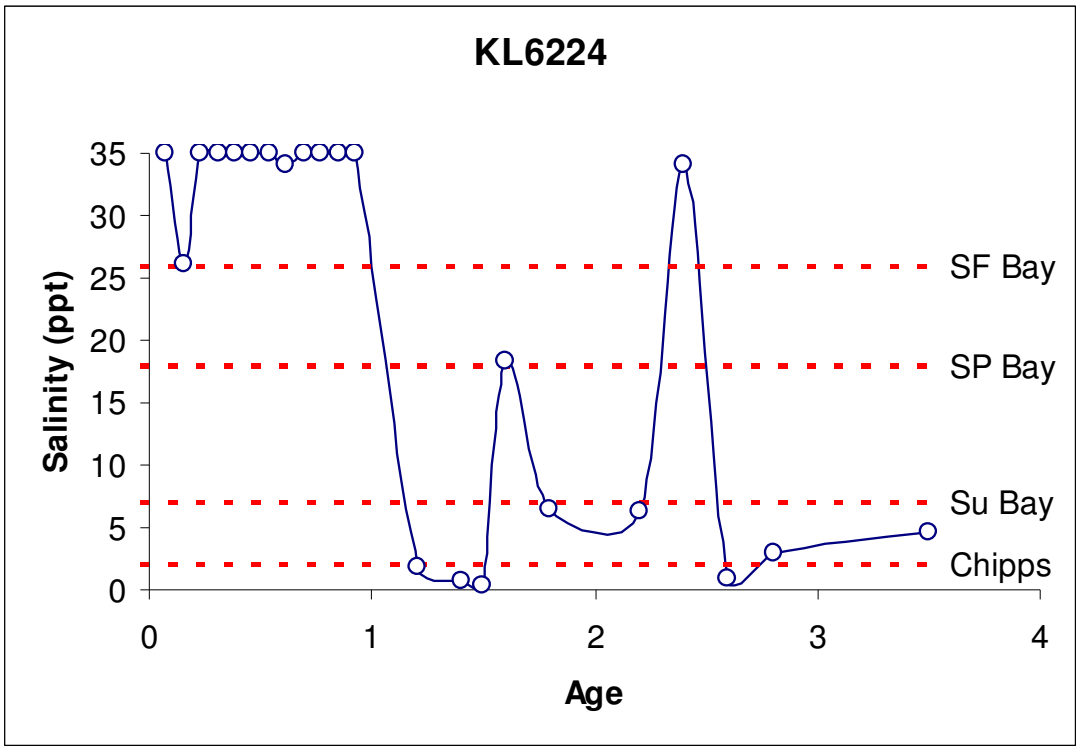


Figure 75a:
Knights Landing 6224 Female, 47.0 cm, 1.4 kg, 4 years

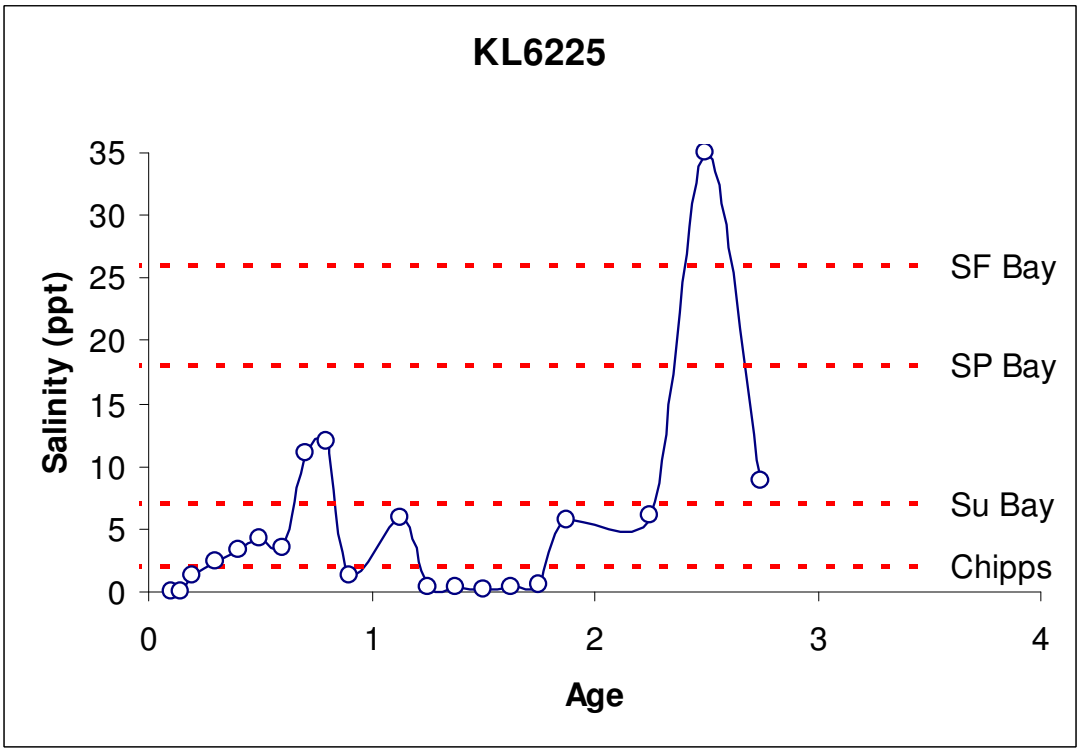


Figure 76a:
Knights Landing 6225 Male, 43.5 cm, 1.1 kg, 3 years

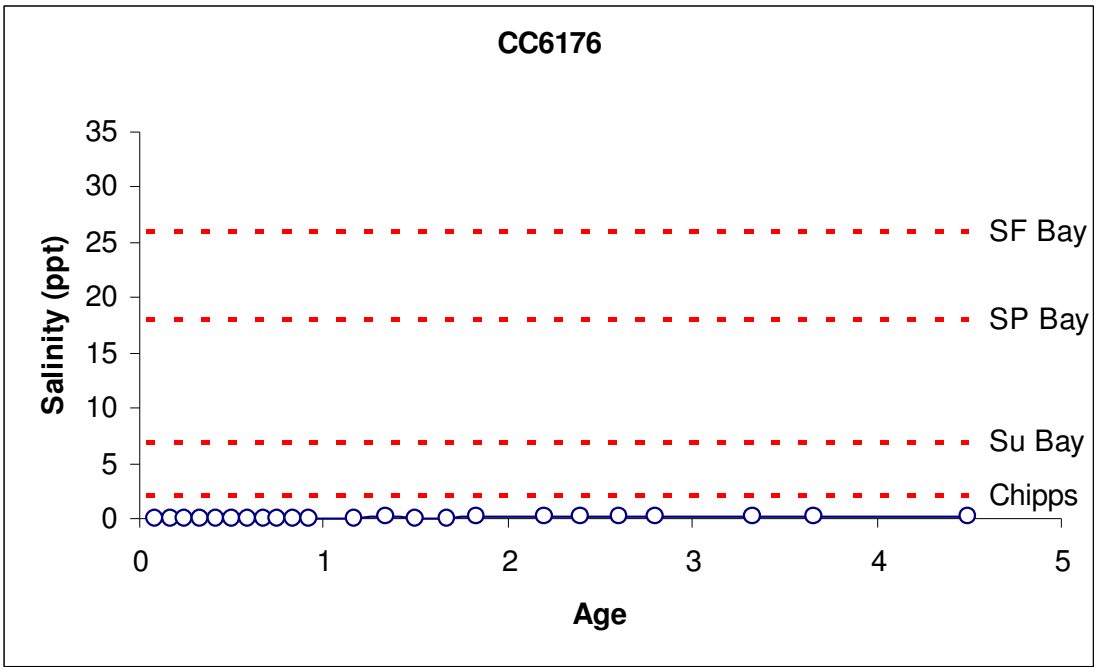


Figure 77a:
Clifton Court 6176, 44.6 cm, 1.5 kg, 5 years

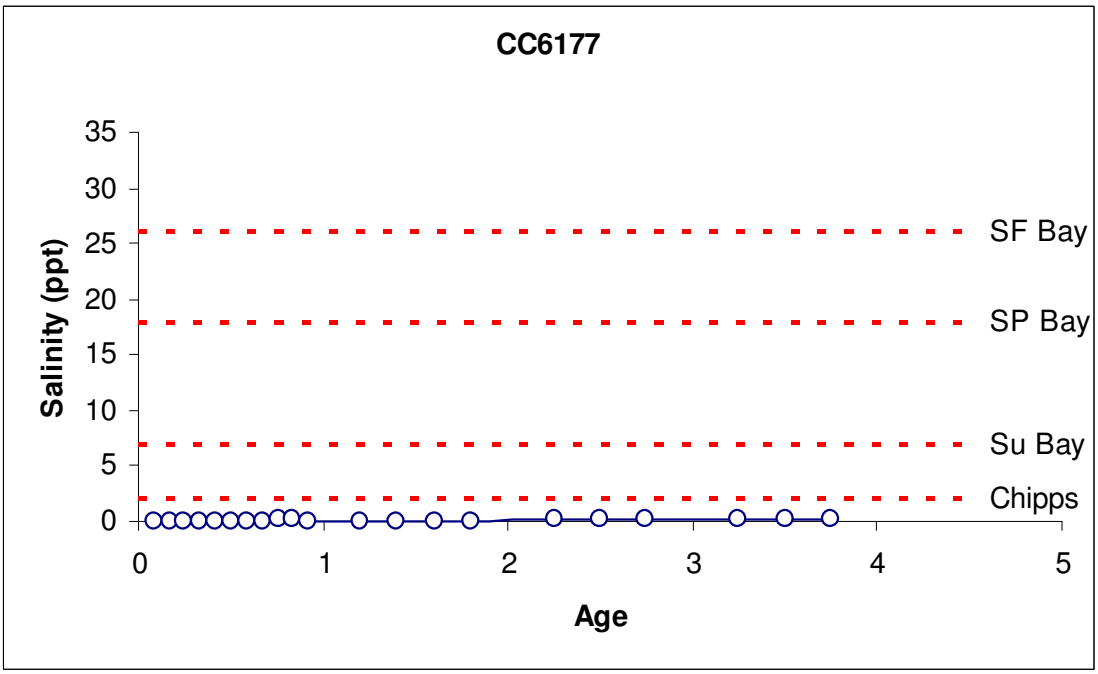


Figure 78a:
Clifton Court 6177, 46.3 cm, 1.4 kg, 4 years

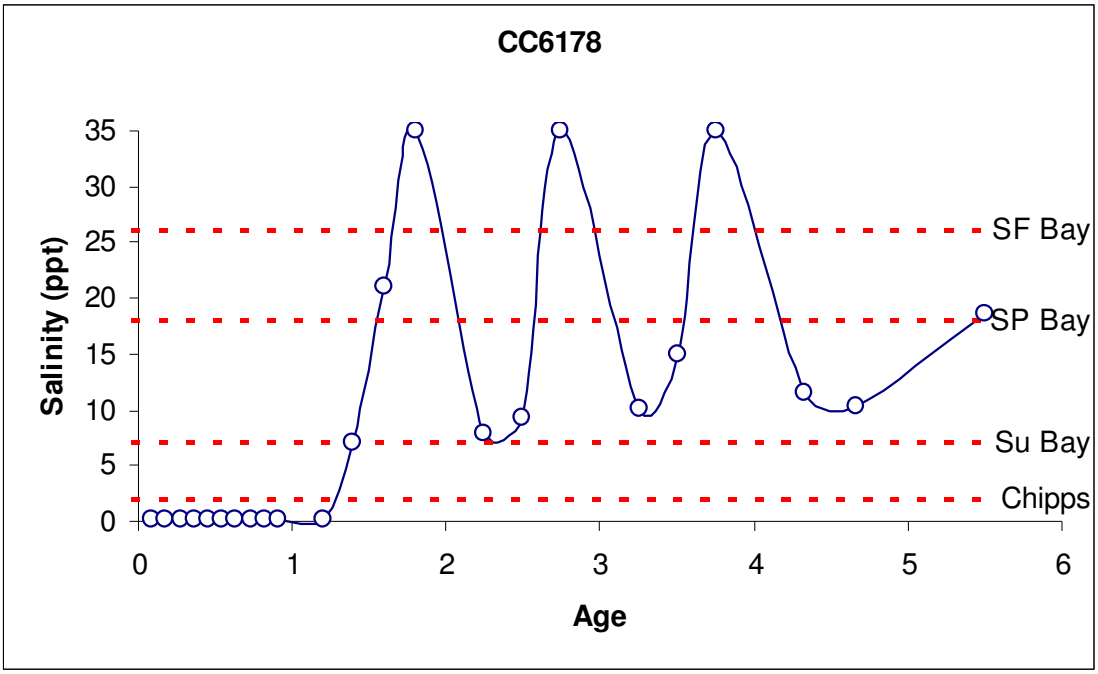


Figure 79a:
Clifton Court 6178 Female, 69.1 cm, 4.1 kg, 6 years

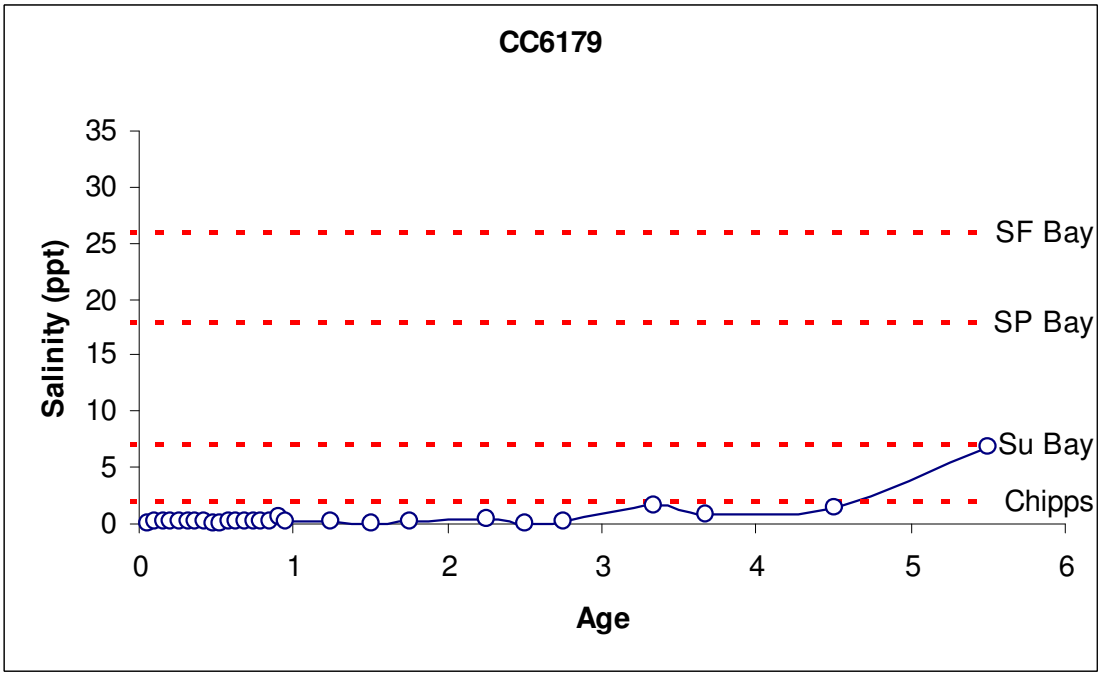


Figure 80a:
Clifton Court 6179 Female, 66.9 cm, 4.2 kg, 6 years

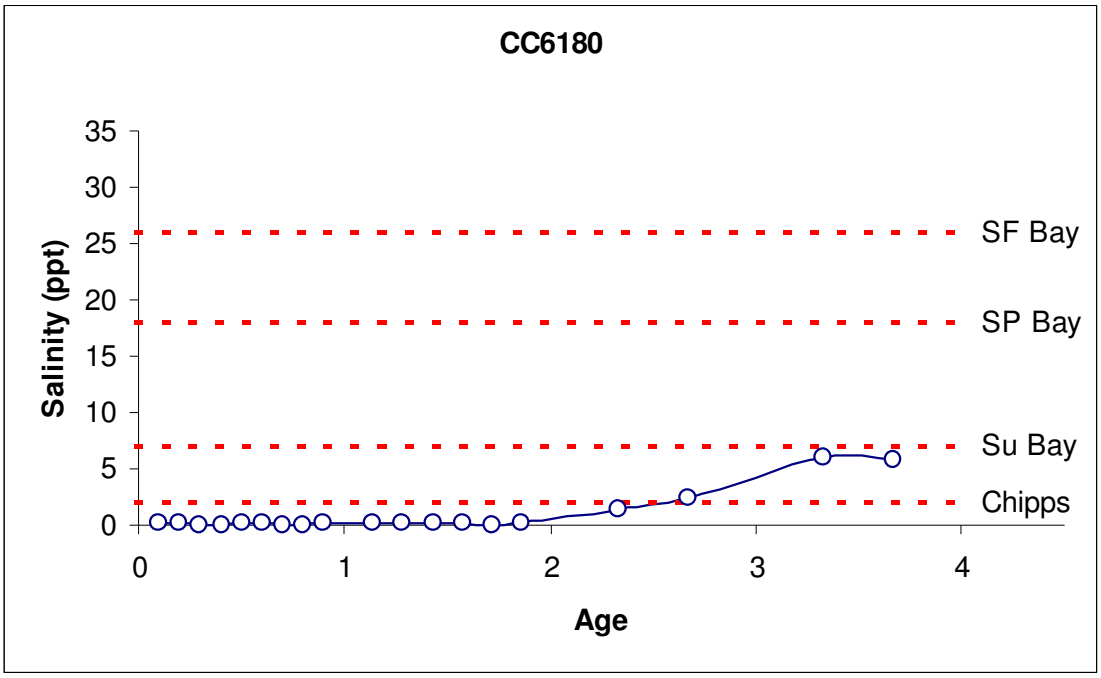


Figure 81a:
Clifton Court 6180 Male, 44.3 cm, 1.2 kg, 4 years

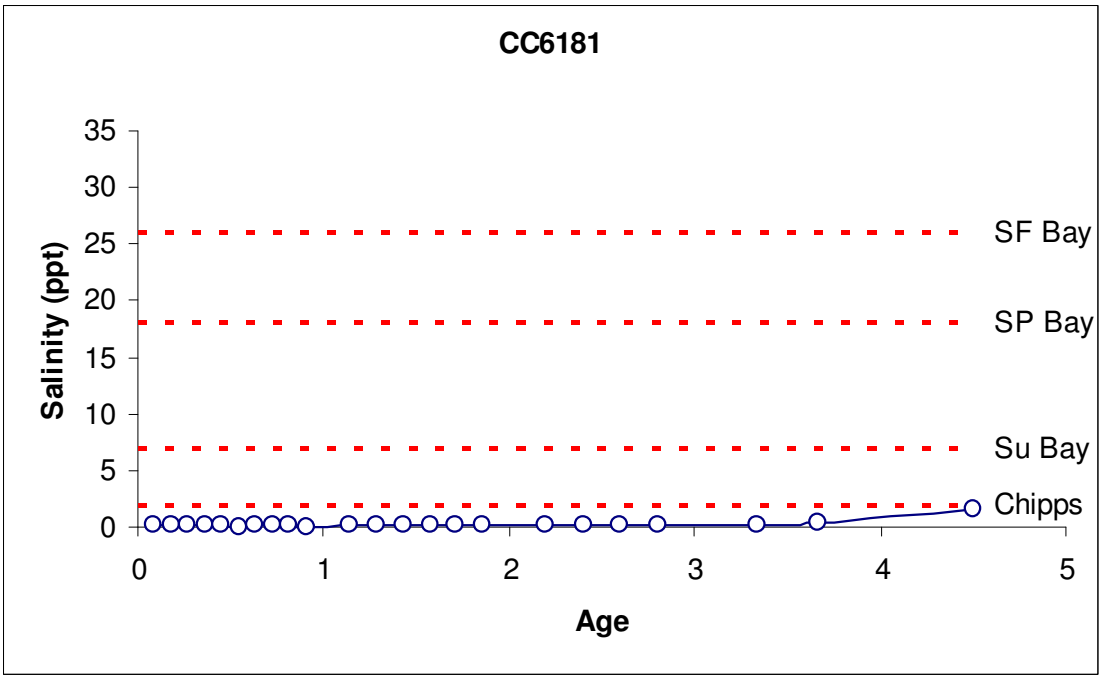


Figure 82a:
Clifton Court 6181 Female, 60.1 cm, 2.3 kg, 5 years

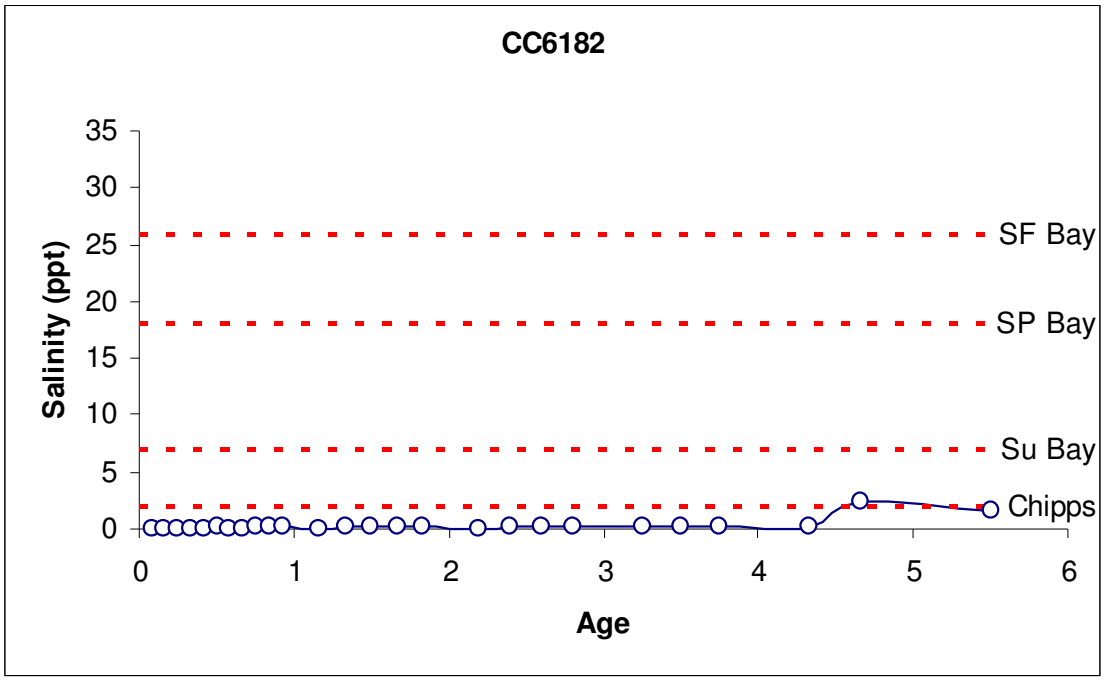


Figure 83a:
Clifton Court 6182 Female, 65.9 cm, 3.6 kg, 6 years

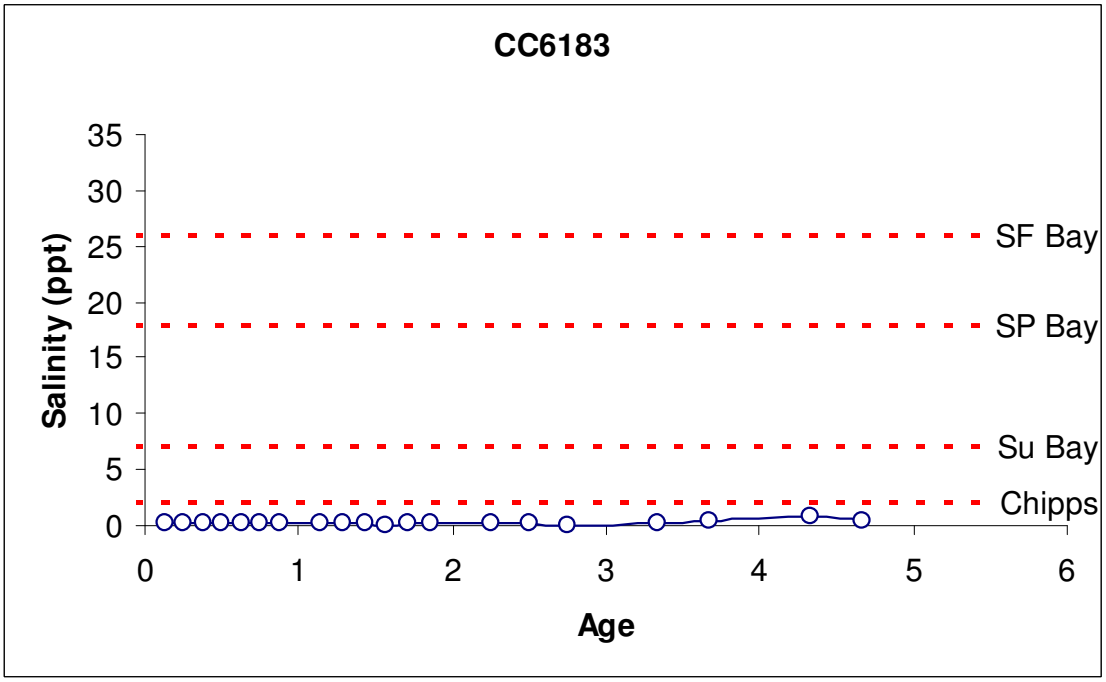


Figure 84a:
Clifton Court 6183 Male, 48.3 cm, 1.3 kg, 5 years

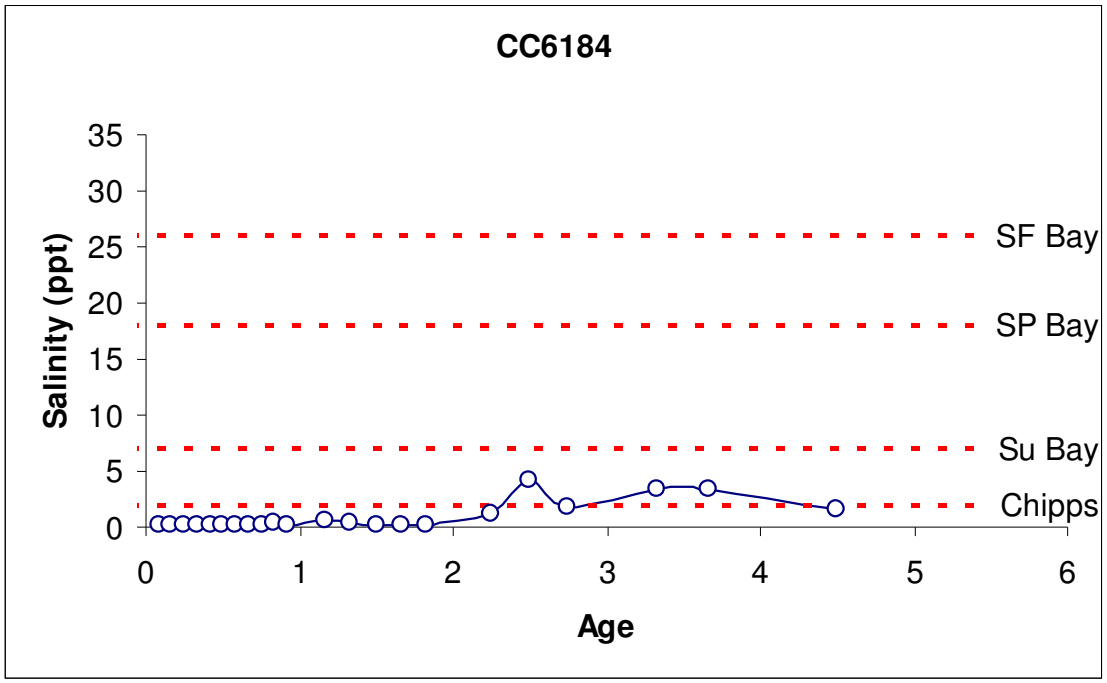


Figure 85a:
Clifton Court 6184 Female, 68.6 cm, 1.6 kg, 5 years

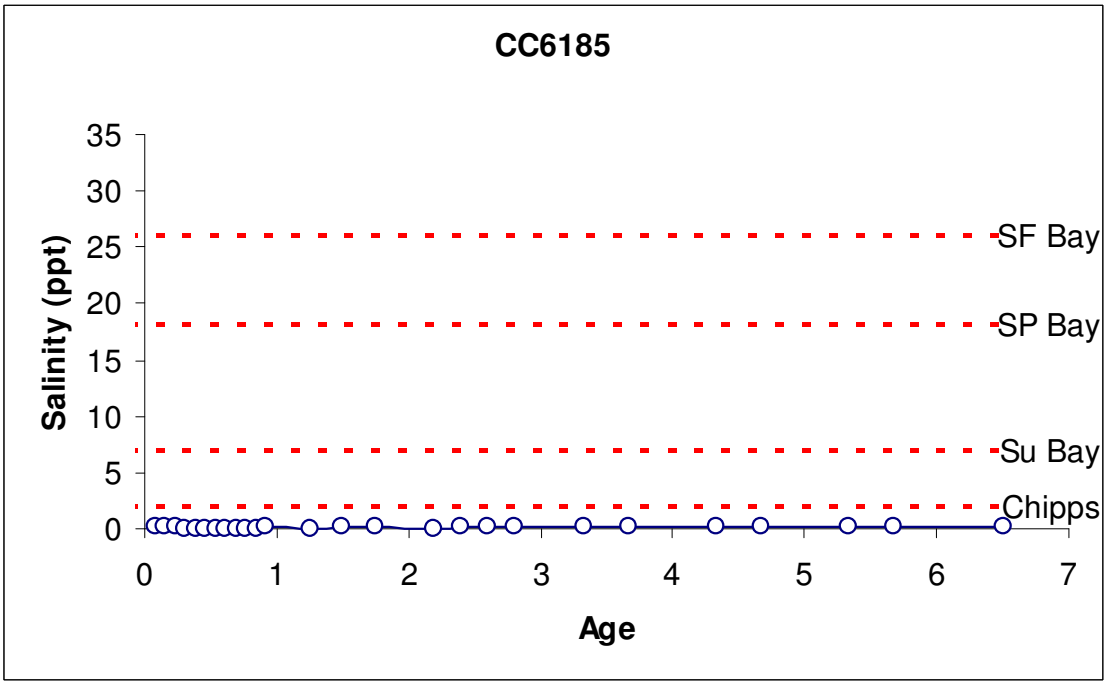


Figure 86a:
Clifton Court 6185 Female, 67.8 cm, 4.6 kg, 7 years

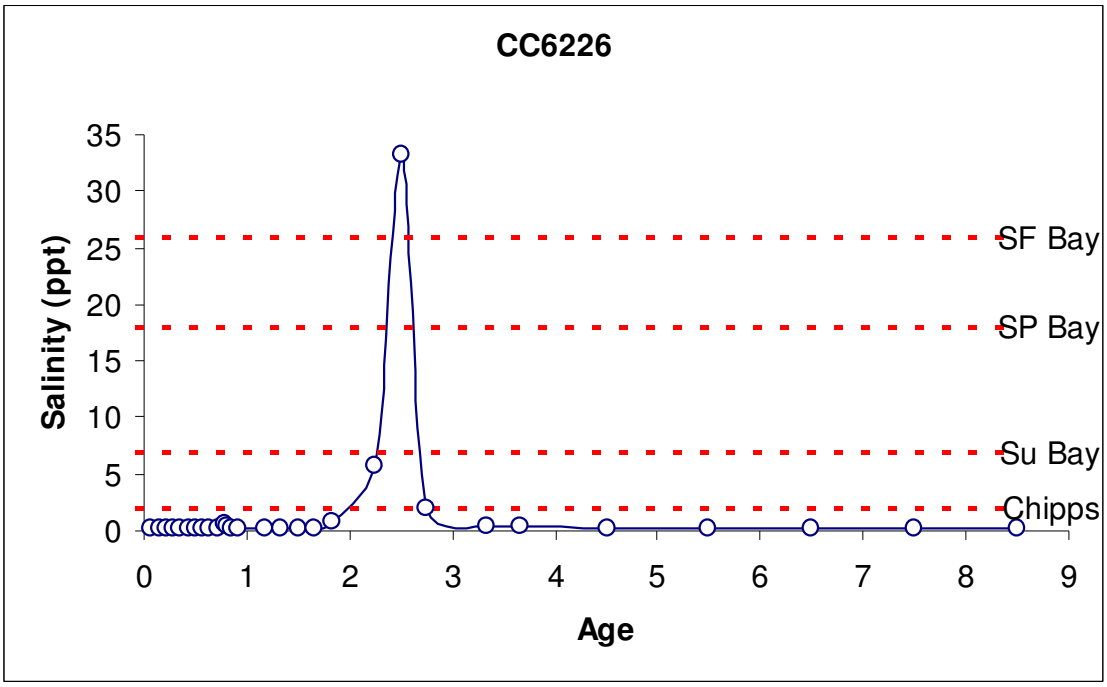


Figure 87a:
Clifton Court 6226 Male, 79.0 cm, 7.3 kg, 9 years

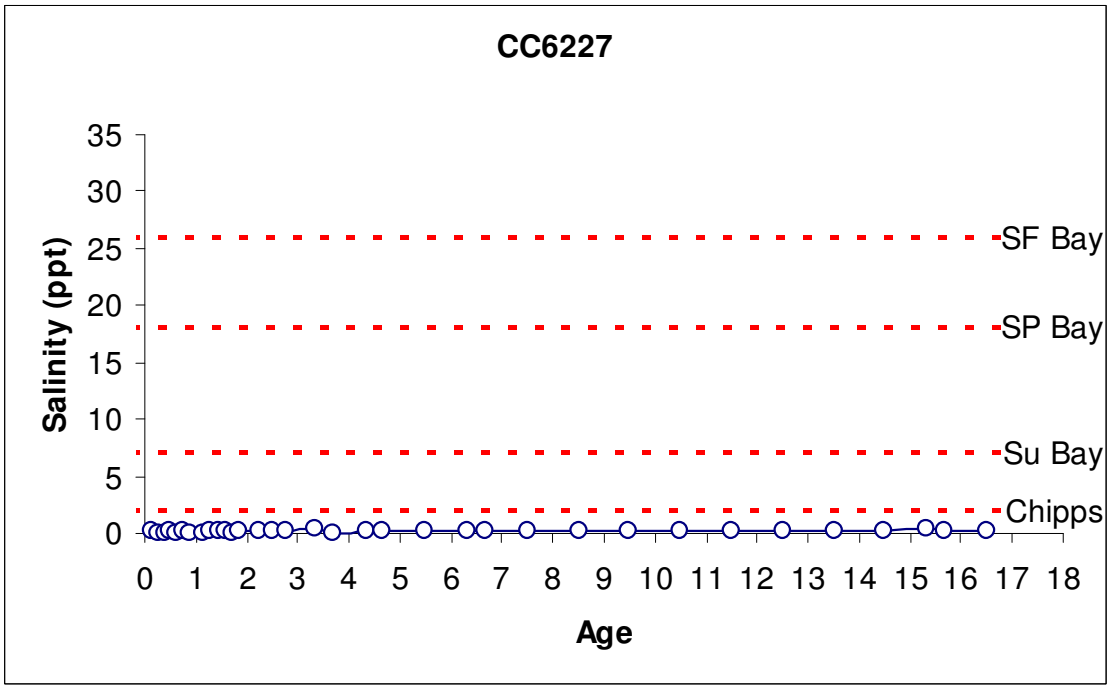


Figure 88a:
Clifton Court 6227 Female, 108.0 cm, 15.0 kg, 17 years

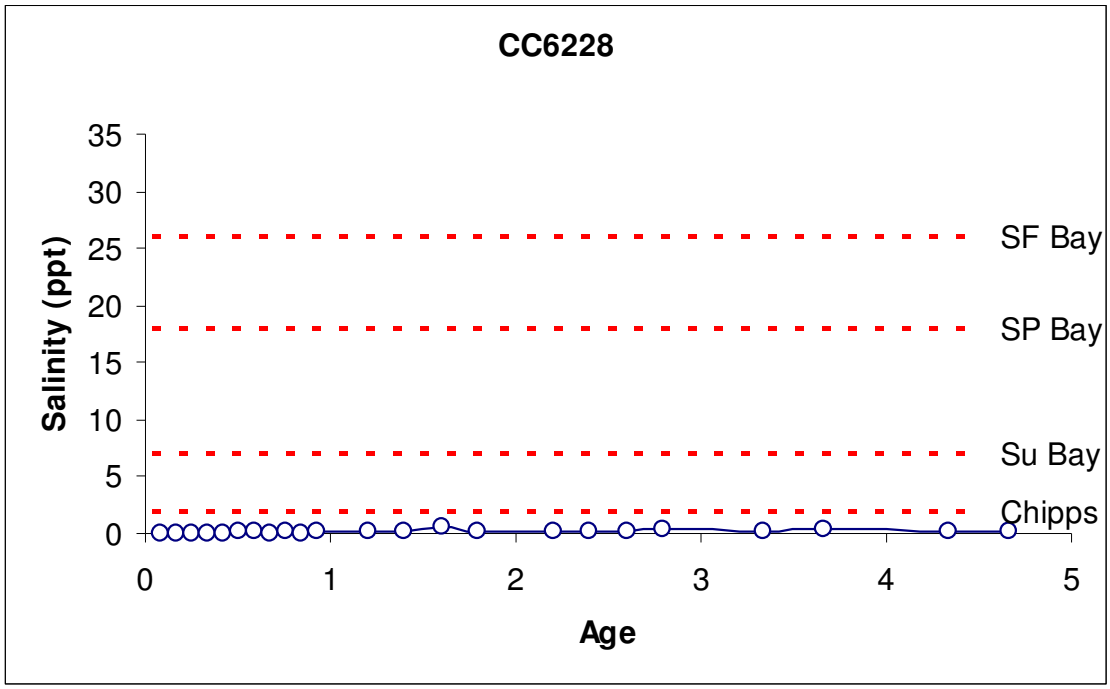


Figure 89a:
Clifton Court 6228 Female, 47.8 cm, 1.3 kg, 5 years

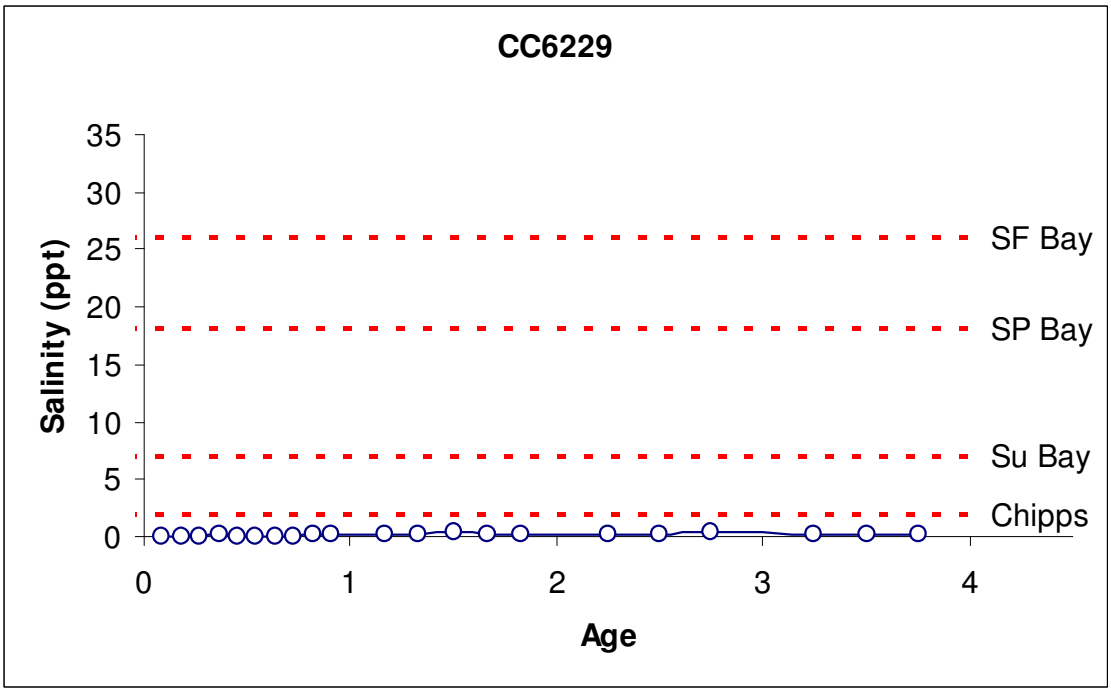


Figure 90a:
Clifton Court 6229 Male, 48.3 cm, 1.2 kg, 4 years

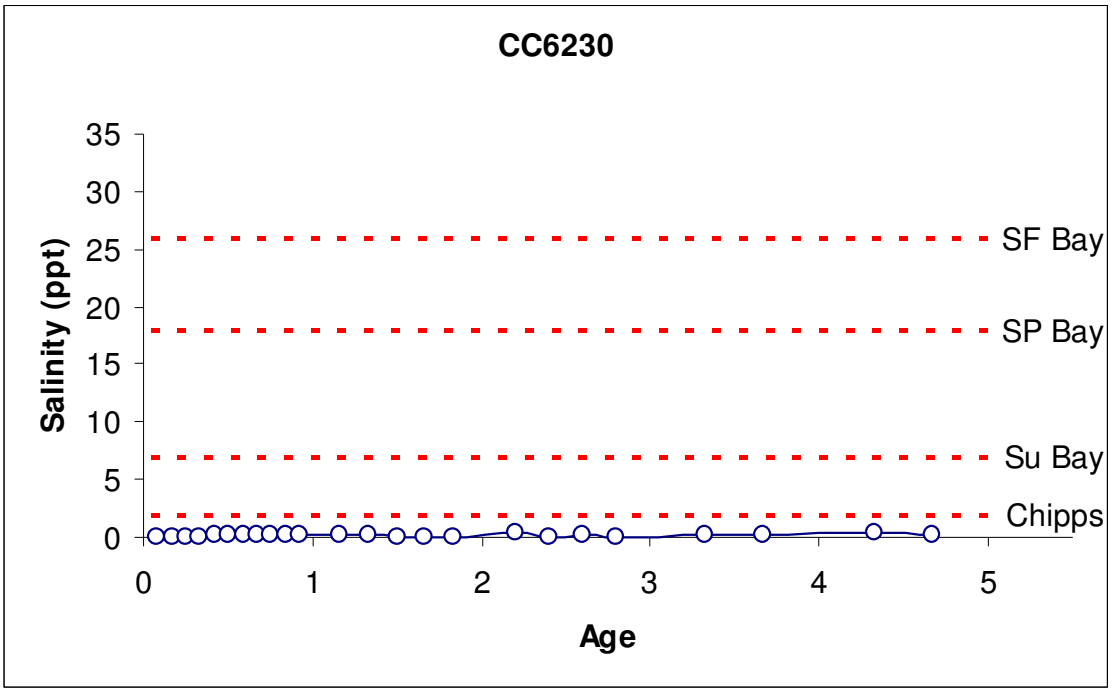


Figure 91a:
Clifton Court 6230 Female, 51.9 cm, 1.6 kg, 5 years

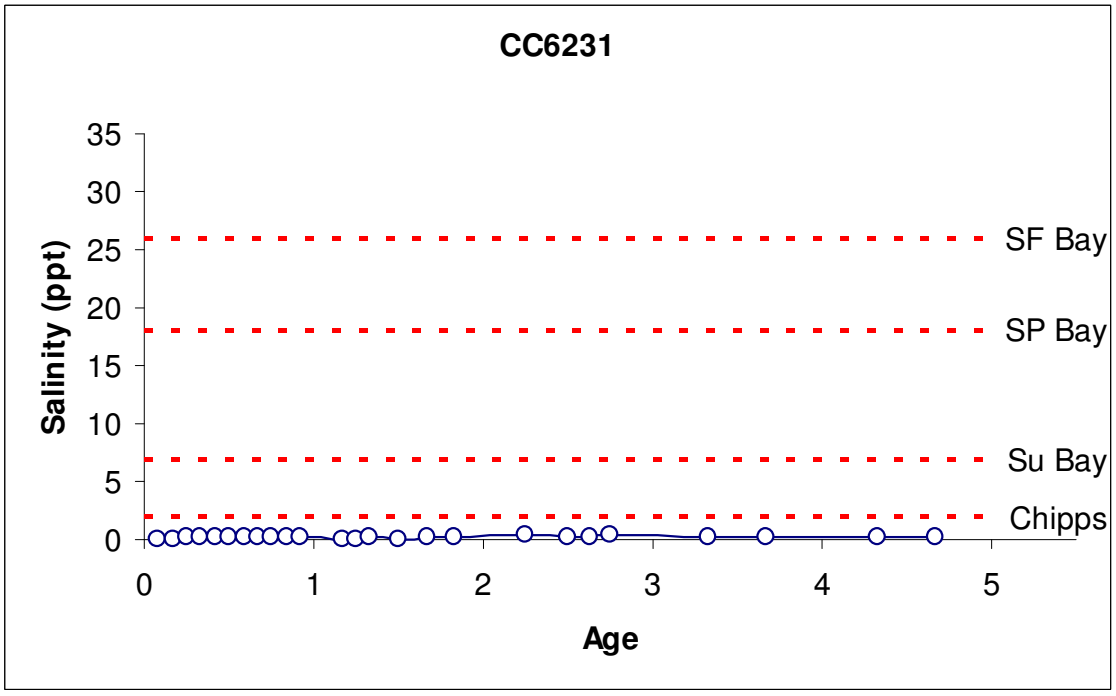


Figure 92a:
Clifton Court 6231 Female, 59.1 cm, 2.8 kg, 5 years

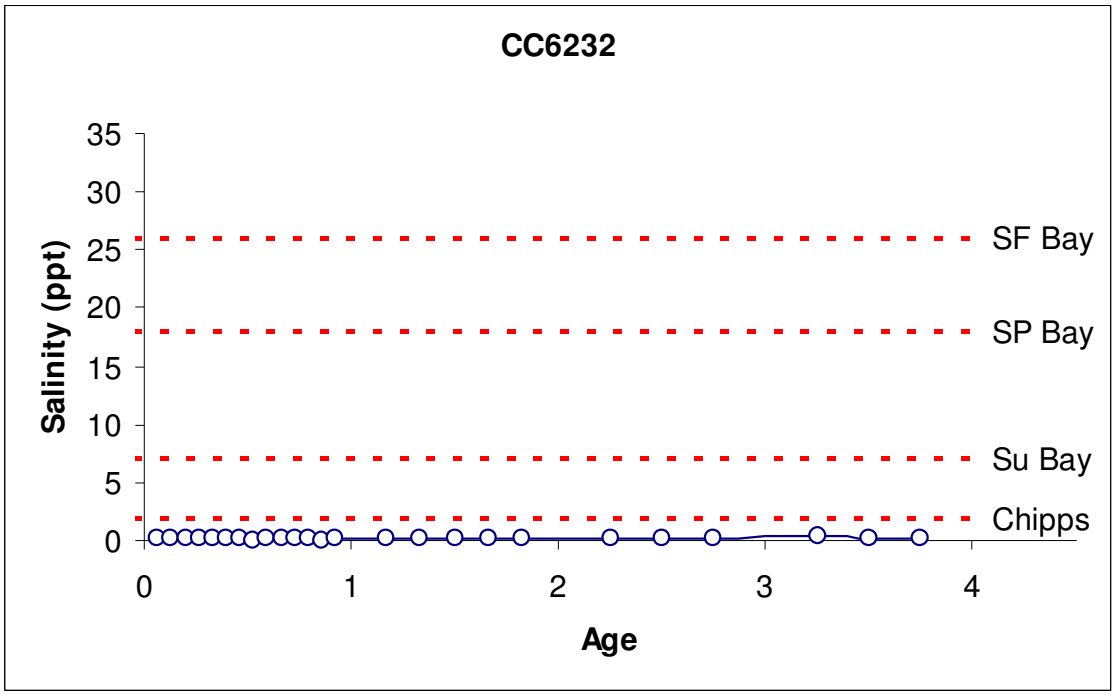


Figure 93a:
Clifton Court 6232 Male, 49.7 cm, 1.0 kg, 4 years

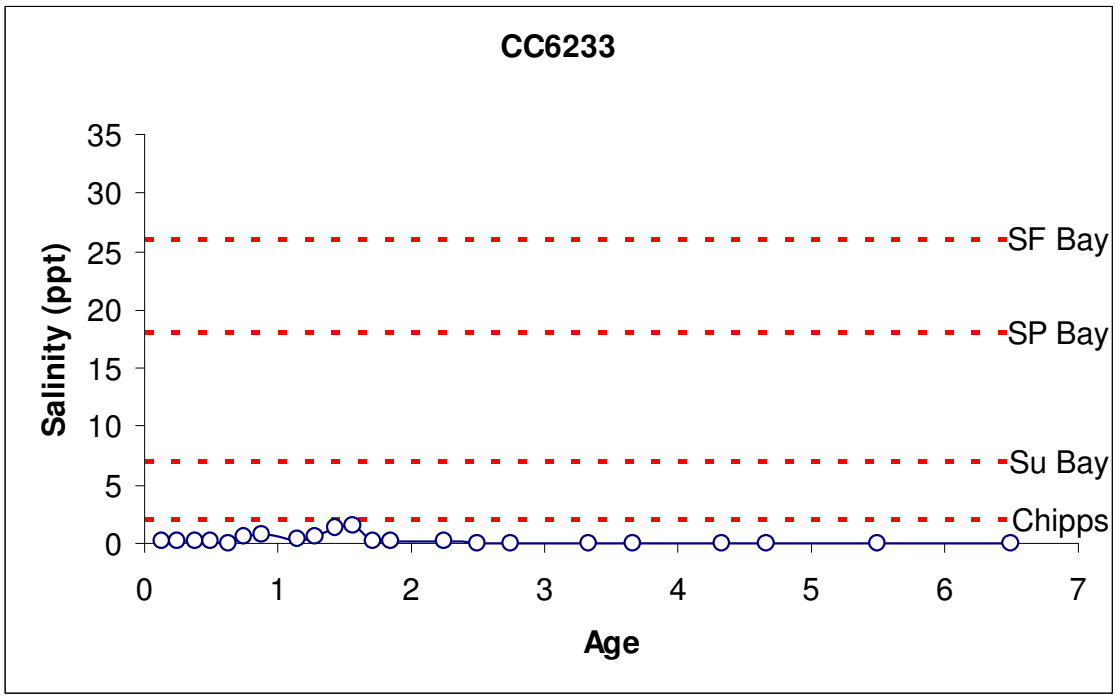


Figure 94a:
Clifton Court 6233 Male, 57.0 cm, 2.3 kg, 7 years

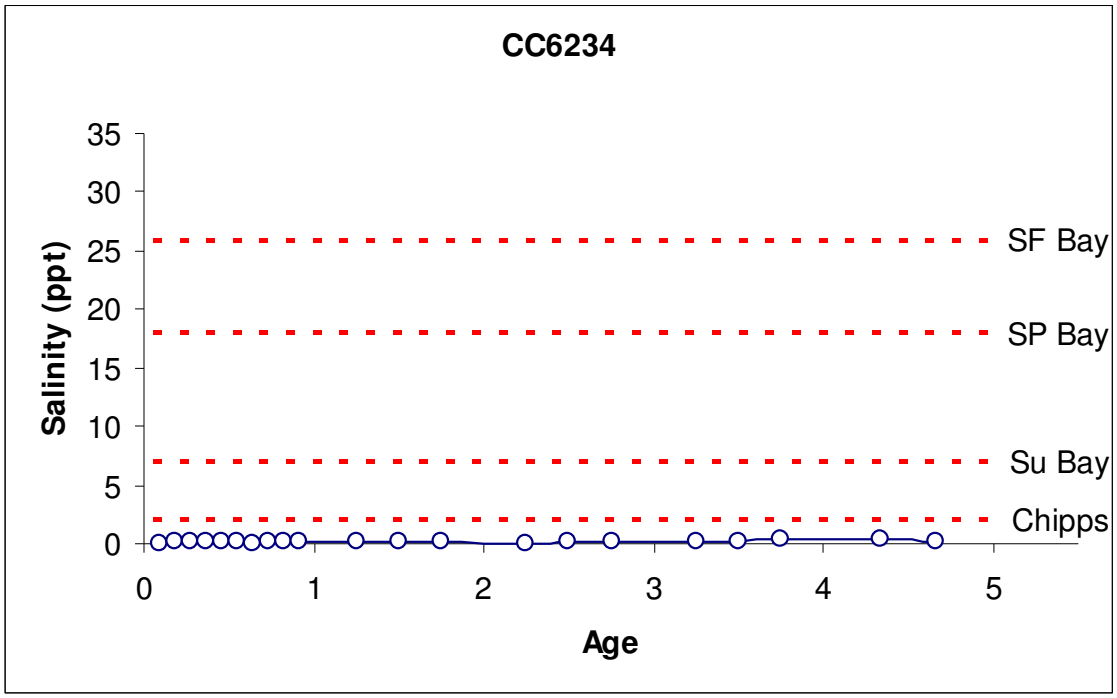


Figure 95a:
Clifton Court 6234 Female, 50.3 cm, 0.9 kg, 5 years

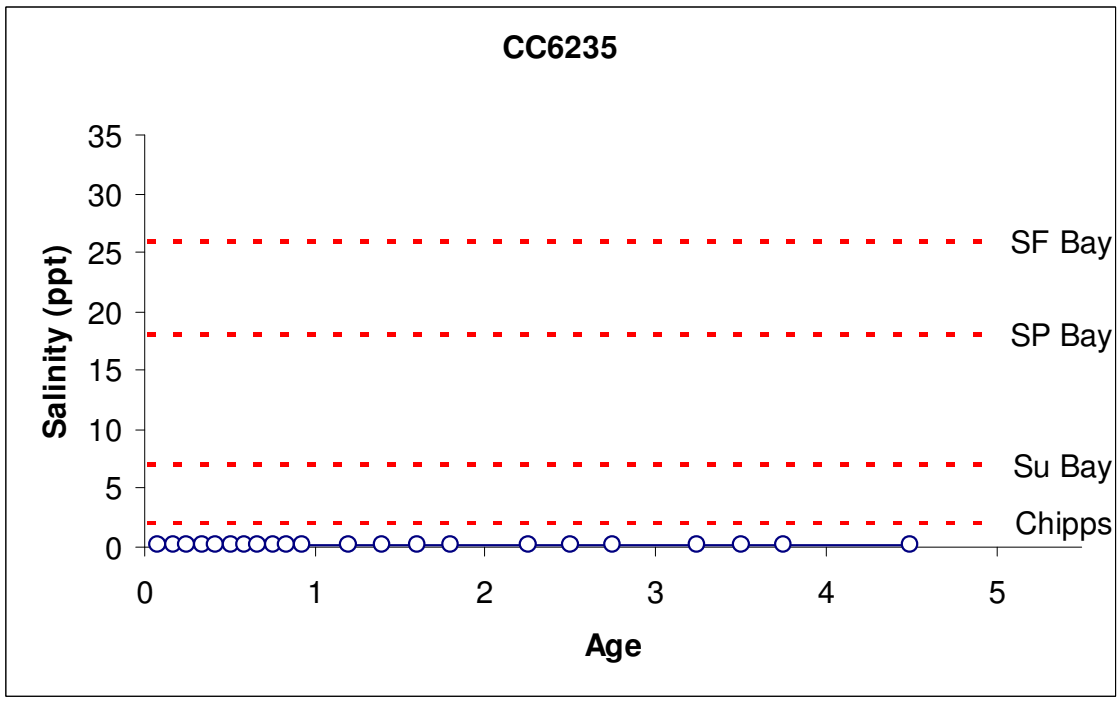


Figure 96a:
Clifton Court 6235 Male, 48.5 cm, 1.3 kg, 5 years

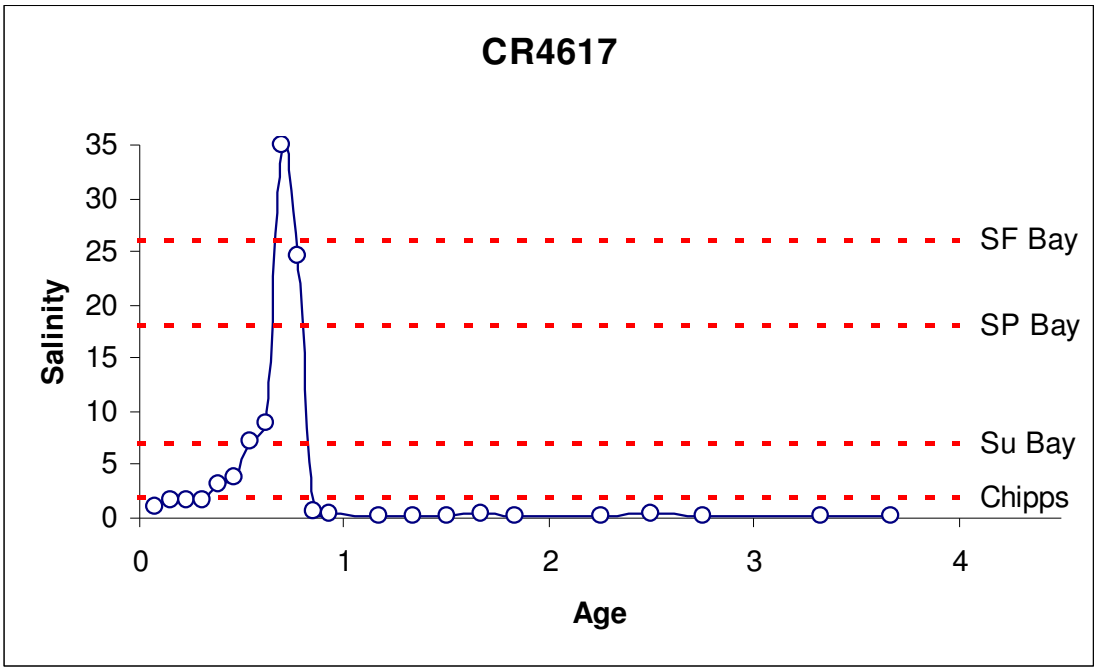


Figure 97a:
Cosumnes River 4617 Female, 56.5 cm, 2.2 kg, 4 years

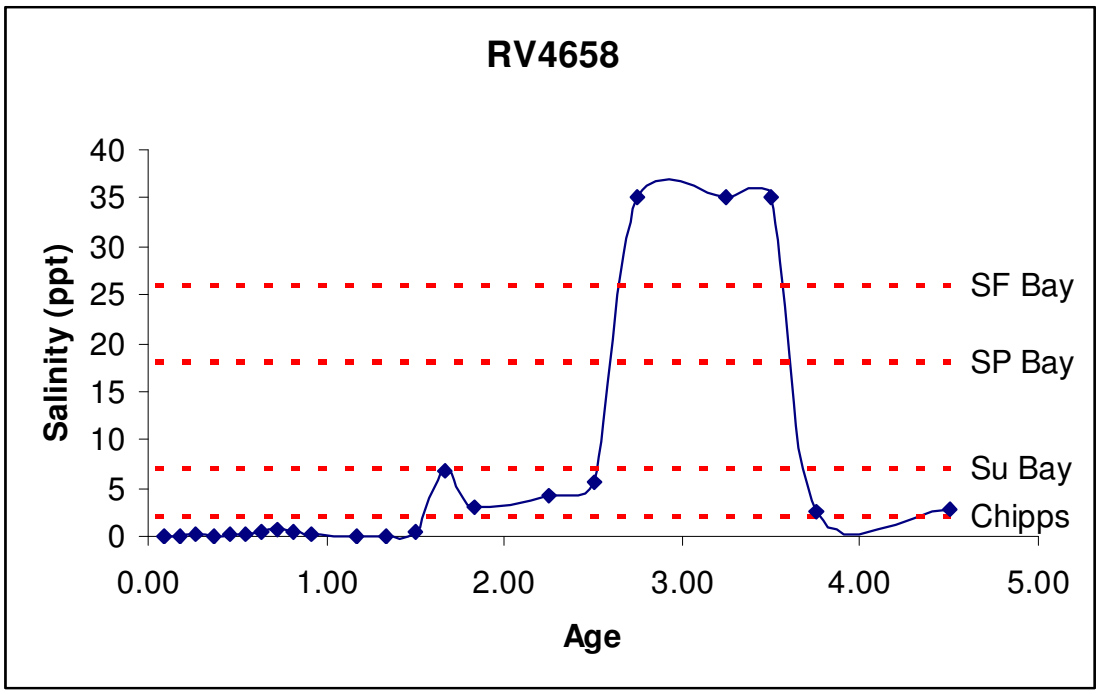


Figure 98a:
Rio Vista Fish Derby 4658 Male, 55.5 cm, 1.9 kg, 5 years

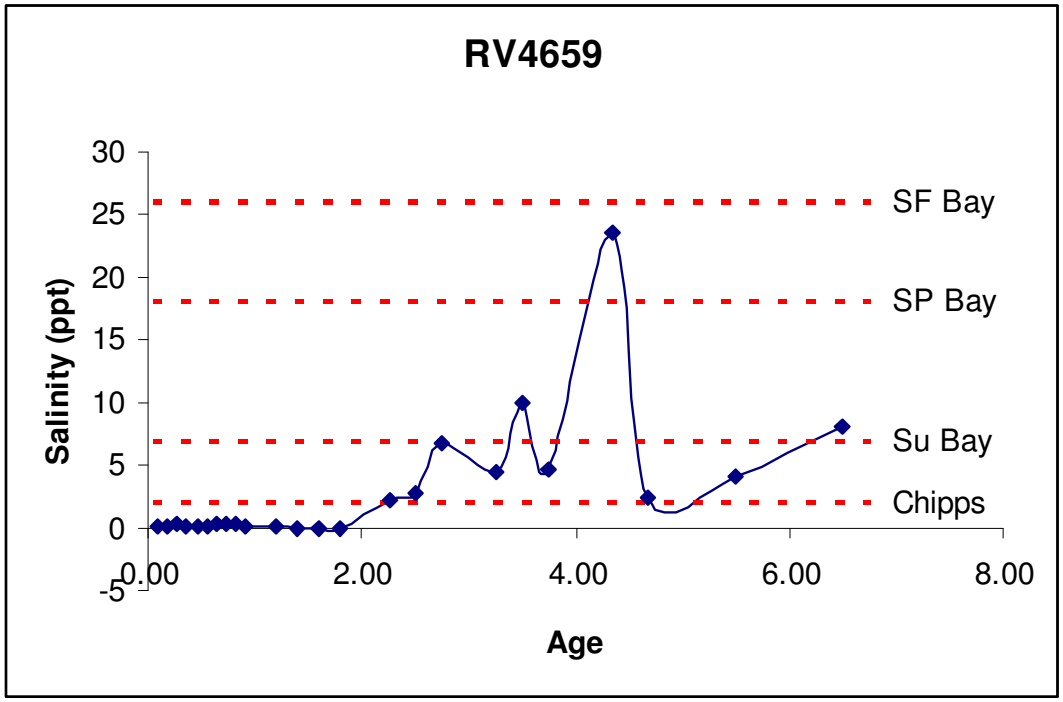


Figure 99a:
Rio Vista Fish Derby 4659 Male, 70.5 cm, 4.1 kg, 7 years

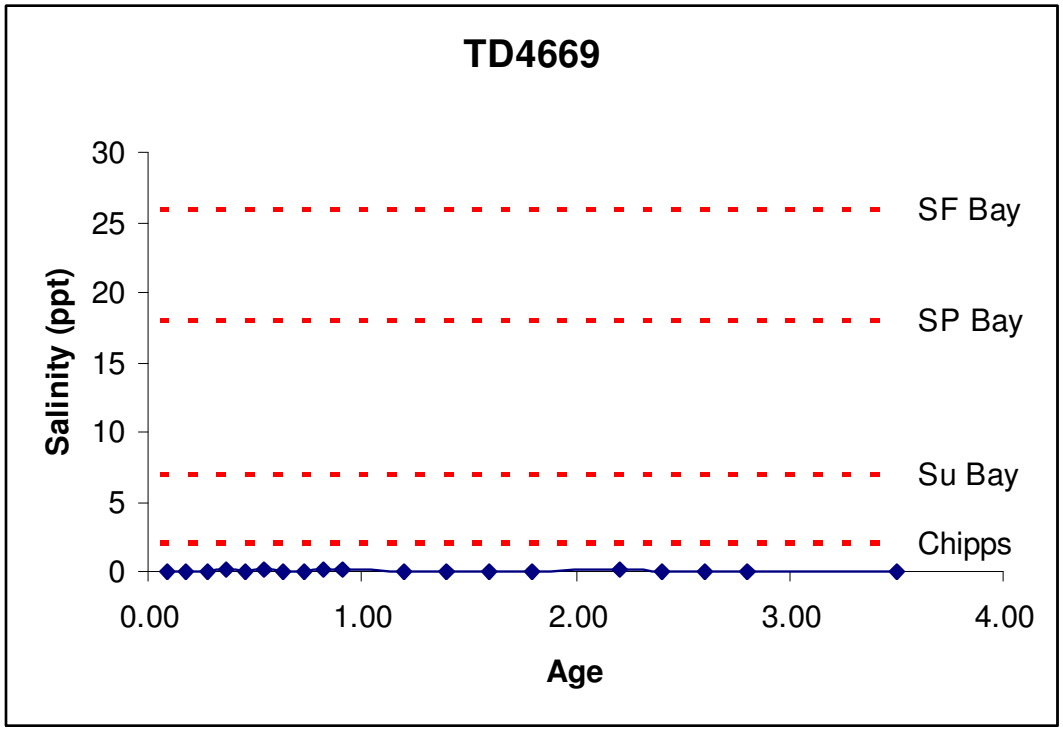


Figure 100a:
Toe Drain 4669 Male, 55.5 cm, 1.9 kg, 4 years

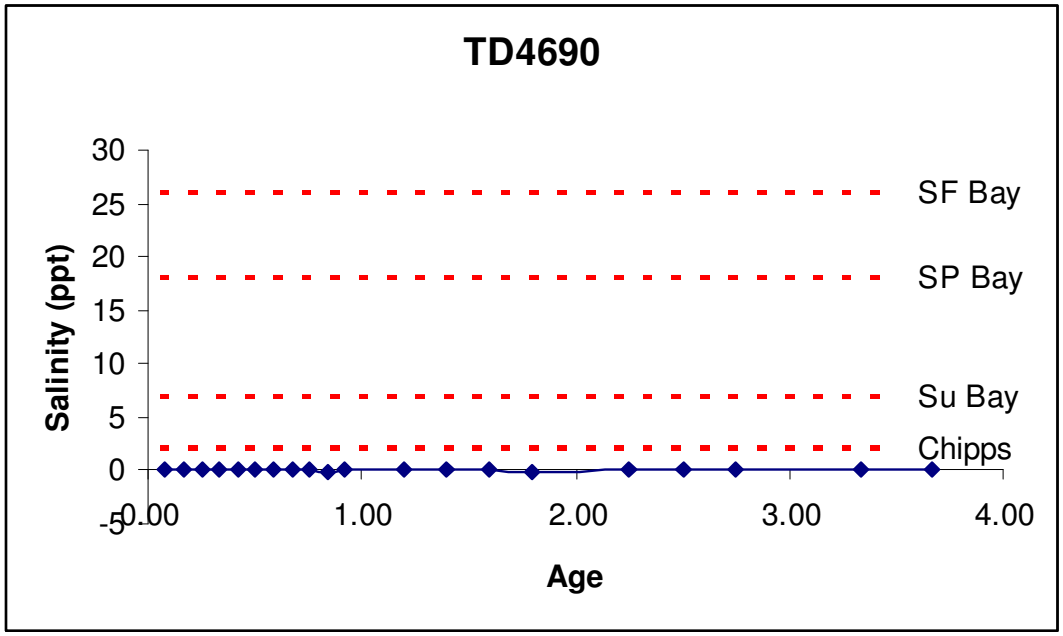


Figure 101a:
Toe Drain 4690 Male, 54.0 cm, 2.3 kg, 4 years

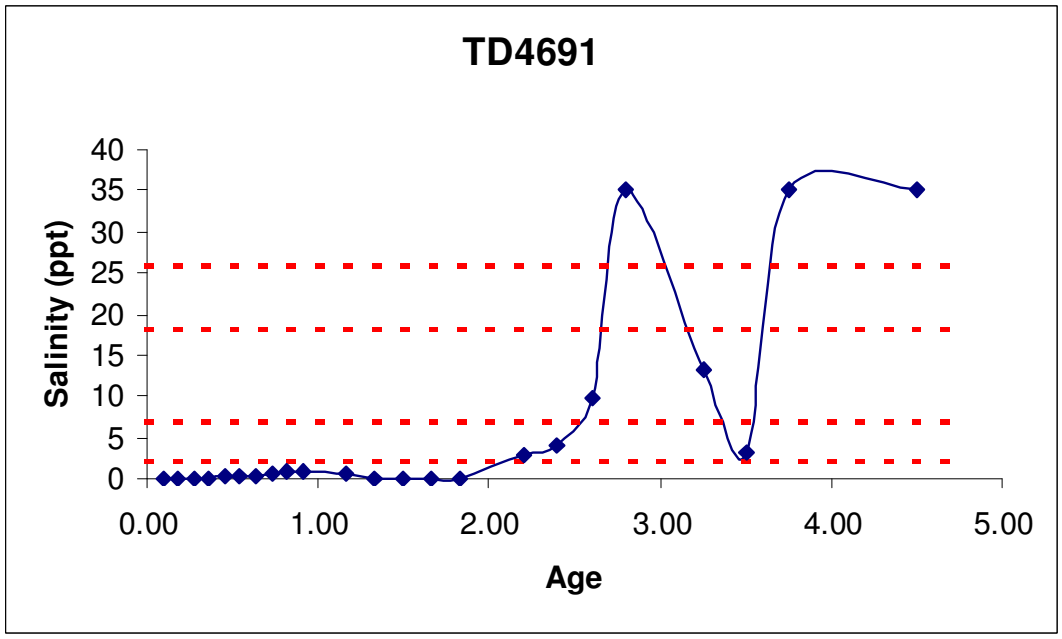


Figure 102a:
Toe Drain 4691 Male, 64.0 cm, 2.8 kg, 5 years

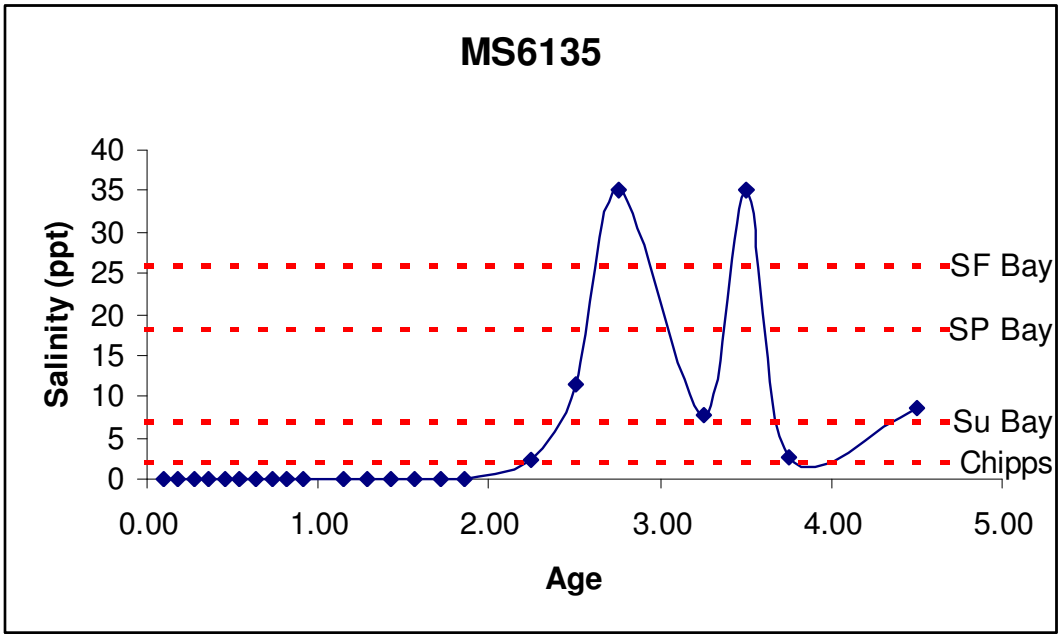


Figure 103a:
Miner Slough 6135 Female, 61.6 cm, 2.8 kg, 5 years

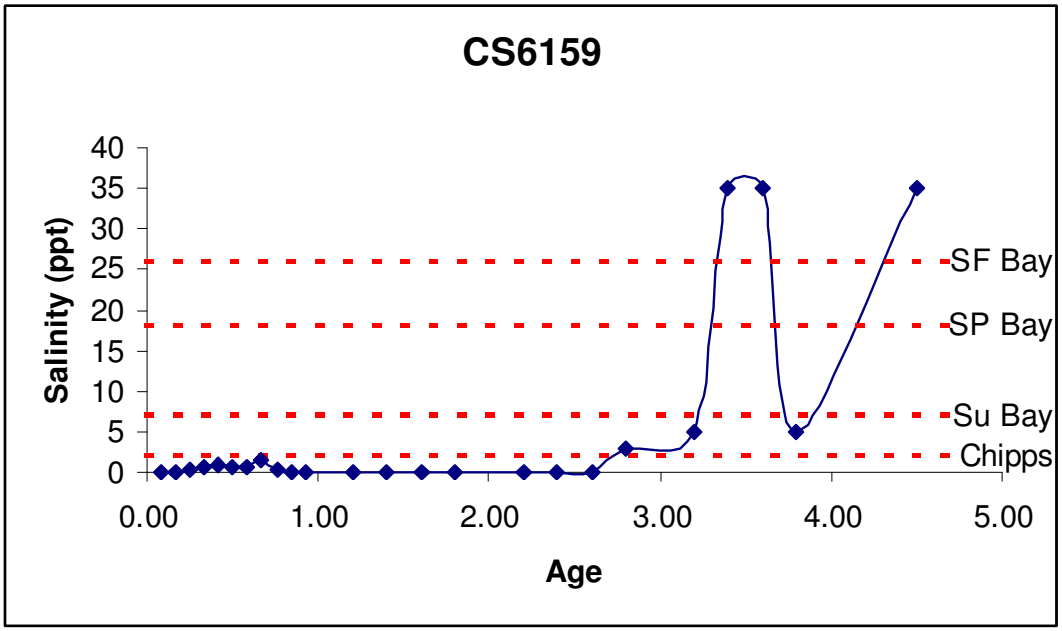


Figure 104a:
Cache Slough 6159 Female, 55.5 cm, 2.0 kg, 5 years

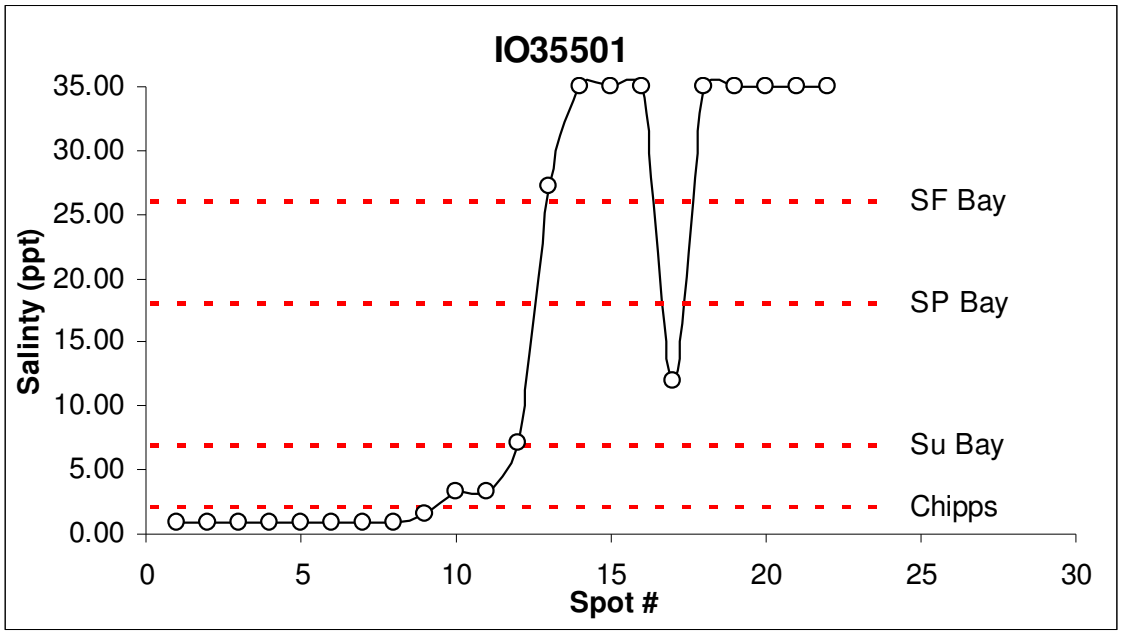


Figure 105a:
San Pablo Bay IO35501, 7 years

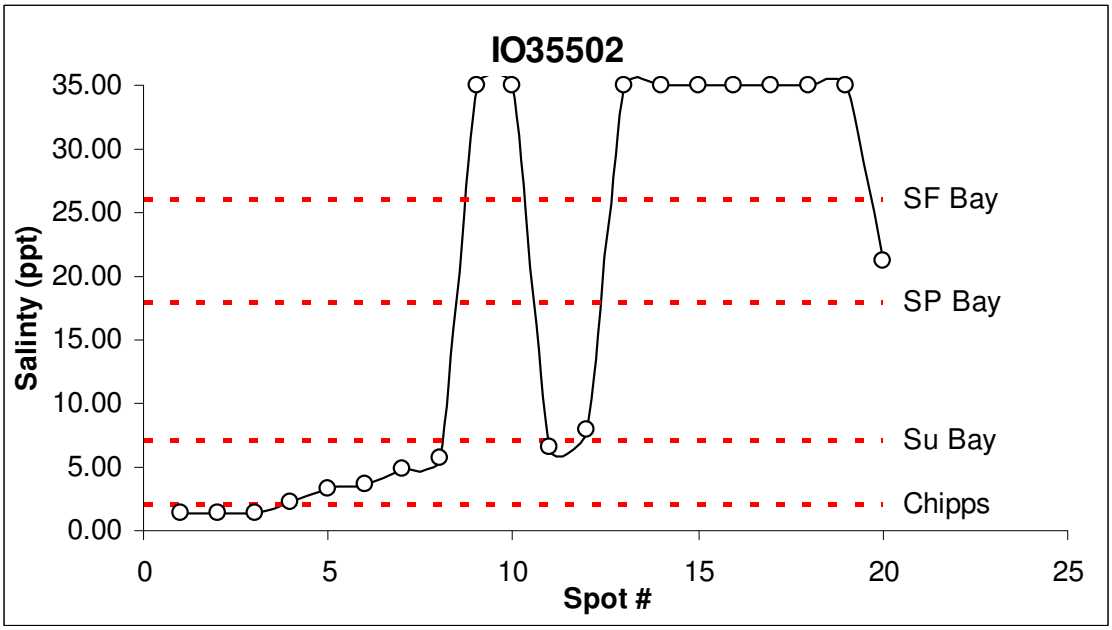


Figure 106a:
San Pablo Bay IO35502, 5 years

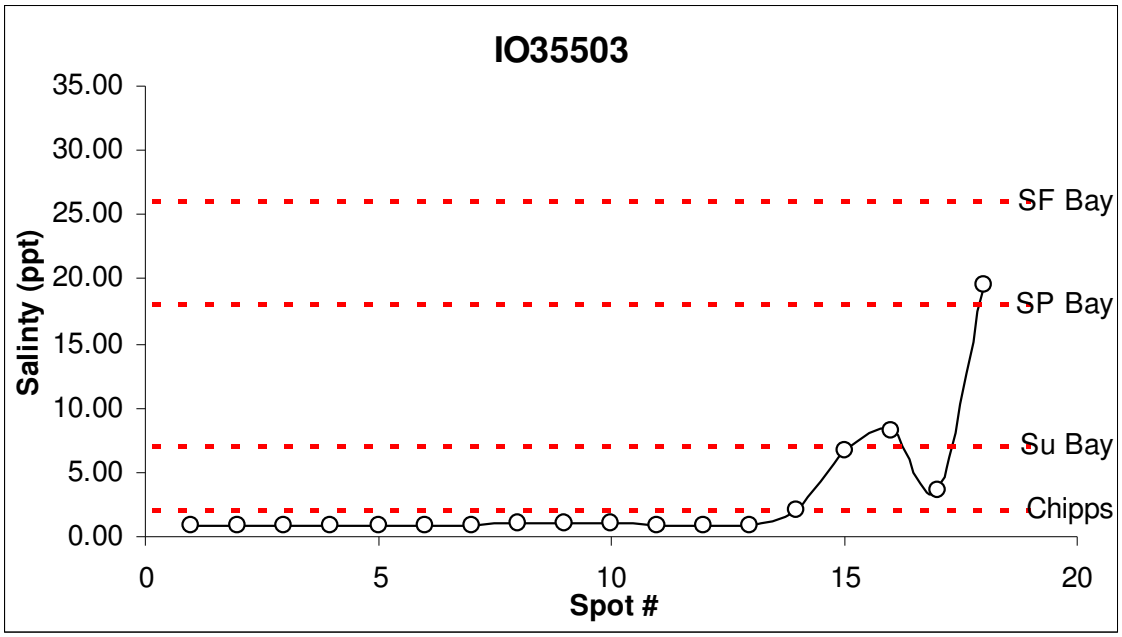


Figure 107a:
San Pablo Bay IO35503, 6 years

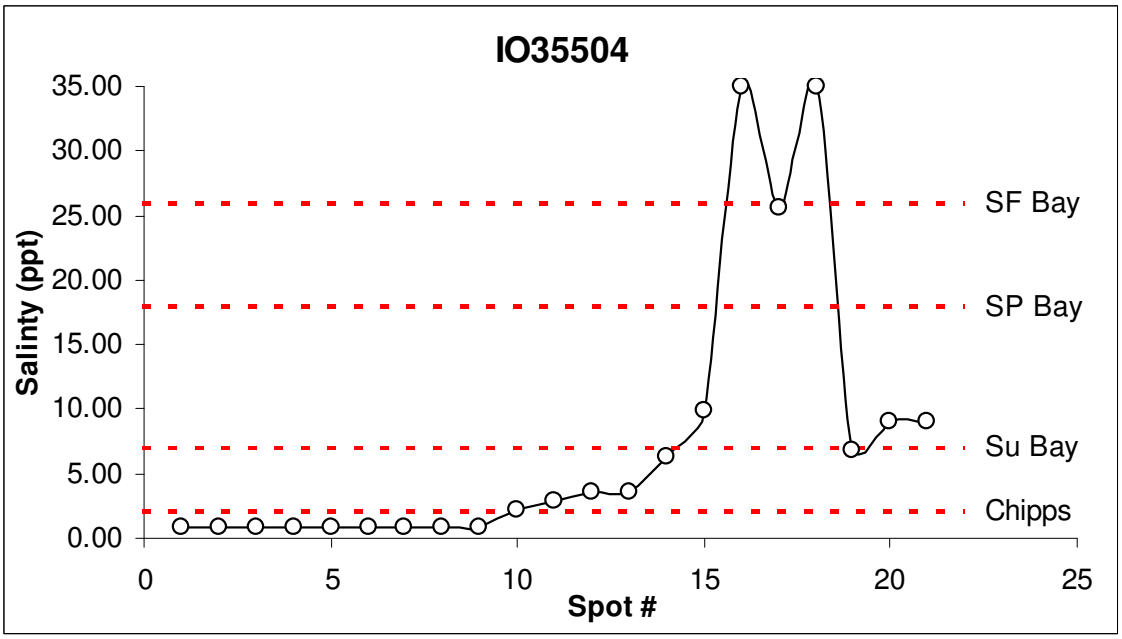


Figure 108a:
San Pablo Bay IO35504, 5 years

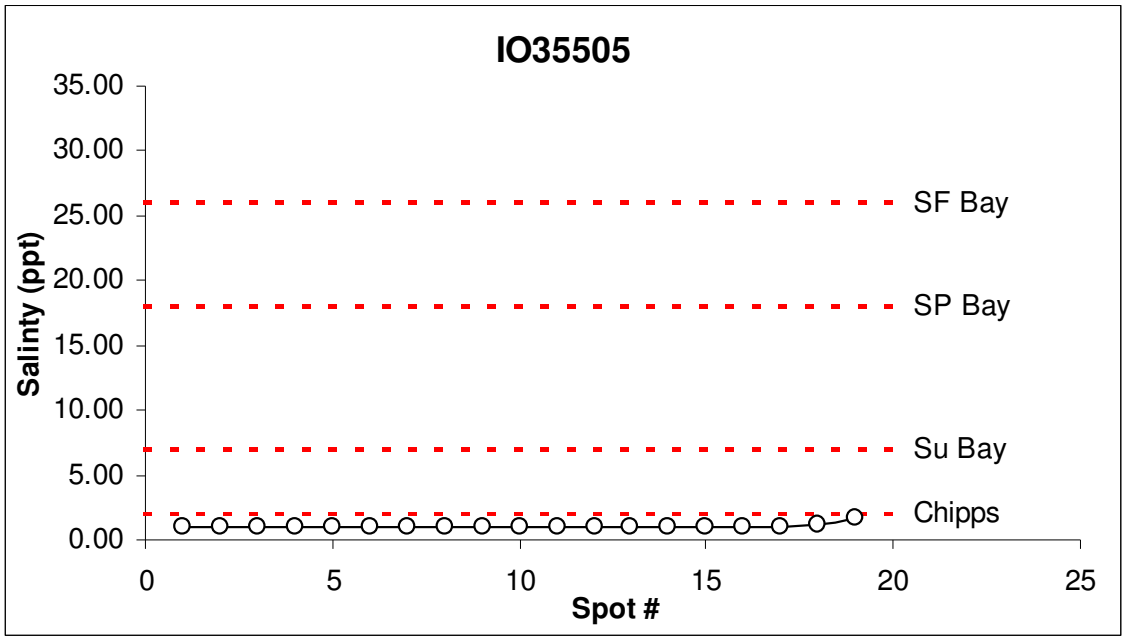


Figure 109a:
San Pablo Bay IO35505, 4 years

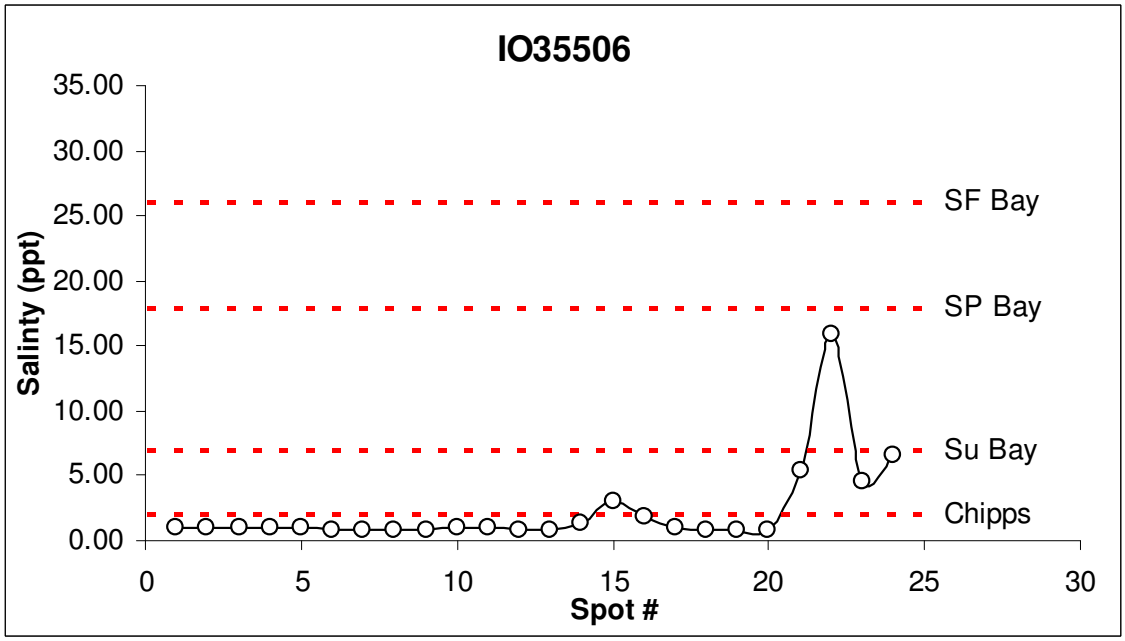


Figure 110a:
San Pablo Bay IO35506, 6 years

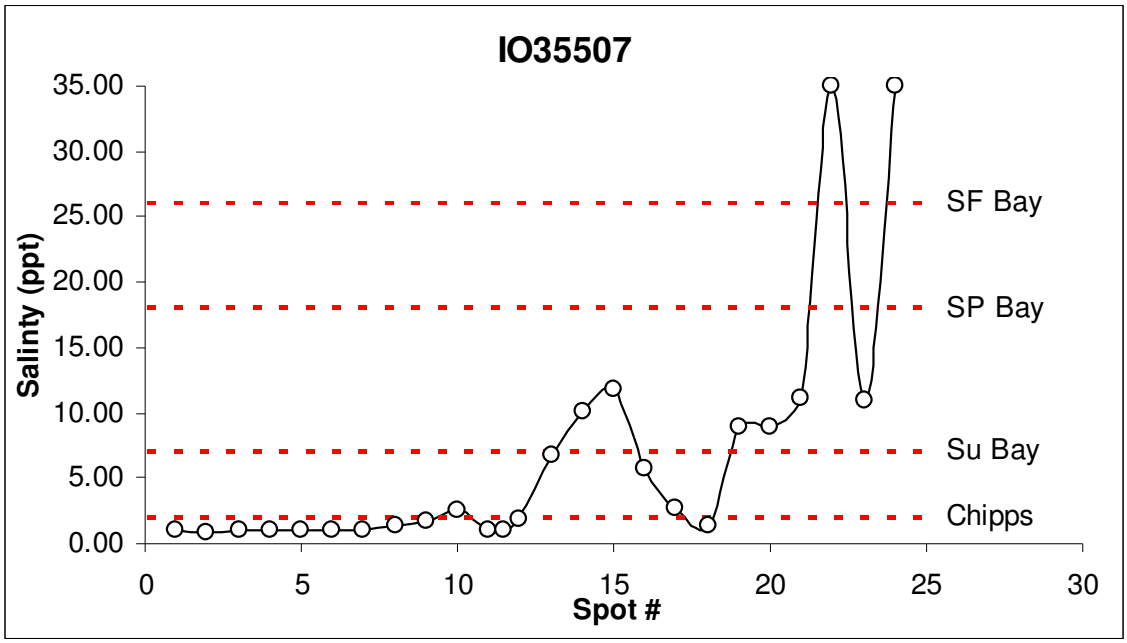


Figure 111a:
San Pablo Bay IO35507, 5 years

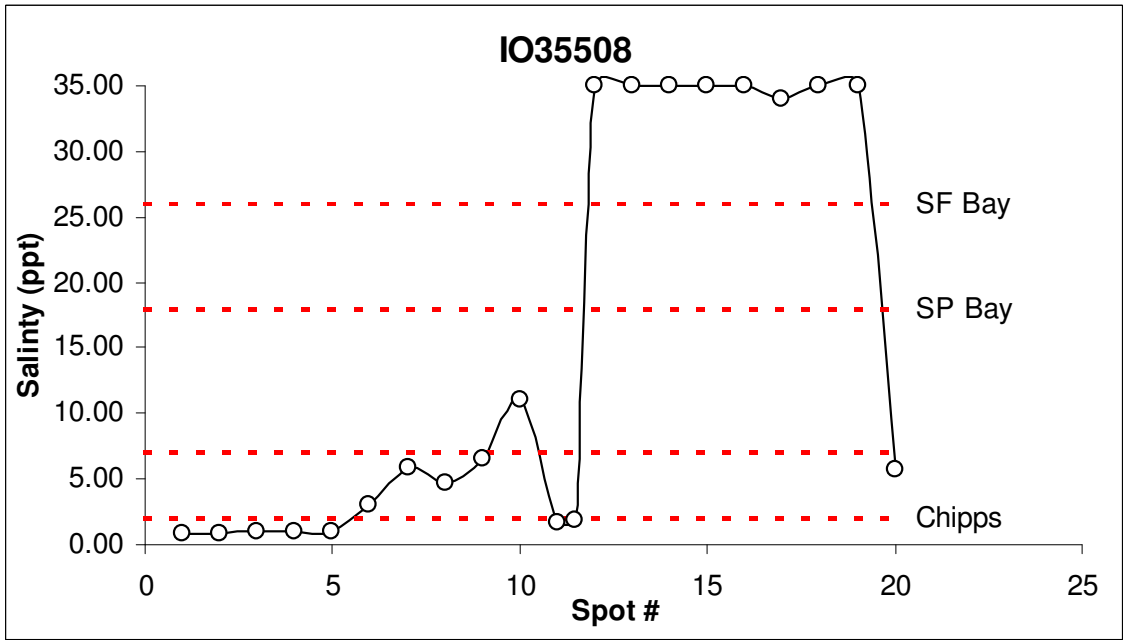


Figure 112a:
San Pablo Bay IO35508, 4 years

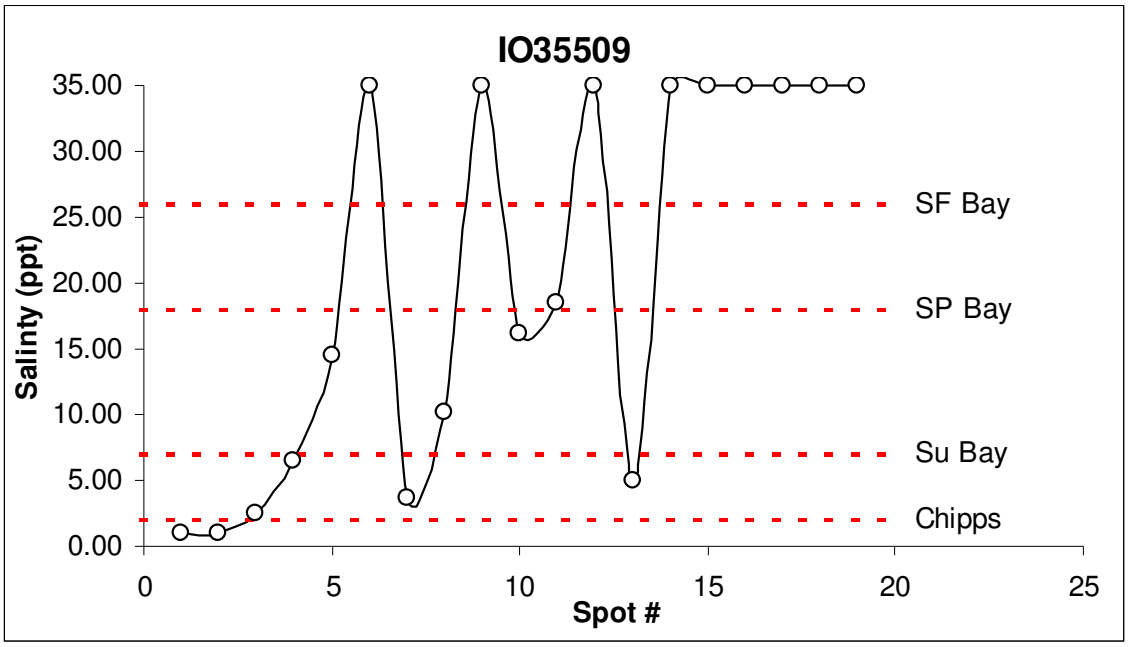


Figure 113a:
San Pablo Bay IO35509, 6 years

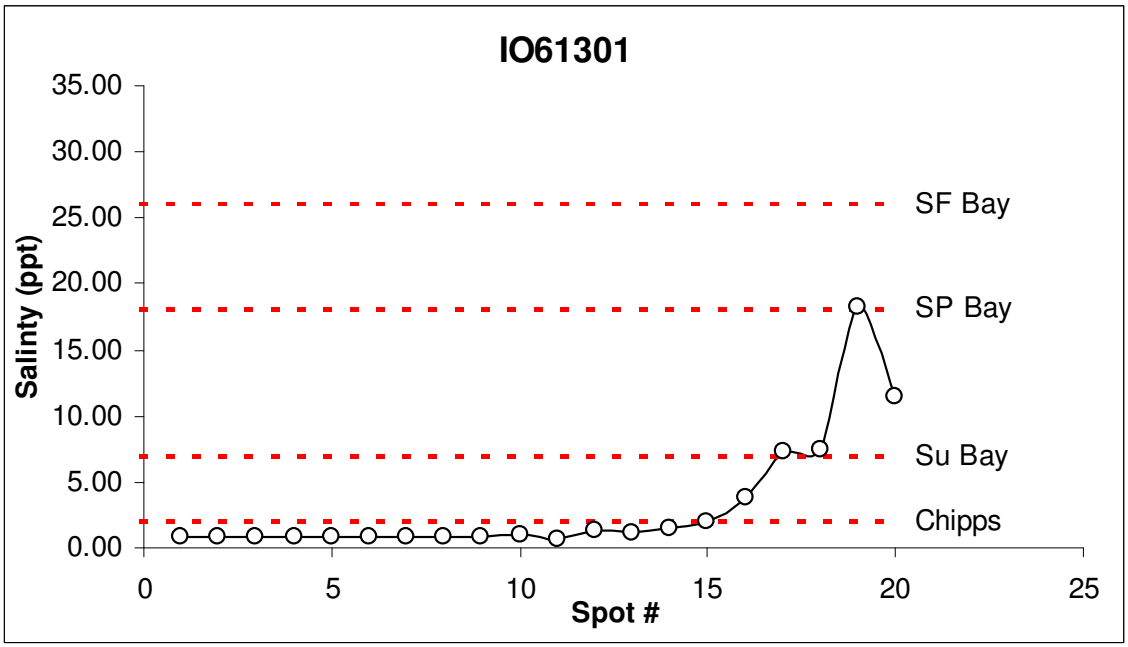


Figure 114a:
South Bay IO61301, 5 years

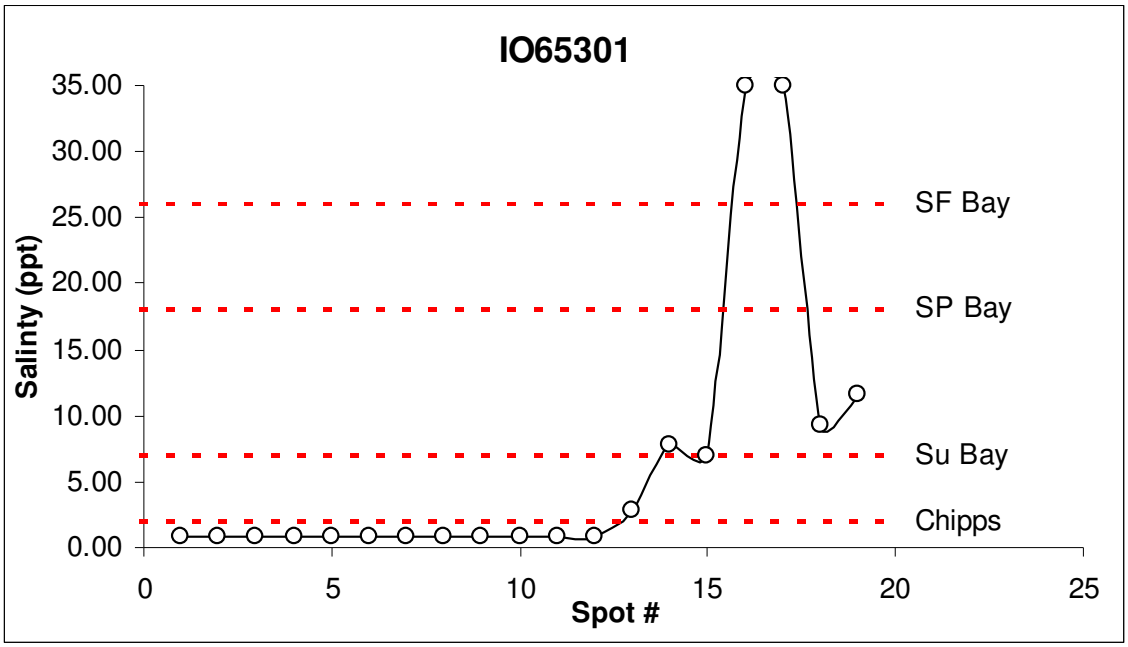


Figure 115a:
San Pablo Bay IO65301, 5 years

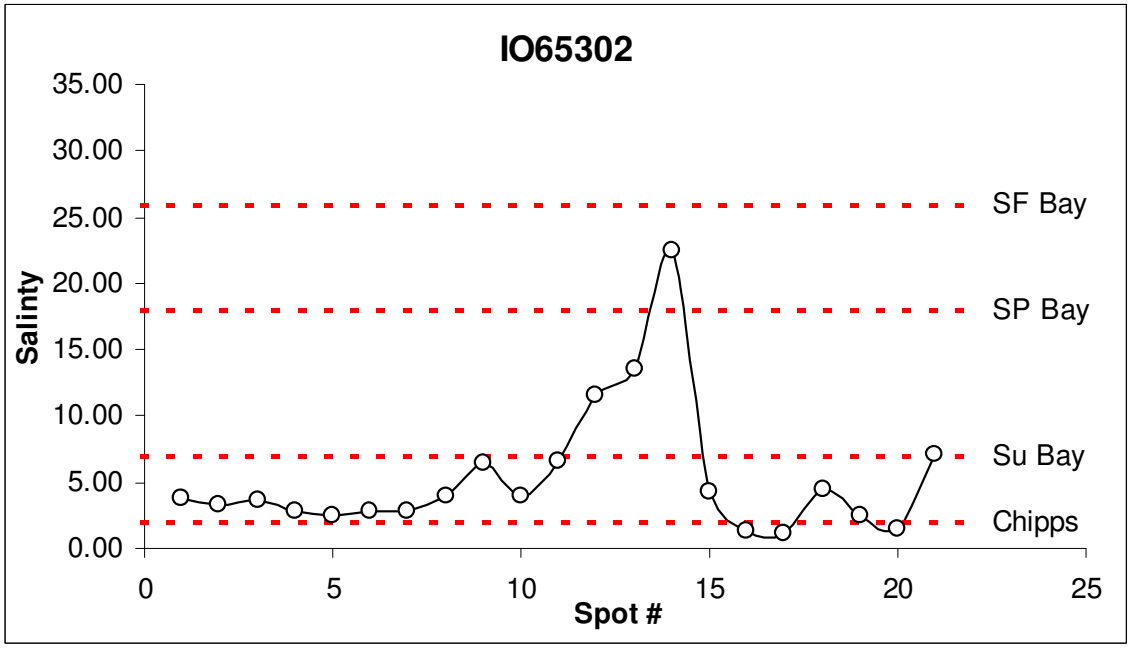


Figure 116a:
San Pablo Bay IO65302, 5 years

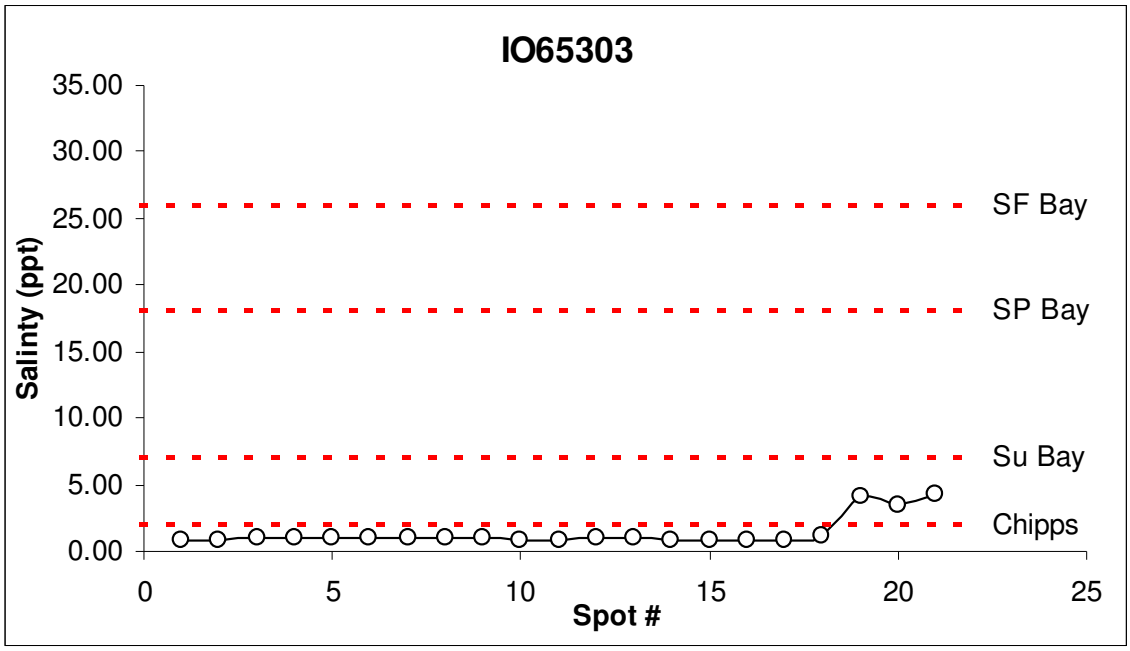


Figure 117a:
San Pablo Bay IO65303, 5 years

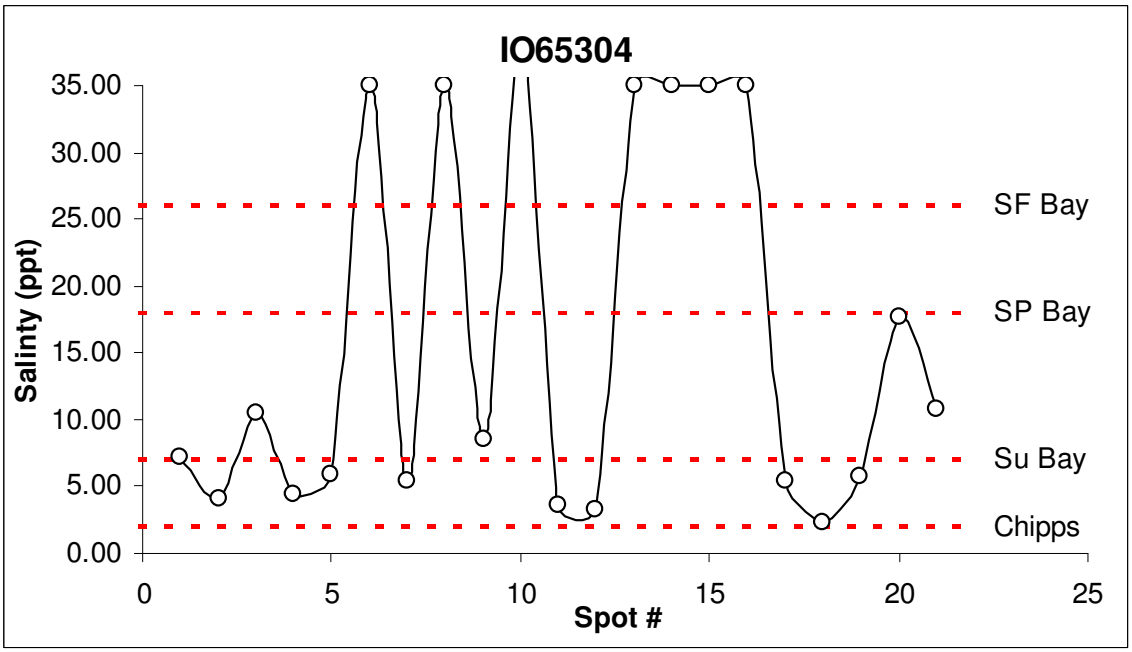


Figure 118a:
San Pablo Bay IO65304, 4 years

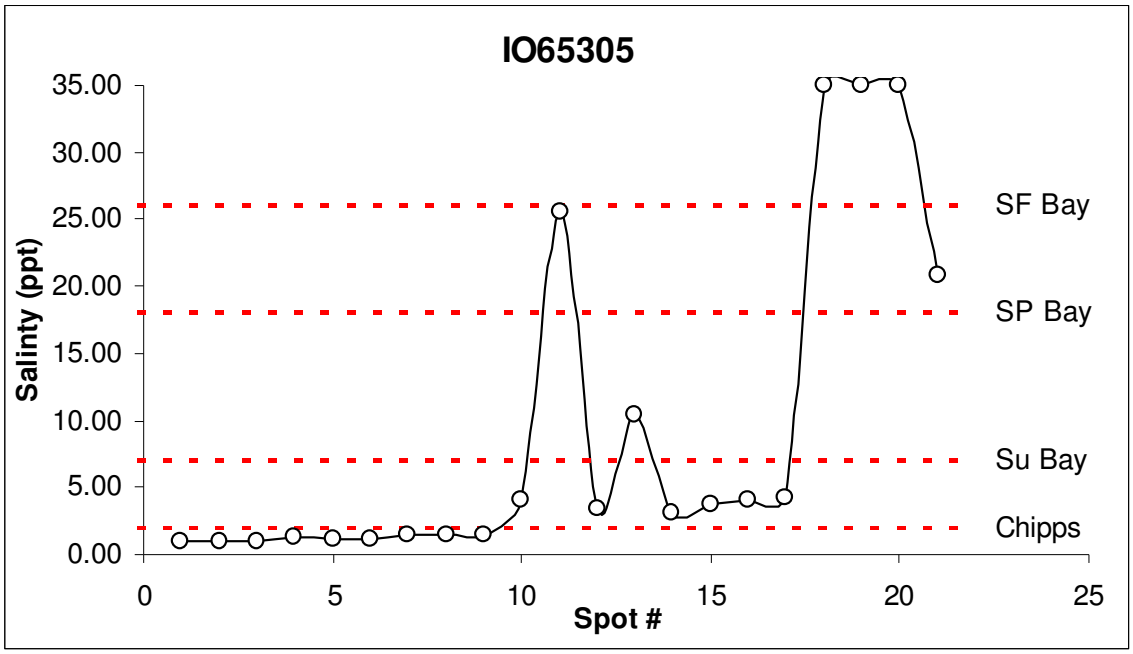


Figure 119a:
San Pablo Bay IO65305, 5 years

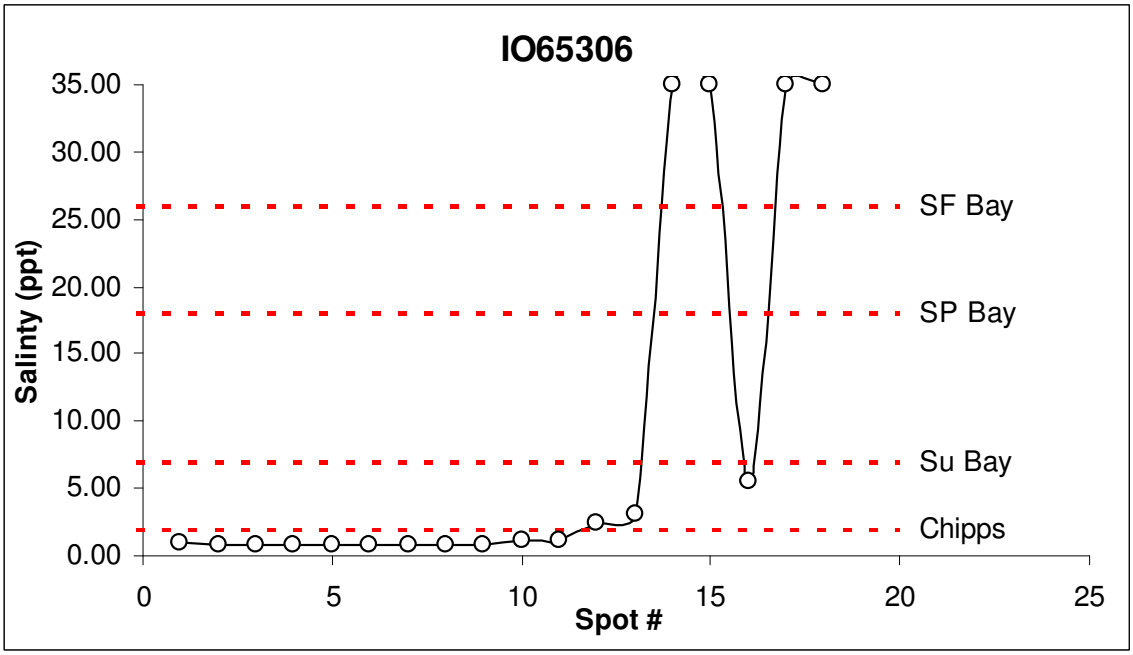


Figure 120a:
San Pablo Bay IO65306, 9 years

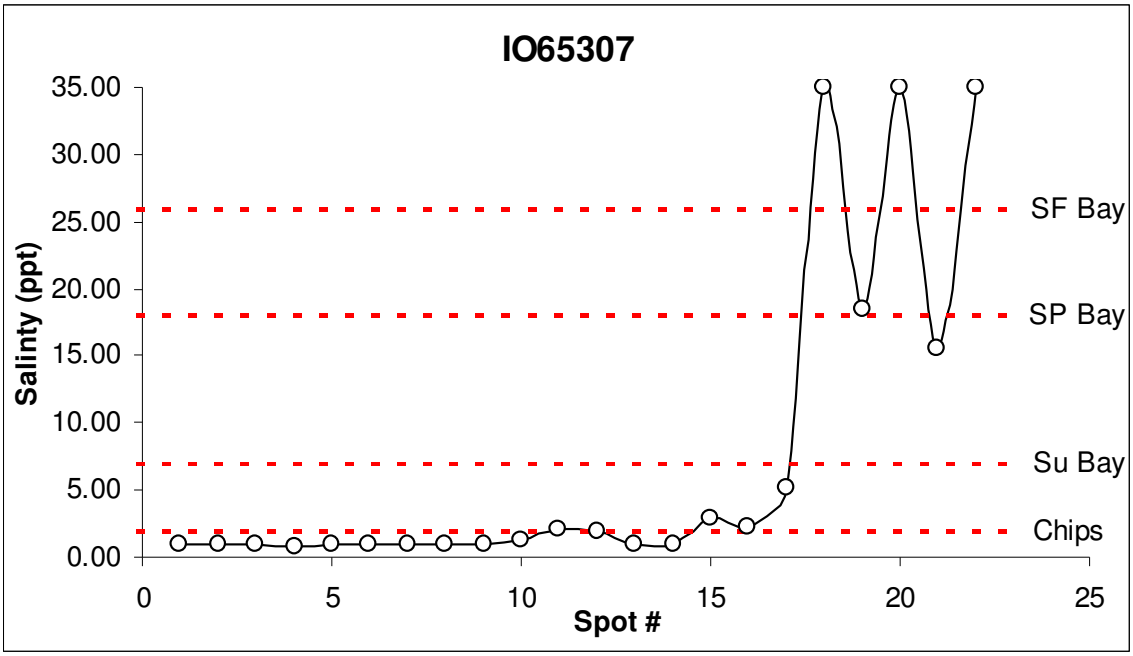


Figure 121a:
San Pablo Bay IO65307, 5 years

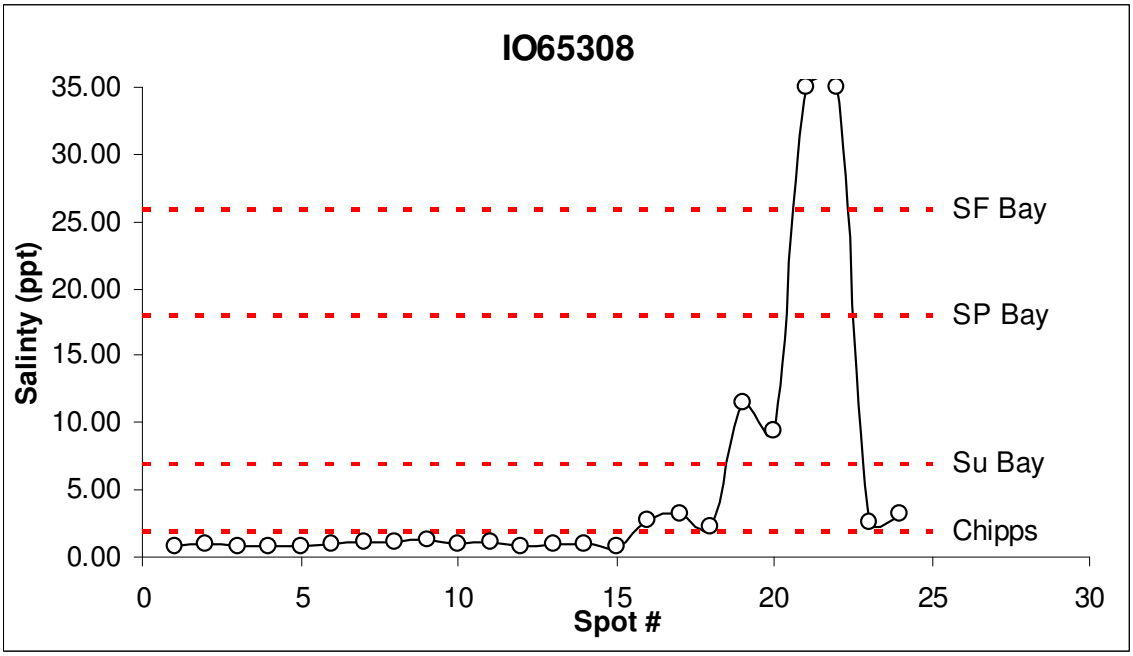


Figure 122a:
San Pablo Bay IO65308, 5 years

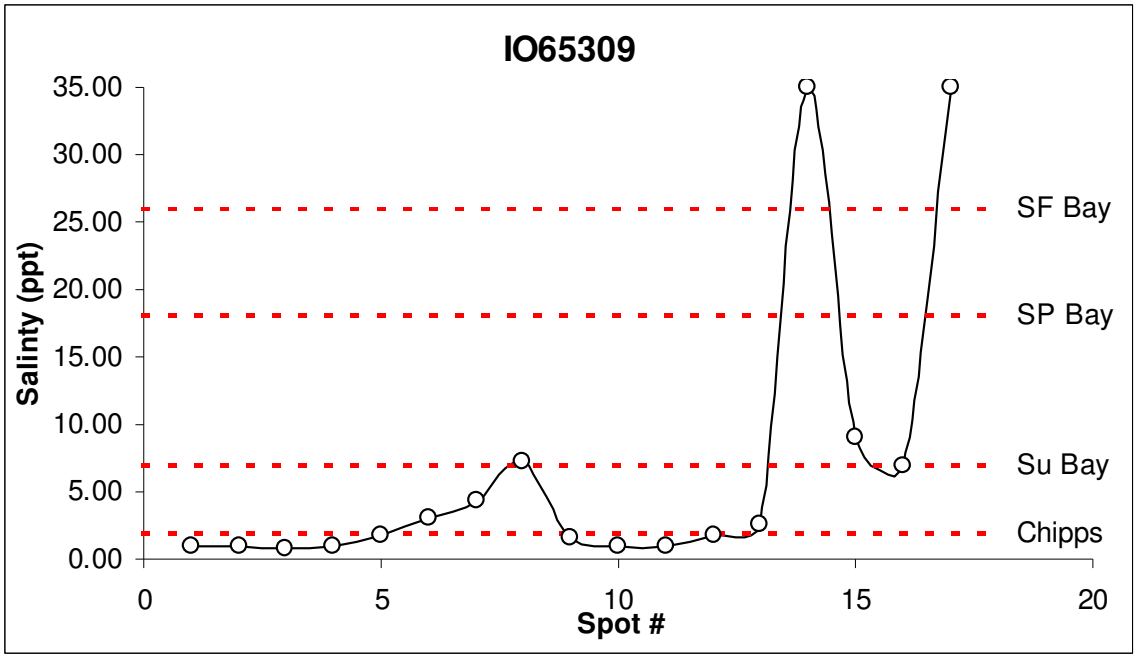


Figure 123a:
San Pablo Bay IO65309, 5 years

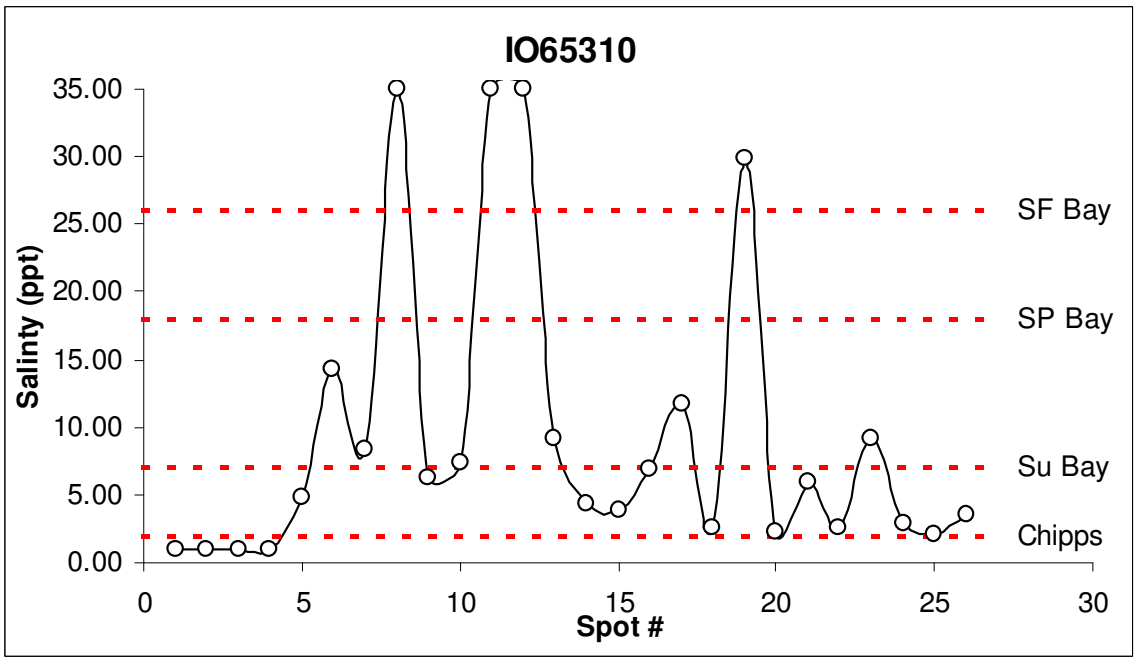


Figure 124a:
San Pablo Bay IO65310, 8 years

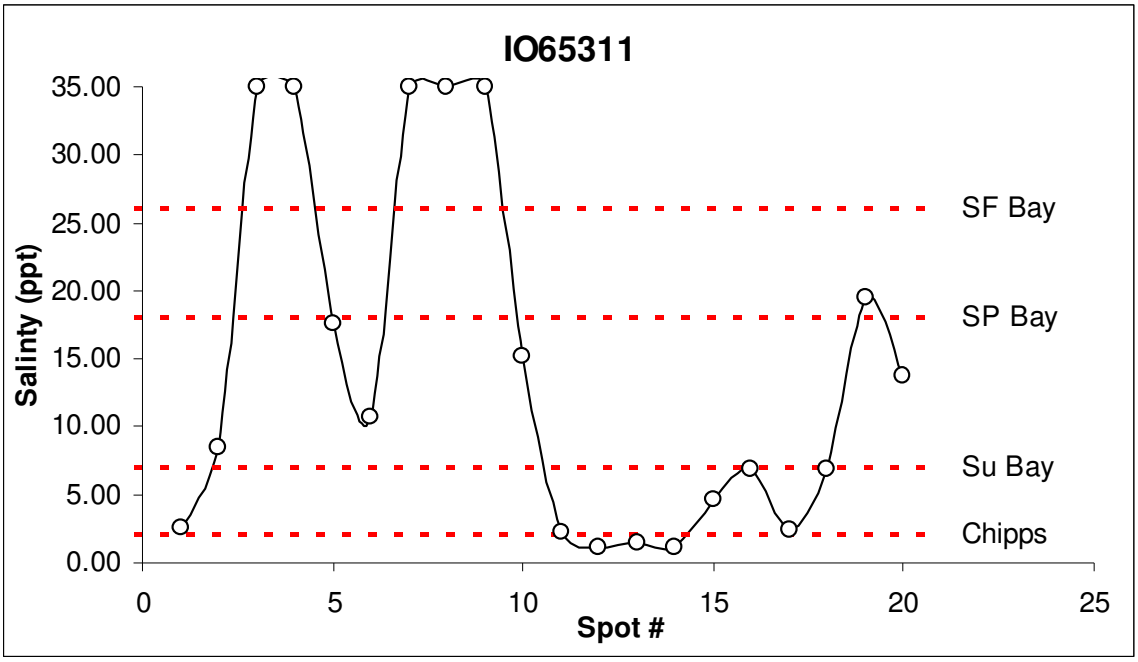


Figure 125a:
San Pablo Bay IO65311, 5 years

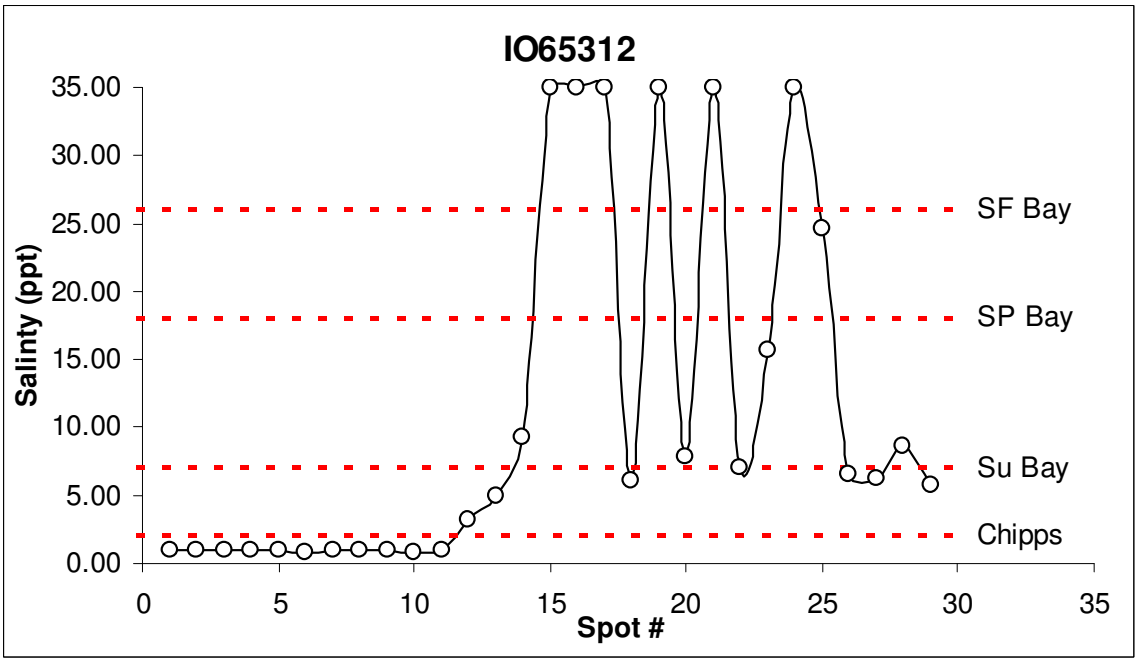


Figure 126a:
San Pablo Bay IO65312, 11 years

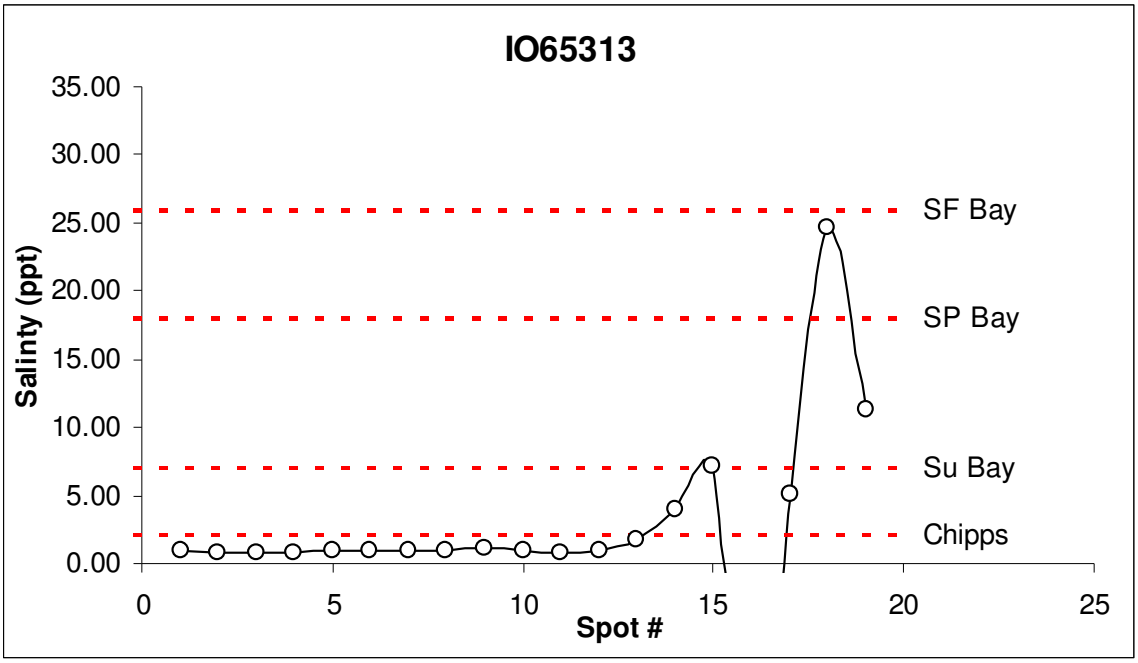


Figure 127a:
San Pablo Bay IO65313, 4 years

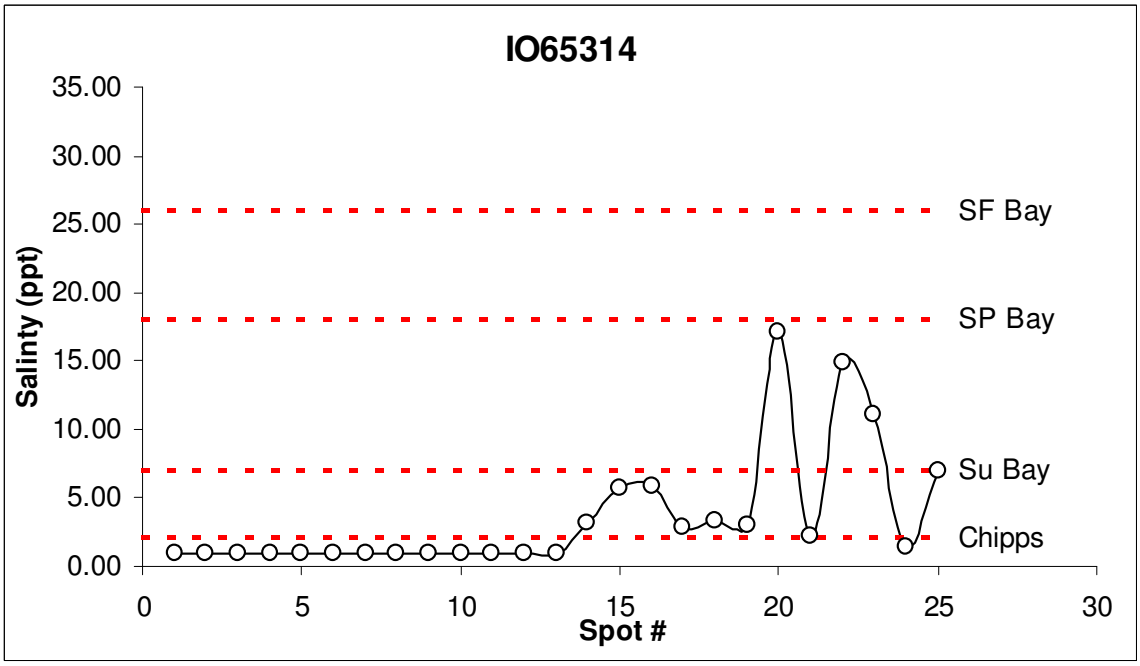


Figure 128a:
San Pablo Bay IO65314, 5 years

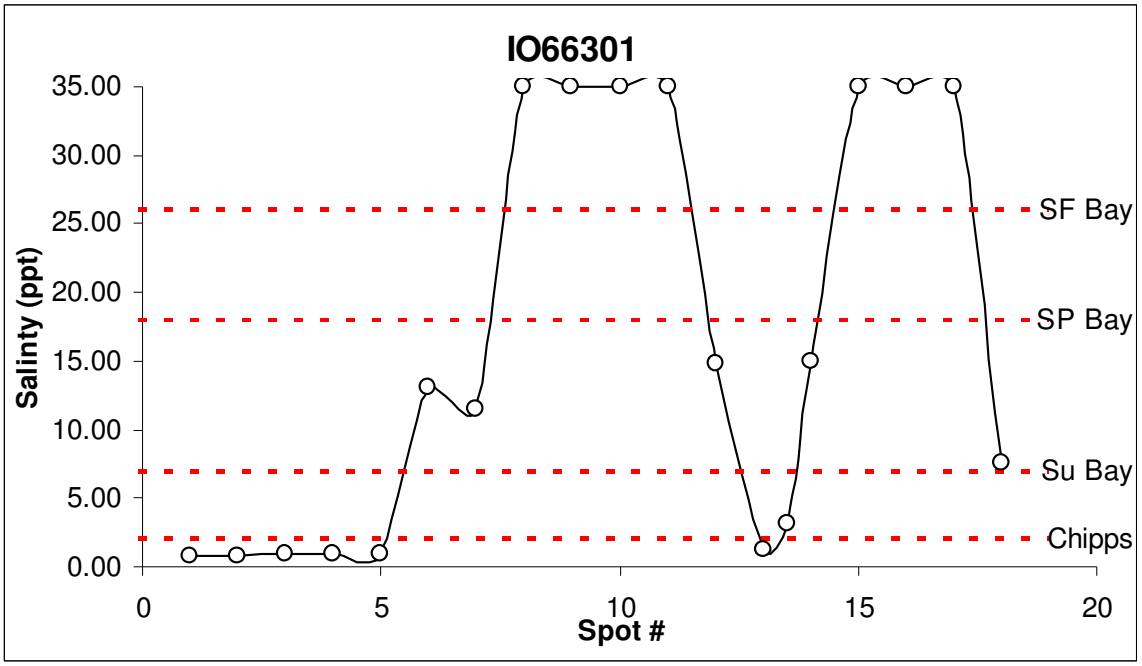


Figure 129a:
Central Bay IO66301, 4 years

Codes for data table below:

H = Domestic/Hatchery female
R = River collected female

Letter is female type, next number is female number followed by day of development and the number of that fish.

So for the code: R2-1-1= River female #2, day 1 post hatch, fish #1
For R4-3-8 = River female #4, day 3 post hatch, fish # 8.

Combined lesion scores for developing striped bass larvae. Lesions scored were: axial/spinal deformities, abdominal edema and skin blistering/edema and necrosis. 0 = no lesion, 1 = minor lesion, 2 = moderate lesion & 3 = severe lesion. N=15 larvae/female/sample period

Table 11a.

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	comments	Total lesion score
H3-1-1	0	0	0		0
H3-1-2	0	0	0		0
H3-1-3	0	0	0		0
H3-1-4	0	0	0		0
H3-1-5	0	0	0		0
H3-1-6	0	0	0		0
H3-1-7	0	0	0		0
H3-1-8	0	0	0		0
H3-1-9	0	0	0		0
H3-1-10	0	0	0		0
H3-1-11	0	0	0		0
H3-1-12	0	0	0		0
H3-1-13	0	0	0		0
H3-1-14	0	0	0		0
H3-1-15	0	0	0		0
H3-3-1	0	0	0		0
H3-3-2	0	0	1		1
H3-3-3	0	0	0		0
H3-3-4	0	0	0		0
H3-3-5	0	0	0		0
H3-3-6	0	0	0		0
H3-3-7	0	0	0		0
H3-3-8	0	0	1		1
H3-3-9	0	0	0		0
H3-3-10	0	0	0		0
H3-3-11	0	0	0		0
H3-3-12	0	0	0		0
H3-3-13	0	0	0		0
H3-3-14	0	0	0		0
H3-3-15	0	0	0		0
H3-5-1	0	0	0		0
H3-5-2	0	0	0		0
H3-5-3	0	0	0		0
H3-5-4	0	0	0		0
H3-5-5	1	0	0		1
H3-5-6	0	0	0		0
H3-5-7	0	0	0		0
H3-5-8	0	0	0		0
H3-5-9	0	0	0		0
H3-5-10	0	0	0		0
H3-5-11	0	0	0		0
H3-5-12	0	0	0		0
H3-5-13	0	0	0		0
H3-5-14	0	0	0		0
H3-5-15	0	0	1		1

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	comments	Total lesion score
H4-1-1	0	0	0		0
H4-1-2	0	0	0		0
H4-1-3	0	0	0		0
H4-1-4	0	0	0		0
H4-1-5	0	0	0		0
H4-1-6	1	0	0		1
H4-1-7	0	0	0		0
H4-1-8	0	0	0		0
H4-1-9	0	0	0		0
H4-1-10	0	0	0		0
H4-1-11	0	0	0		0
H4-1-12	0	0	0		0
H4-1-13	0	0	0		0
H4-1-14	0	0	0		0
H4-1-15	0	0	0		0
H4-3-1	0	0	0		0
H4-3-2	0	0	0		0
H4-3-3	0	0	0		0
H4-3-4	0	0	0		0
H4-3-5	0	0	0		0
H4-3-6	0	0	0		0
H4-3-7	0	0	0		0
H4-3-8	0	0	0		0
H4-3-9	0	0	0		0
H4-3-10	0	0	0		0
H4-3-11	0	0	0		0
H4-3-12	0	0	0		0
H4-3-13	0	0	0		0
H4-3-14	0	0	0		0
H4-3-15	0	0	0		0
H4-5-1	0	0	0		0
H4-5-2	0	0	0		0
H4-5-3	2	0	0		2
H4-5-4	0	0	0		0
H4-5-5	0	0	0		0
H4-5-6	1	0	0		1
H4-5-7	0	0	0		0
H4-5-8	0	0	0		0
H4-5-9	0	0	0		0
H4-5-10	0	0	0		0
H4-5-11	0	0	0		0
H4-5-12	0	0	0		0
H4-5-13	0	0	0		0
H4-5-14	1	0	0		1
H4-5-15	0	0	0		0

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	comments	Total lesion score
H5-1-1	0	0	0		0
H5-1-2	0	0	0		0
H5-1-3	0	0	0		0
H5-1-4	0	0	0		0
H5-1-5	0	0	1		1
H5-1-6	0	0	0		0
H5-1-7	0	0	1		1
H5-1-8	0	0	0		0
H5-1-9	0	0	0		0
H5-1-10	0	0	0		0
H5-1-11	0	0	0		0
H5-1-12	0	0	0		0
H5-1-13	0	0	0		0
H5-1-14	0	0	0		0
H5-1-15	0	0	1		1
H5-3-1	0	0	0		0
H5-3-2	0	0	0		0
H5-3-3	0	0	0		0
H5-3-4	0	0	0		0
H5-3-5	0	0	0		0
H5-3-6	0	0	0		0
H5-3-7	0	0	0		0
H5-3-8	0	0	0		0
H5-3-9	0	0	0		0
H5-3-10	1	0	0		0
H5-3-11	0	0	0		0
H5-3-12	0	0	0		0
H5-3-13	0	0	0		0
H5-3-14	0	0	0		0
H5-3-15	0	0	0		0
H5-5-1	0	0	0		0
H5-5-2	0	0	0		0
H5-5-3	0	0	0		0
H5-5-4	0	0	0		0
H5-5-5	0	0	0		0
H5-5-6	0	0	0		0
H5-5-7	0	0	0		0
H5-5-8	0	0	0		0
H5-5-9	0	0	0		0
H5-5-10	0	0	0		0
H5-5-11	0	0	0		0
H5-5-12	0	0	0		0
H5-5-13	1	0	0		1
H5-5-14	0	0	0		0
H5-5-15	0	0	0		0

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	comments	Total lesion score
R31-1-1	0	1	1		2
R31-1-2	0	1	1		2
R31-1-3	0	1	2		3
R31-1-4	3	2	3		8
R31-1-5	0	1	3		4
R31-1-6	0	1	2		3
R31-1-7	0	3	2		5
R31-1-8	0	1	1		2
R31-1-9	1	1	2		4
R31-1-10	1	3	1		5
R31-1-11	1	1	1		3
R31-1-12	2	3	2		7
R31-1-13	0	2	0		2
R31-1-14	2	3	1		6
R31-1-15	2	2	1		4
R31-3-1	0	0	1		1
R31-3-2	0	0	1		1
R31-3-3	0	1	3		4
R31-3-4	0	1	2		3
R31-3-5	0	0	0		0
R31-3-6	0	0	2		2
R31-3-7	0	0	0		0
R31-3-8	0	0	2		2
R31-3-9	0	1	1		2
R31-3-10	0	0	1		1
R31-3-11	0	0	1		1
R31-3-12	0	1	2		3
R31-3-13	0	0	1		1
R31-3-14	0	1	1		2
R31-3-15	0	0	1		1
R31-5-1	1	1	3		5
R31-5-2	1	1	2	Abnormal yolk: dissociated	4
R31-5-3	1	1	2	Abnormal yolk: dissociated	4
R31-5-4	0	1	2		3
R31-5-5	0	0	1		1
R31-5-6	1	1	2		4
R31-5-7	1	1	1	Abnormal yolk: dissociated	3
R31-5-8	0	1	2	Abnormal yolk: dissociated	3
R31-5-9	2	1	3		6
R31-5-10	0	0	1	Abnormal yolk: dissociated	1
R31-5-11	0	0	2		2
R31-5-12	1	1	1	Abnormal yolk: dissociated	3
R31-5-13	1	1	3		
R31-5-14	2	0	2	Abnormal yolk: dissociated	4
R31-5-15	1	1	3		5

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	Comments	Total lesion score
R33-1-1	0	0	2		2
R33-1-2	0	1	2		3
R33-1-3	1	1	1		1
R33-1-4	2	1	1		4
R33-1-5	0	1	3		4
R33-1-6	0	0	0		0
R33-1-7	0	0	1		1
R33-1-8	0	0	0		0
R33-1-9	0	1	1		2
R33-1-10	0	1	1		2
R33-1-11	0	0	0		0
R33-1-12	0	0	0		0
R33-1-13	2	0	2		4
R33-1-14	0	1	1		2
R33-1-15	1	1	1		3
R33-3-1	1	1	1	Abnormal yolk: dissociated	3
R33-3-2	0	1	2		3
R33-3-3	0	0	2		2
R33-3-4	0	2	3		5
R33-3-5	0	0	2	Abnormal yolk: dissociated	2
R33-3-6	0	1	2		3
R33-3-7	0	0	3		3
R33-3-8	1	1	1	Abnormal yolk: dissociated	3
R33-3-9	0	1	2		3
R33-3-10	1	1	2	Abnormal yolk: dissociated	4
R33-3-11	1	1	3		5
R33-3-12	0	1	2	Abnormal yolk: dissociated	3
R33-3-13	1	1	3	Abnormal yolk: dissociated	5
R33-3-14	0	1	3	Abnormal yolk: dissociated	4
R33-3-15	0	1	3	Abnormal yolk: dissociated	4
R33-5-1	0	1	1	Abnormal yolk: dissociated	2
R33-5-2	0	1	2	Abnormal yolk: dissociated	3
R33-5-3	1	0	2	Abnormal yolk: dissociated	3
R33-5-4	1	1	3	Abnormal yolk: dissociated	5
R33-5-5	0	1	3		4
R33-5-6	0	1	2		3
R33-5-7	1	1	3		5
R33-5-8	0	1	2		3
R33-5-9	1	1	2	Abnormal yolk: dissociated	4
R33-5-10	1	1	2		4
R33-5-11	0	1	2	Abnormal yolk: dissociated	3
R33-5-12	0	0	3		3
R33-5-13	2	1	2	Abnormal yolk: dissociated	5
R33-5-14	2	1	2		5
R33-5-15	0	0	2	Abnormal yolk: dissociated	2

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	comments	Total lesion score
R35-1-1	0	2	3	R35 -Worst edema	5
R35-1-2	0	2	2	of all groups	4
R35-1-3	0	3	2		5
R35-1-4	0	2	2		4
R35-1-5	0	2	3		5
R35-1-6	0	3	2		5
R35-1-7	0	3	3		6
R35-1-8	0	2	1		3
R35-1-9	0	2	2		4
R35-1-10	0	3	3		6
R35-1-11	0	3	2		5
R35-1-12	0	2	2		4
R35-1-13	0	2	2		4
R35-1-14	0	3	1		4
R35-1-15	0	2	1		3
R35-3-1	0	2	3		5
R35-3-2	0	1	2		3
R35-3-3	0	1	3		4
R35-3-4	0	2	3		5
R35-3-5	0	2	3		5
R35-3-6	0	1	3		4
R35-3-7	1	1	1		3
R35-3-8	0	1	2		3
R35-3-9	0	1	3		4
R35-3-10	0	2	3		5
R35-3-11	0	1	3		4
R35-3-12	0	1	1		2
R35-3-13	0	1	3		4
R35-3-14	0	1	3		4
R35-3-15	0	1	2		3
R35-5-1	2	2	2		6
R35-5-2	2	2	2		6
R35-5-3	0	1	2		3
R35-5-4	0	1	3		4
R35-5-5	1	1	2		4
R35-5-6	2	1	3		6
R35-5-7	0	2	3		5
R35-5-8	2	2	2		6
R35-5-9	0	1	3		4
R35-5-10	3	1	3		7
R35-5-11	1	2	3		6
R35-5-12	3	1	2		6
R35-5-13	0	1	2		3
R35-5-14	3	1	3		7
R35-5-15	0	1	1		2

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	comments	Total lesion score
R37-1-1	0	1	2		3
R37-1-2	0	2	2		4
R37-1-3	0	2	3		5
R37-1-4	0	3	2		5
R37-1-5	0	3	2		5
R37-1-6	0	2	3		5
R37-1-7	0	3	2		5
R37-1-8	0	1	2		3
R37-1-9	0	2	2		4
R37-1-10	1	2	2		5
R37-1-11	0	3	2		5
R37-1-12	0	2	2		4
R37-1-13	0	1	2		3
R37-1-14	0	1	2		3
R37-1-15	0	2	3		5
R37-3-1	0	3	3		6
R37-3-2	0	1	3		4
R37-3-3	0	2	2		4
R37-3-4	1	2	3		6
R37-3-5	0	2	2		4
R37-3-6	1	2	1		4
R37-3-7	0	3	2		5
R37-3-8	0	2	1		3
R37-3-9	0	2	3		5
R37-3-10	0	2	2		4
R37-3-11	0	1	2		3
R37-3-12	1	2	3		6
R37-3-13	1	2	2		5
R37-3-14	0	2	2		4
R37-3-15	1	3	2		6
R37-5-1				No survival to	
R37-5-2				Day 5	
R37-5-3					
R37-5-4					
R37-5-5					
R37-5-6					
R37-5-7					
R37-5-8					
R37-5-9					
R37-5-10					
R37-5-11					
R37-5-12					
R37-5-13					
R37-5-14					
R37-5-15					

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	comments	Total lesion score
R38-1-1	0	2	2		4
R38-1-2	0	2	3		5
R38-1-3	0	1	2		3
R38-1-4	0	3	3		6
R38-1-5	0	2	3		5
R38-1-6	0	1	2		3
R38-1-7	0	2	2		4
R38-1-8	0	3	2		5
R38-1-9	0	3	3		6
R38-1-10	1	2	2		5
R38-1-11	0	1	2		3
R38-1-12	1	1	2		4
R38-1-13	1	2	2		5
R38-1-14	0	1	2		3
R38-1-15	0	1	1		2
R38-3-1	0	0	2		2
R38-3-2	0	1	2		3
R38-3-3	0	1	1		2
R38-3-4	0	1	0		1
R38-3-5	0	0	0		0
R38-3-6	0	0	0		0
R38-3-7	0	1	1		2
R38-3-8	0	1	2		3
R38-3-9	0	2	3		5
R38-3-10	1	1	2		4
R38-3-11	0	1	2		3
R38-3-12	1	1	2		4
R38-3-13	0	1	3		4
R38-3-14	2	1	3		6
R38-3-15	0	1	2		3
R38-5-1				No survival to	
R38-5-2				Day 5	
R38-5-3					
R38-5-4					
R38-5-5					
R38-5-6					
R38-5-7					
R38-5-8					
R38-5-9					
R38-5-10					
R38-5-11					
R38-5-12					
R38-5-13					
R38-5-14					
R38-5-15					

Fish ID	Lordosis/Kyphosis	abdominal edema	fin necrosis/edema	comments	Total lesion score
R39-1-1	0	2	2		4
R391-2	3	3	2		8
R39-1-3	0	2	2		4
R39-1-4	0	0	0		0
R39-1-5	0	3	2		5
R39-1-6	0	2	2		4
R39-1-7	0	3	2		5
R39-1-8	3	2	2		7
R39-1-9	0	2	2		4
R39-1-10	0	2	2		4
R39-1-11	0	1	2		3
R39-1-12	0	1	2		3
R39-1-13	0	1	1		2
R39-1-14	0	3	2		5
R39-1-15	0	0	0		0
R39-3-1	0	2	2		4
R39-3-2	0	1	2		3
R39-3-3	0	2	2		4
R39-3-4	0	2	1		3
R39-3-5	0	2	3		5
R39-3-6	0	2	3		5
R39-3-7	0	2	3		5
R39-3-8	0	2	2		4
R39-3-9	0	2	3		5
R39-3-10	0	2	3		5
R39-3-11	0	2	2		4
R39-3-12	0	2	2		4
R39-3-13	0	2	3		5
R39-3-14	0	0	0		0
R39-3-15	0	2	3		5
R39-5-1				No survival to	
R39-5-2				Day 5	
R39-5-3					
R39-5-4					
R39-5-5					
R39-5-6					
R39-5-7					
R39-5-8					
R39-5-9					
R39-5-10					
R39-5-11					
R39-5-12					
R39-5-13					
R39-5-14					
R39-5-15					

Figure 130a
Live domestic/control: Normal 3 days post-hatching larvae

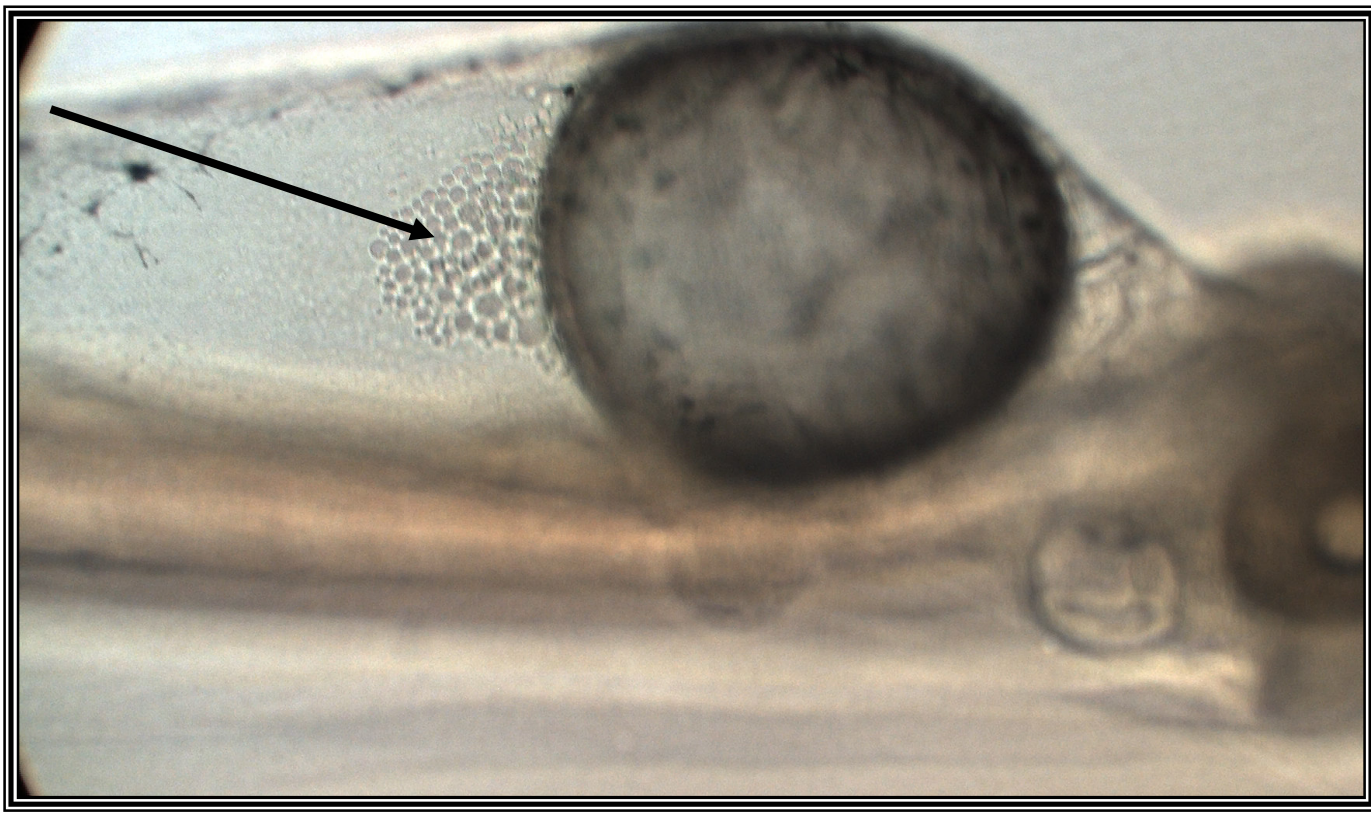


Figure 131a
Live river larvae: Abnormal yolk (arrow) 3 days post-hatching

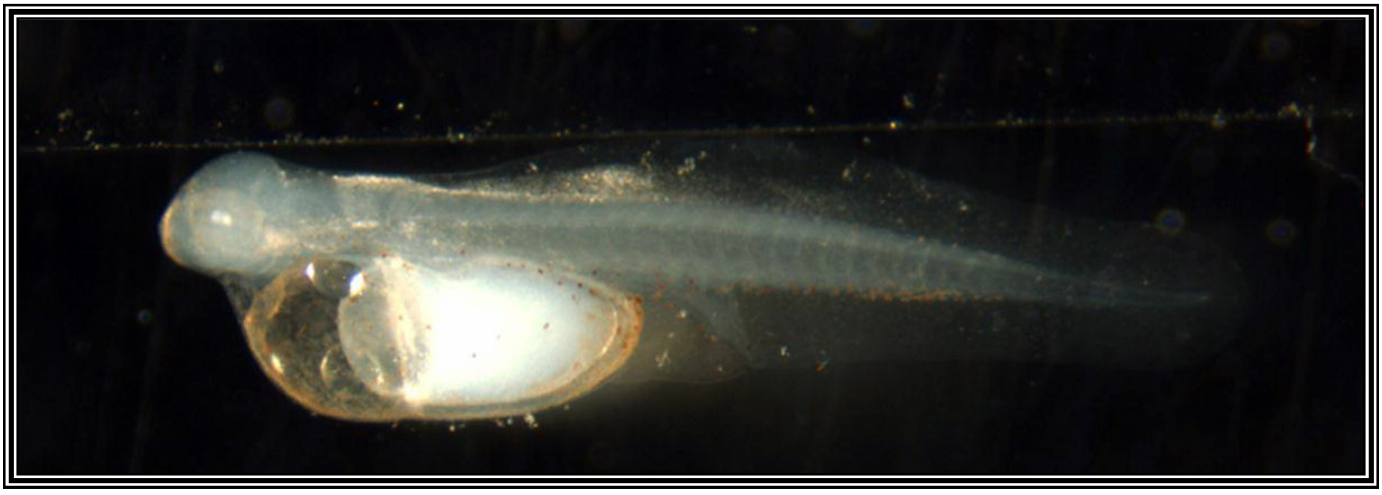


Figure 132a.
Preserved domestic/control: Normal 1 day post-hatching larvae

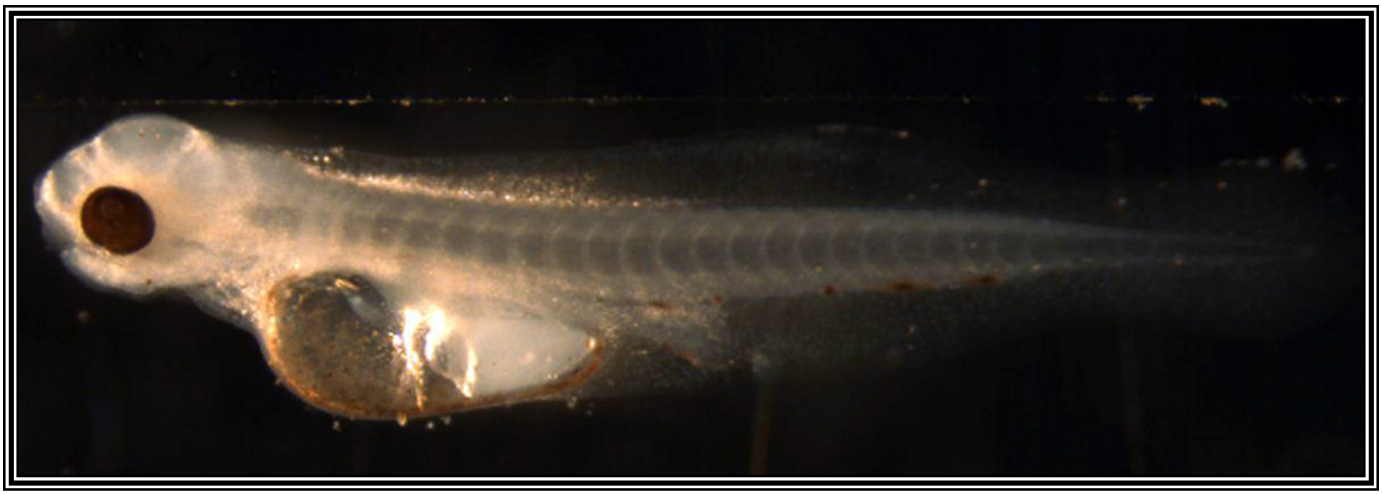


Figure 133a
Preserved domestic/control: Normal 3 day post-hatching larvae

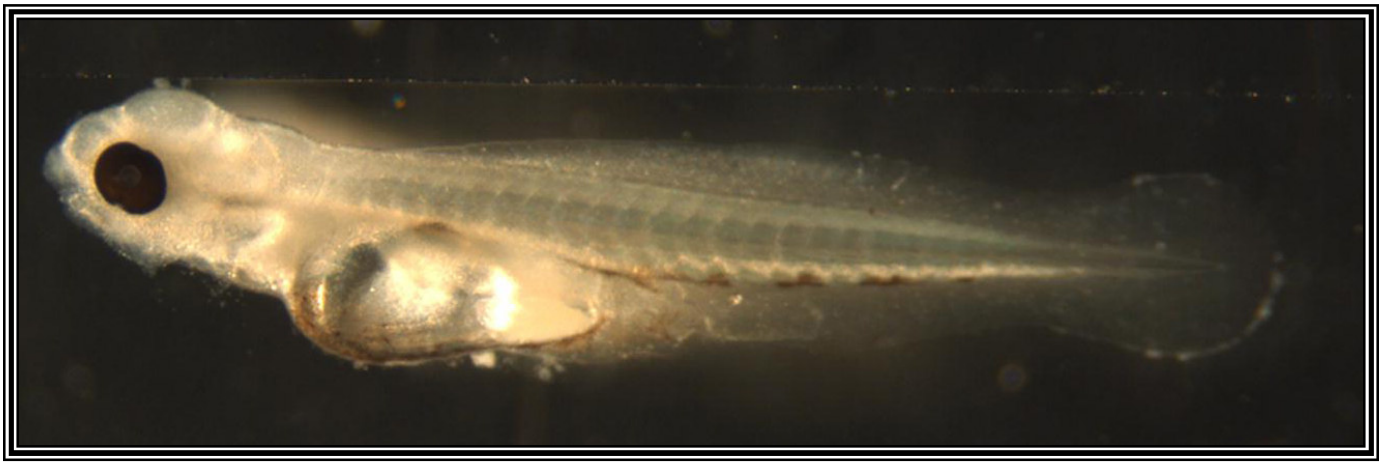


Figure 134a
Preserved domestic/control: Normal 5 day post-hatching larvae

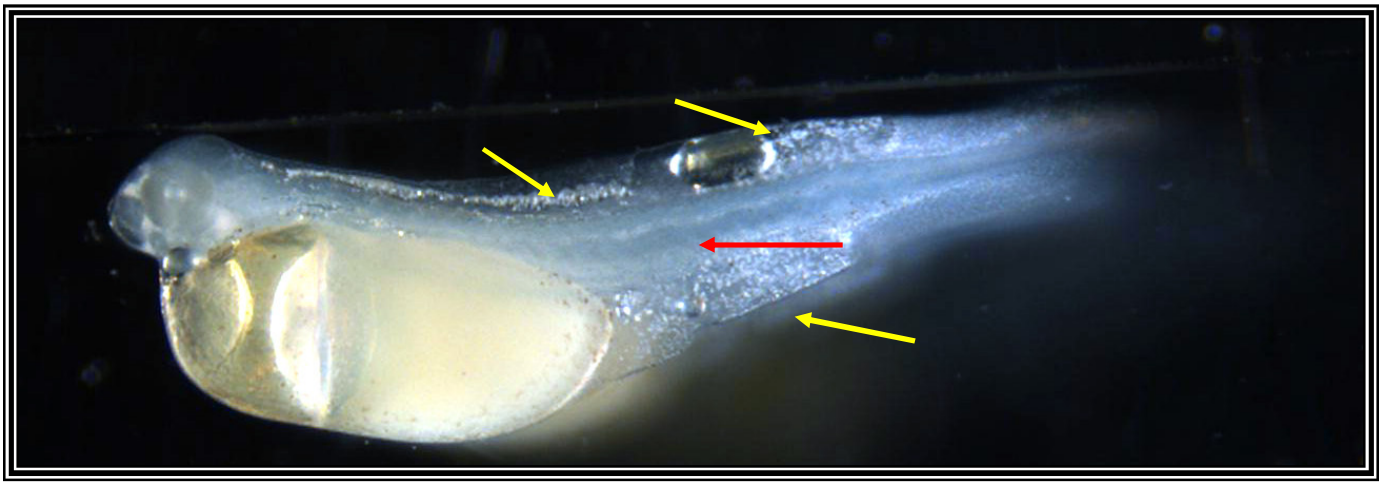


Figure 135a

Preserved river: Abnormal 1 day post-hatching larvae yellow arrows indicate fin blistering, edema, necrosis and red arrow indicates spinal abnormality/lordosis

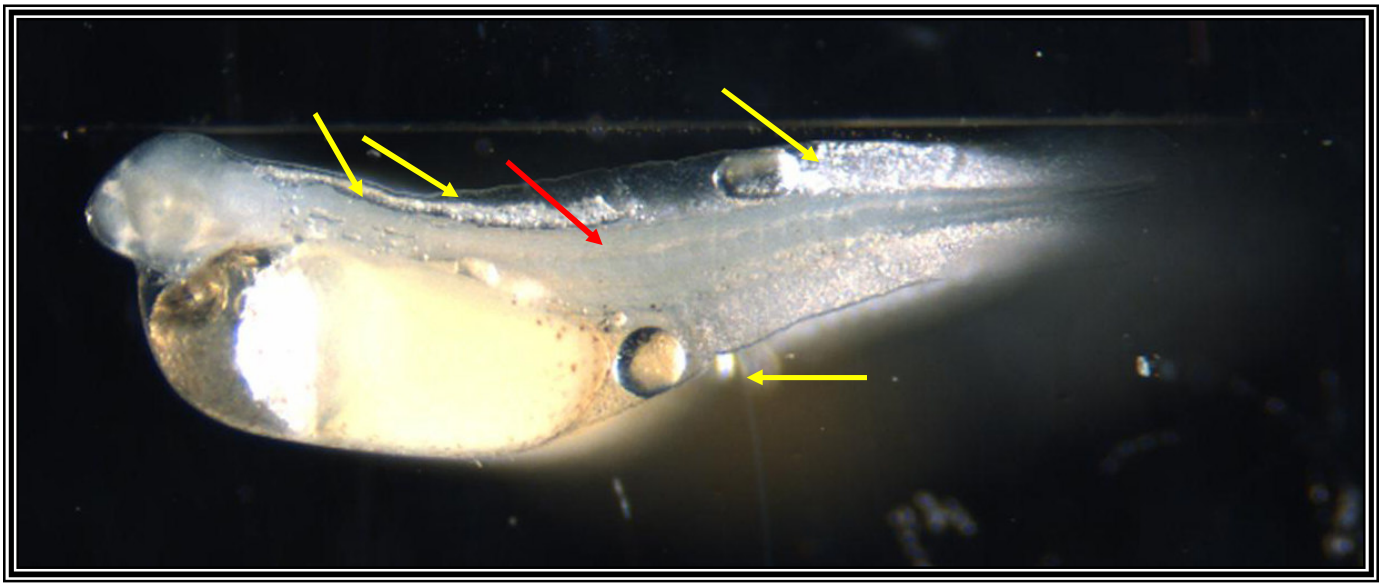


Figure 136a

Preserved river: Abnormal 1 day post-hatching larvae; yellow arrows indicate fin blistering, edema, necrosis and red arrow indicates spinal abnormality/lordosis

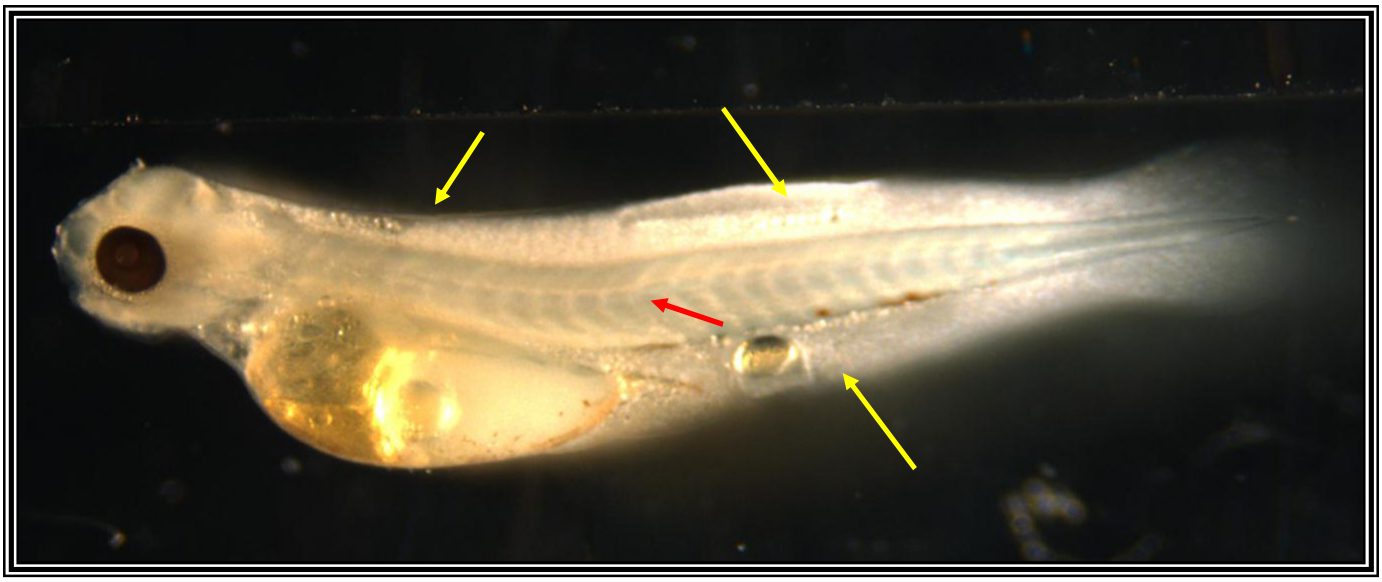


Figure 137a
Preserved river: Abnormal 3 day post-hatching larvae; yellow arrows indicate fin blistering, edema, necrosis and red arrow indicates spinal abnormality/lordosis

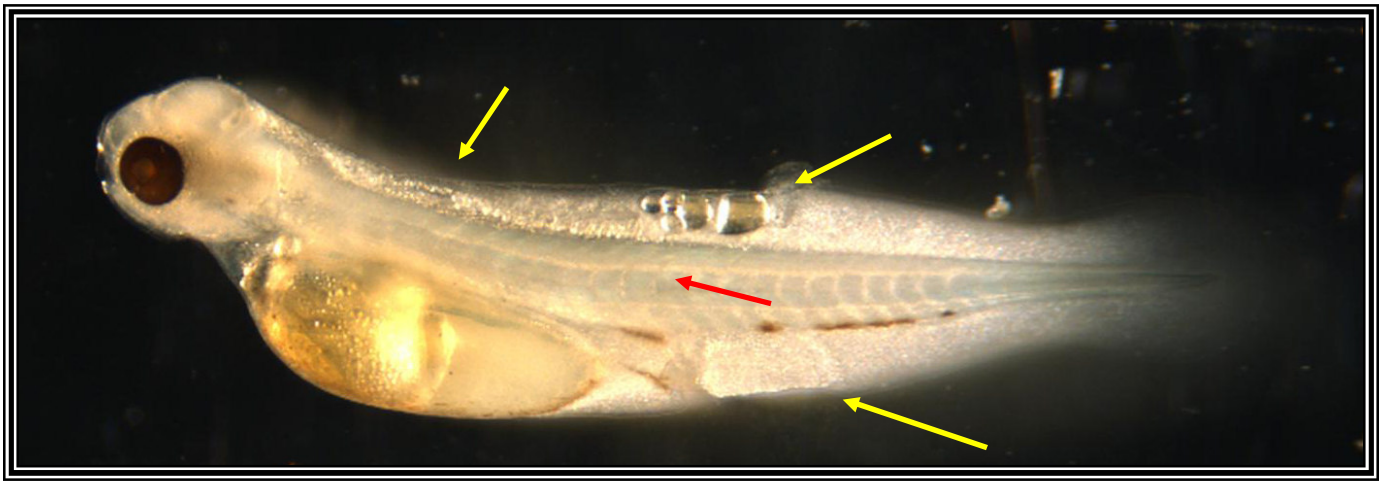


Figure 138a
Preserved river: Abnormal 3 day post-hatching larvae; yellow arrows indicate fin blistering, edema, necrosis and red arrow indicates spinal abnormality/lordosis

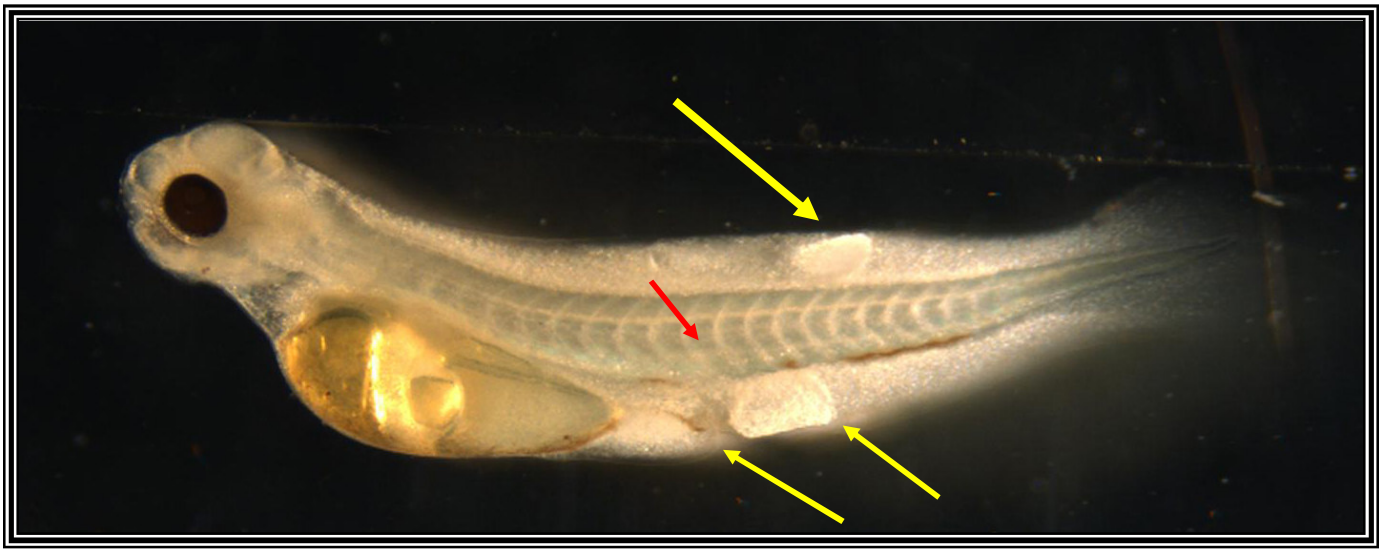


Figure 139a

Preserved river: Abnormal 3 day post-hatching larvae; yellow arrows indicate fin blistering, edema, necrosis and red arrow indicates spinal abnormality/lordosis

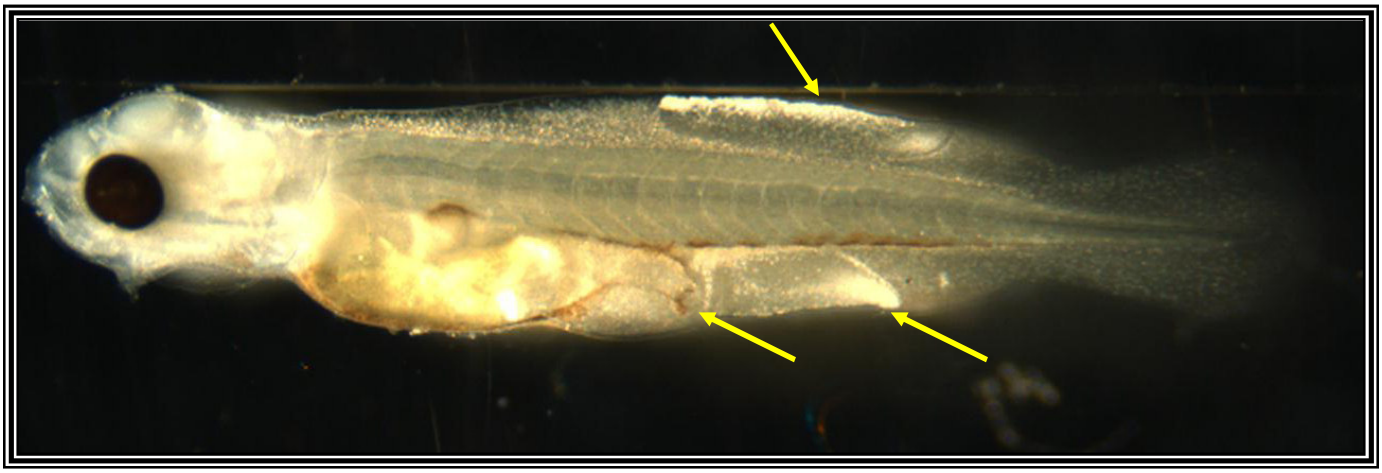


Figure 140a

Preserved river: Abnormal 5 day post-hatching larvae; yellow arrows indicate fin blistering, edema, necrosis

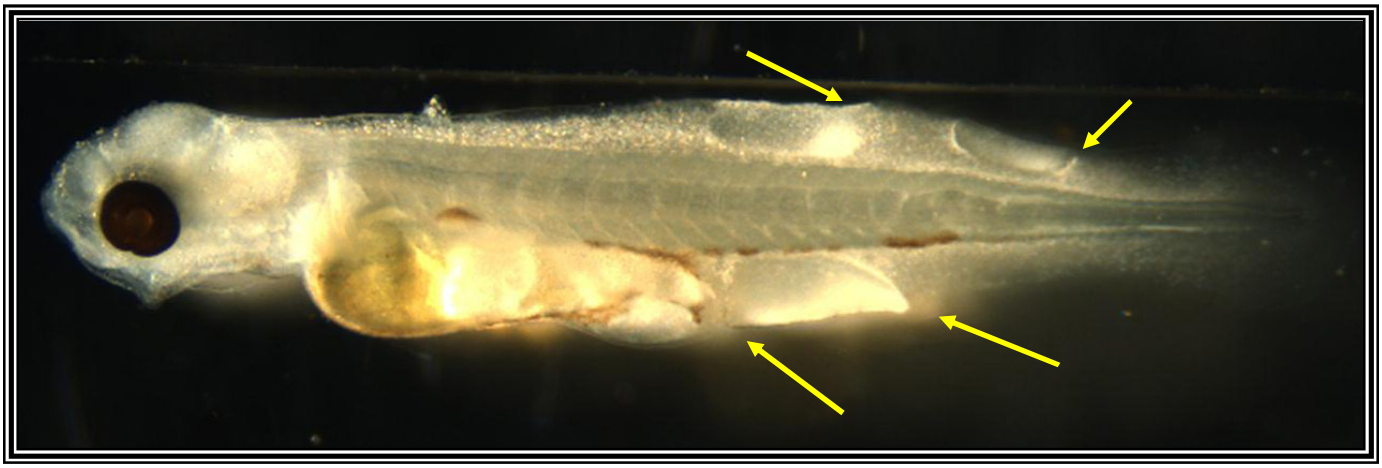


Figure 141a

Preserved River: Abnormal 5 day post-hatching larvae; yellow arrows indicate fin blistering, edema, necrosis

PATHOLOGY REPORT

Group: TNS 2007

Date of Report: April 6, 2009

Species: Striped Bass

Diagnoses:
1) Coelomitis, variable, granulomatous, verminous; with the formation of discrete granulomas; due to trematode infection (34%) (see narrative and comment)
2) Coccidiosis, mild to moderate, enteric (see narrative and comment)

History: Multiple whole juvenile striped bass (112) that were collected from the Sacramento-San Joaquin Delta in 2007 were submitted in formalin for microscopic examination.

Microscopic Examination: Tissues from 112 juvenile striped bass were examined microscopically; please refer to Table X that contains the identification numbers and data for each fish.

The most common finding in these fish was an intracoelomic trematode infection that resulted in the formation of discrete trematode granulomas. Specifically, 34% of the fish (38/112) were infected, whereas 3% (1/38) had a rare infection (Fish #12); 74% (28/38 had a mild infection (Fish #16, 18-21, 49, 56-58, 61-64, 70, 75, 83, 89, 93-94, 96, 99, 101, 103, 112, 118, 124, 126, 162); 18% (7/38 had a mild to moderate infection (Fish #15, 68, 84, 147, 100, 114, 122); 3% (1/38) had a moderate infection (Fish #31); and 3% (1/38) had a moderate to severe infection (Fish #97). However, there was no significant difference among the various subgroups of these fish including the collection station or time of collection.

Finally, four (4) fish had an enteric coccidian infection of variable severity (Fish # 114, 147, 149, and 162), although this was not considered a significant finding.

Comment: The primary finding in these fish was the variable occurrence of occasional discrete verminous granulomas within the coelomic cavity of several fish that was not an unexpected finding, since trematode infections are not an uncommon finding in wild fishes. However, to have 34% of this group infected is a higher than normal incidence of infection and therefore constitutes a significant finding

The rare enteric coccidiosis in these fish was considered a conservative estimate since mild and/or early infections are often difficult to detect in histological sections. Although the enteric coccidiosis was not considered a significant finding, severe infection may compromise enteric function in an affected fish.

The absence of branchial parasitic infections in these fish was due to the absence of the heads in the fish submitted for microscopic examination. Given the nature and severity of branchial parasitic infections seen in fish from other DFG surveys (where branchial tissue was available) it must be noted that it is likely these fish also had high levels of branchial parasitic infections. In summary the nature and severity of these infections are not considered to be a normal finding. The fish evaluated are suffering from an abnormal incidence of parasitic infections and as such under significant physiological stress. Please contact for additional information as necessary.

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PATHOLOGY REPORT

Group: SKT 2007

Date of Report: April 6, 2009

Species: Striped Bass

Diagnosis: Branchitis, mild, ciliated protozoan; striped bass (Fish#056, 320 and 326)

History: Multiple whole juvenile striped bass (11) that were collected from the Sacramento-San Joaquin Delta in 2007 were submitted in formalin for microscopic examination.

Microscopic Examination and Comment: Tissues from 11 juvenile striped bass were examined microscopically; please refer to Table X that contains the identification numbers and data for each fish.

Three (3) fish (Fish#056, 320 and 326 had a mild branchial parasitic infection. Otherwise, there were no additional findings. Please contact for additional information as necessary.

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PATHOLOGY REPORT

Group: POD 2007

Date of Report: April 6, 2009

Species: Striped Bass

Diagnoses:
1) Branchitis, variable severity (79%), multifocal, epithelial, lamellar, protozoal, ciliate (primarily trichodinids with other ciliated protozoans); striped bass (see narrative and comment)
2) Coelomitis, mild, granulomatous, verminous; with the formation of discrete granulomas; due to trematode infection (see comment)
3) Branchitis, rare, epithelial, lamellar; presumptive bacterial (=chlamydial) etiology (see narrative and comment)
4) Coccidiosis, mild to moderate, enteric (see narrative)

History: Multiple whole juvenile striped bass (130) that were collected from the Sacramento-San Joaquin Delta in 2007 were submitted in formalin for microscopic examination.

Microscopic Examination: Tissues from 130 juvenile striped bass were examined microscopically; please refer to Table X that contains the identification numbers and data for each fish.

The primary significant finding in these fish was a branchial ciliated protozoan parasitic infection of variable severity. Specifically, 103 of 130 were affected (79%), whereas 10% (10/103) had a rare infection (Fish #020, 025-026, 036, 217, 243, 264, 314, 333, and no #); 53% (55/103) had a mild infection (Fish #017, 035, 060, 088, 091-092, 103-104, 111-112, 129, 131-133, 143, 145-146, 156, 168, 200-203, 229, 231, 239, 241, 246, 248, 258, 265, 267, 274, 277, 279, 290, 294, 296,312, 318, 320-321, 334-335, 338, 343, 345, 354, 359-360, 363-365, and 372-373); 16% (16/103) had a mild to moderate infection (Fish #038, 144, 205, 247, 266, 291, 293, 316, 336-337, 358, 362, 367, 370, and 375-376); 10% (10/103) had a moderate infection (Fish #62, 232, 250, 280, 292, 317, 341-342, 353, and 368); 6% (6/103) had a moderate to severe infection (Fish #130, 204, 242, 249, 287, and 371); and 6% had a severe infection (Fish #278, 295, 297, 332, and 349-350).

There was also a rare (Fish #230, 247, 290, 320, and 332) to mild (Fish #017, 023, 278, 017, 318, and 297) to moderate (Fish #091 and 143) or moderate to severe (Fish #131) epitheliocystis infection in several fish, although this was not considered a significant finding.

Likewise, only three (3) fish (Fish #229, 321, and 345) had a mild intracoelomic trematode infection that resulted in the formation of discrete trematode granulomas, although this was considered an incidental, but not a significant finding.

Finally, five (5) fish had a mild enteric coccidian infection (Fish #112, 258, 168, 316, and 367), whereas one (1) fish had a moderate enteric coccidian infection (Fish #372), although this was not considered a significant finding.

Comment: The most significant finding in these fish was the branchial protozoan infection in several fish that was consistent with a trichodinid infection, but also included other ciliated protozoan such as sessile ciliates. There was no significant difference among the various subgroups of these fish including the collection station or time of collection. Regardless, it should be understood that the antemortem severity of external parasitic infections cannot be definitively determined by histological examination, since external parasitic agents will generally leave the host following death of the host or will be removed from the tissue following fixation of the tissues. In this context, a more definitive determination of the severity of infection can only be determined by the cytological examination of branchial preparations using branchial tissue obtained from live fish or fish immediately following euthanasia. In addition, a definitive identification of the protozoan parasites cannot be performed on histological sections but also requires cytological preparations. However, Trichodiniasis can result in significant, chronic morbidity and mortality especially in juvenile fish and is often associated with factors or stressors that further predispose fish to infection.

The occurrence of occasional discrete verminous granulomas within the coelomic cavity of several fish was not an unexpected finding, since trematode infections are not an uncommon finding in wild fishes.

The occurrence of enlarged lamellar epithelial cells in occasional fish that were further characterized by an abundance of dense basophilic cytoplasm, which was consistent with an intracellular bacterial (=chlamydial) infection that is often referred to as epitheliocystis infection, was an interesting finding but was not considered a significant finding in these individual fish due to the rare or mild occurrence of these cells. Regardless of the significance, a definitive etiological diagnosis requires electron-microscopic examination of these cells, which can be performed for completeness as necessary.

Finally, the enteric coccidiosis in these fish was considered a conservative estimate since mild and/or early infections are often difficult to detect in histological sections. Although the enteric coccidiosis was not considered a significant finding, severe infection may compromise enteric function in an affected fish. Please contact for additional information as necessary.

_____

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PATHOLOGY REPORT

Group: FMWT 2007

Date of Report: April 6, 2009

Species: Striped Bass

Diagnoses:
1) Branchitis, variable severity (65%), multifocal, epithelial, lamellar, protozoal, ciliate (primarily trichodinids with other ciliated protozoans); striped bass (see narrative and comment)
2) Coelomitis, variable, granulomatous, verminous; with the formation of discrete granulomas; due to trematode infection (see comment)
3) Branchitis, rare, epithelial, lamellar; presumptive bacterial (=chlamydial) etiology (see narrative and comment)
4) Coccidiosis, enteric (see narrative and comment)
5) Intracytoplasmic accumulation of eosinophilic droplets, multifocal, proximal renal tubules (see narrative and comment)

History: Multiple juvenile striped bass (62) that were collected from the Sacramento-San Joaquin Delta in 2007 were submitted in formalin for microscopic examination.

Microscopic Examination: Tissues from 62 juvenile striped bass were examined microscopically; please refer to Table X that contains the identification numbers and data for each fish.

The primary significant finding in these fish was a branchial ciliated protozoan parasitic infection of variable severity. Specifically, 40 of 62 fish were affected (65%), whereas 20% (8/40) had a rare infection (Fish#17, 25, 31-32, 35, 44, 54, and 63); 40% (16/40) had a mild infection (Fish#6, 8, 15, 22-23, 27-28, 30, 37-38, 41, 45, 49, 53, 56, and 60); 13% (5/40) had a mild to moderate infection (Fish#5, 10, 12, 16, and 58); 23% (9/40) had a moderate infection (Fish#7, 9, 11, 24, 40, 48, 55, 57, and 74); and 5% (2/40) had a moderate to severe infection (Fish#14 and 46).

Two (2) fish (Fish#35 and 38) has a rare epitheliocystis infection.

There was a rare (Fish#12, 34, 36, 39, 45, 64, and 67) to mild (Fish#5, 16, 44, 59, 56-57, 68, 71, and 73) or mild to moderate (Fish#35, 55 and 70) to moderate (Fish#31 and 69) intracoelomic trematode infection with the formation of discrete trematode granulomas.

Occasional fish had a rare (Fish#25 and 57) to mild (Fish#5-7, 17, 23, 27, 31, 67, 69) or mild to moderate (Fish #38) or moderate to severe (Fish #24) enteric coccidiosis.

Three (3) fish had eosinophilic droplets (Fish#48-50).

Comment: The most significant finding in these fish was the branchial protozoan infection in several fish that was consistent with a trichodinid infection, but also included other ciliated protozoan such as sessile ciliates. There was no significant difference among the various subgroups of these fish including the collection station or time of collection. Regardless, it should be understood that the antemortem severity of external parasitic infections cannot be definitively determined by histological examination, since external parasitic agents will generally leave the host following death of the host or will be removed from the tissue following fixation of the tissues. In this context, a more definitive determination of the severity of infection can only be determined by the cytological examination of branchial preparations using branchial tissue obtained from live fish or fish immediately following euthanasia. In addition, a definitive identification of the protozoan parasites cannot be performed on histological sections but also requires cytological preparations. However, Trichodiniasis can result in significant, chronic morbidity and mortality especially in juvenile fish and is often associated with factors or stressors that further predispose fish to infection.

The occurrence of occasional discrete verminous granulomas within the coelomic cavity of several fish was not an unexpected finding, since trematode infections are not an uncommon finding in wild fishes.

The occurrence of enlarged lamellar epithelial cells in occasional fish that were further characterized by an abundance of dense basophilic cytoplasm, which was consistent with an intracellular bacterial (=chlamydial) infection that is often referred to as epitheliocystis infection, was an interesting finding but was not considered a significant finding due to the rare occurrence of these cells. Regardless of the significance, a definitive etiological diagnosis requires electron-microscopic examination of these cells, which can be performed for completeness as necessary.

The enteric coccidiosis in these fish was considered a conservative estimate since mild and/or early infections are often difficult to detect in histological sections. Although the enteric coccidiosis was not considered a significant finding, severe infection may compromise enteric function in an affected fish.

Finally, the intracytoplasmic accumulation of eosinophilic droplets is not an uncommon finding in various fishes (wild or captive and freshwater or marine species) and is generally considered a normal finding due to protein absorption of the proximal renal tubules from the glomerular filtrate. However, in higher vertebrates (mammals), the presence of intracytoplasmic protein droplets is generally associated with protein absorption due to a glomerulopathy that results in the loss of protein in the glomerular filtrate, whereas in fishes the accumulation of eosinophilic droplets within the proximal renal tubules is generally not associated with glomerular lesions. However, some pathologists have considered that the loss of protein within the glomerular filtrate and the subsequent absorption of this protein in the proximal renal tubules may occasionally be associated with exposure to increased ammonia concentrations or exposure to toxicants. In this context, the observation of eosinophilic droplets within the proximal renal tubules of a few juvenile striped bass should not be dismissed as a normal finding, but should be considered as a possible indicator of a toxic or environmental insult in these fish. Please contact for additional information as necessary.

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Appendix B: Bibliography

Ackermann, G. E., J. Schwaiger, et al. (2002). "Effects of long-term nonylphenol exposure on gonadal development and biomarkers of estrogenicity in juvenile rainbow trout Oncorhynchus mykiss." Aquat Toxicol **60**(3-4): 203-21.

Arsenault, J. T., W. L. Fairchild, et al. (2004). "Effects of water-borne 4-nonylphenol and 17beta-estradiol exposures during parr-smolt transformation on growth and plasma IGF-I of Atlantic salmon (Salmo salar L.)." Aquat Toxicol **66**(3): 255-65.

Arzuaga, X., D. Wassenberg, et al. (2006). "The chlorinated AHR ligand 3,3',4,4',5-pentachlorobiphenyl (PCB126) promotes reactive oxygen species (ROS) production during embryonic development in the killifish (Fundulus heteroclitus)." Aquat Toxicol **76**(1): 13-23.

Bailey, H. C., C. DiGiorgio, et al. (1994). "The Effect of Agricultural Discharge on Striped Bass (Morone saxatilis) in California Sacramento San Joaquin Drainage." Ecotoxicology **3**(2): 123-142.

Bailey, H. C., C. DiGiorgio, et al. (1993). "The Effect of Agricultural Discharge on Striped Bass (Morone saxatilis) in California Sacramento San Joaquin Drainage." Ecotoxicology and Environmental Safety.

Bailey, H. C., D. J. Ostrach, et al. (1991). "Toxicity of Agricultural Return Waters to Early Life Stages of Striped Bass (*Morone saxatilis*) and Opossum Shrimp (*Neomysis mercedis*)." Society of Environmental Toxicology and Chemistry **12 Annual Meeting**: 50.

Bennett, W. A., D. J. Ostrach, et al. (1995). "Larval Striped Bass Condition in a Drought-stricken Estuary: Evaluating Pelagic Food-web Limitation." Ecological Applications **5**(3): 680-692.

Billiard, S. M., N. C. Bols, et al. (2004). "In vitro and in vivo comparisons of fish-specific CYP1A induction relative potency factors for selected polycyclic aromatic hydrocarbons." Ecotoxicol Environ Saf **59**(3): 292-9.

Billiard, S. M., A. R. Timme-Laragy, et al. (2006). "The role of the aryl hydrocarbon receptor pathway in mediating synergistic developmental toxicity of polycyclic aromatic hydrocarbons to zebrafish." Toxicol Sci **92**(2): 526-36.

Bozcaarmutlu, A. and E. Arinc (2004). "Inhibitory effects of divalent metal ions on liver microsomal 7-ethoxyresorufin O-deethylase (EROD) activity of leaping mullet." Mar Environ Res **58**(2-5): 521-4.

Cai, L., J. B. Klein, et al. (2000). "Metallothionein inhibits peroxynitrite-induced DNA and lipoprotein damage." J Biol Chem **275**(50): 38957-60.

Campana, S. E. a. C. M. J. (1992). Analysis of otolith microstructure data. Otolith Microstructure and analysis. I. D. K. S. a. S. E. C. [ed], Can. Spec. Publ. Fish. Aquat.Sci. **117:** p. 73-100.

Carney, S. A., R. E. Peterson, et al. (2004). "2,3,7,8-Tetrachlorodibenzo-p-dioxin activation of the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator pathway causes developmental toxicity through a CYP1A-independent mechanism in zebrafish." Mol Pharmacol **66**(3): 512-21.

Chan, P. K. and S. H. Cheng (2003). "Cadmium-induced ectopic apoptosis in zebrafish embryos." Arch Toxicol **77**(2): 69-79.

Chatterjee, A. and I. B. Maiti (1991). "Induction and turnover of catfish (Heteropneustes fossilis) metallothionein." Mol Cell Biochem **108**(1): 29-38.

Chung, M. J., P. A. Walker, et al. (2005). "ZINC-mediated gene expression offers protection against H2O2-induced cytotoxicity." Toxicol Appl Pharmacol **205**(3): 225-36.
Cionna, C., F. Maradonna, et al. (2006). "Effects of nonylphenol on juveniles and adults in the grey mullet, Liza aurata." Reprod Toxicol **22**(3): 449-54.
De Wit CA (2002) "An overview of brominated flame retardants in the environment." *Chemosphere* 46:583–624.
da Silva, M. E. and N. C. Meirelles (2004). "Interaction of non-ionic surfactants with hepatic CYP in Prochilodus scrofa." Toxicol In Vitro **18**(6): 859-67.
Dong, W., H. Teraoka, et al. (2004). "Role of aryl hydrocarbon receptor in mesencephalic circulation failure and apoptosis in zebrafish embryos exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin." Toxicol Sci **77**(1): 109-16.
Drevnick, P. E., M. B. Sandheinrich, et al. (2006). "Increased ovarian follicular apoptosis in fathead minnows (Pimephales promelas) exposed to dietary methylmercury." Aquat Toxicol **79**(1): 49-54.
Ellmann GL, C. K., Andres VJr., Feather-Stone RM (1961). "A new and rapid colorimetric determination of acetylcholinesterase activity." Biochem Pharmacol: 7:88-95.
Fenske, M., G. Maack, et al. (2005). "An environmentally relevant concentration of estrogen induces arrest of male gonad development in zebrafish, Danio rerio." Environ Toxicol Chem **24**(5): 1088-98.
Ferreira, M., P. Antunes, et al. (2004). "Organochlorine contaminants in flounder (Platichthys flesus) and mullet (Mugil cephalus) from Douro estuary, and their use as sentinel species for environmental monitoring." Aquat Toxicol **69**(4): 347-57.
Fukada, H., A. Haga, et al. (2001). "Development and validation of chemiluminescent immunoassay for vitellogenin in five salmonid species." Comp Biochem Physiol A Mol Integr Physiol **130**(1): 163-70.
Fulton MH, K. P. (2001). "Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects." Environ Toxicol Chem: 20:37-45.
George, S., M. Gubbins, et al. (2004). "A comparison of pollutant biomarker responses with transcriptional responses in European flounders (Platicthys flesus) subjected to estuarine pollution." Mar Environ Res **58**(2-5): 571-5.
George, S. G. and P. Young (1986). "The time course of effects of cadmium and 3-methylcholanthrene on activities of enzymes of xenobiotic metabolism and metallothionein levels in the plaice, Pleuronectes platessa." Comp Biochem Physiol C **83**(1): 37-44.
Guiney, P. D., R. M. Smolowitz, et al. (1997). "Correlation of 2,3,7,8-tetrachlorodibenzo-p-dioxin induction of cytochrome P4501A in vascular endothelium with toxicity in early life stages of lake trout." Toxicol Appl Pharmacol **143**(2): 256-73.
Harris, C. A., E. M. Santos, et al. (2001). "Nonylphenol affects gonadotropin levels in the pituitary gland and plasma of female rainbow trout." Environ Sci Technol **35**(14): 2909-16.
Hegelund, T., K. Ottosson, et al. (2004). "Effects of the antifungal imidazole ketoconazole on CYP1A and CYP3A in rainbow trout and killifish." Environ Toxicol Chem **23**(5): 1326-34.
Henegariu, O., P. Bray-Ward, et al. (2000). "Custom fluorescent-nucleotide synthesis as an alternative method for nucleic acid labeling." Nat Biotechnol **18**(3): 345-8.

Heppell, S. A., N. D. Denslow, et al. (1995). "Universal assay of vitellogenin as a biomarker for environmental estrogens." Environ Health Perspect **103 Suppl 7**: 9-15.
Heppell, S. A. and C. V. Sullivan (1999). "Gag (Mycteroperca microlepis) vitellogenin: purification, characterizzation and use for enzyme linked immunosorbent assay (ELISA) of female maturity in three species of grouper." Fish Physiology and Biochemistry **20**: 361-374.
Hill, R. L., Jr. and D. M. Janz (2003). "Developmental estrogenic exposure in zebrafish (Danio rerio): I. Effects on sex ratio and breeding success." Aquat Toxicol **63**(4): 417-29.
Hodson, P. V., S. Efler, et al. (1996). "Measuring the potency of pulp mill effluents for induction of hepatic mixed-function oxygenase activity in fish." J Toxicol Environ Health **49**(1): 83-110.
Incardona, J. P., M. G. Carls, et al. (2005). "Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development." Environ Health Perspect **113**(12): 1755-62.
Incardona, J. P., H. L. Day, et al. (2006). "Developmental toxicity of 4-ring polycyclic aromatic hydrocarbons in zebrafish is differentially dependent on AH receptor isoforms and hepatic cytochrome P4501A metabolism." Toxicol Appl Pharmacol **217**(3): 308-21.
Ingram, B. L. and D. Sloan (1992). "Strontium Isotopic Composition of Estuarine Sediments as Paleosalinity-Paleoclimate Indicator." Science **255**(5040): 68-72.
Ishibashi, H., N. Matsumura, et al. (2004). "Effects of triclosan on the early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin." Aquat Toxicol **67**(2): 167-79.
Ishibashi, H., N. Watanabe, et al. (2005). "Toxicity to early life stages and an estrogenic effect of a bisphenol A metabolite, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene on the medaka (Oryzias latipes)." Life Sci **77**(21): 2643-55.
Jobling, S., S. Coey, et al. (2002). "Wild intersex roach (Rutilus rutilus) have reduced fertility." Biol Reprod **67**(2): 515-24.
Jobling, S., R. Williams, et al. (2006). "Predicted exposures to steroid estrogens in U.K. rivers correlate with widespread sexual disruption in wild fish populations." Environ Health Perspect **114 Suppl 1**: 32-9.
Kang, I. J., H. Yokota, et al. (2003). "Effects of 4-nonylphenol on reproduction of Japanese medaka, Oryzias latipes." Environ Toxicol Chem **22**(10): 2438-45.
Kester, M. H., S. Bulduk, et al. (2002). "Potent inhibition of estrogen sulfotransferase by hydroxylated metabolites of polyhalogenated aromatic hydrocarbons reveals alternative mechanism for estrogenic activity of endocrine disrupters." J Clin Endocrinol Metab **87**(3): 1142-50.
Kidd, K. A., P. J. Blanchfield, et al. (2007). "Collapse of a fish population after exposure to a synthetic estrogen." Proc Natl Acad Sci U S A **104**(21): 8897-901.
Kirby, M. F., A. J. Smith, et al. (2007). "Ethoxyresorufin-O-deethylase (EROD) and vitellogenin (VTG) in flounder (Platichthys flesus): system interaction, crosstalk and implications for monitoring." Aquat Toxicol **81**(3): 233-44.
Kruner, G. and Westernhagen (1999). "Sources of measurement error in assays of EROD activity of fish for biological effects monitoring." Helgol Mar Res **53**: 250-256.
Kuiper, R. V., A. Bergman, et al. (2004). "Some polybrominated diphenyl ether (PBDE) flame retardants with wide environmental distribution inhibit TCDD-induced EROD activity in primary cultured carp (CYPrinus carpio) hepatocytes." Aquat Toxicol **68**(2): 129-39.

Lacorn, M., A. Lahrssen, et al. (2001). "Quantification of metallothionein isoforms in fish liver and its implications for biomonitoring." Environ Toxicol Chem **20**(1): 140-5.
Lionetto, M., Caricato, R., Giordano, M., and Schettino, T. 2005. Acetylcholinesterase as biomarker in environmental biomonitoring. *In* Recent trends in the acetylcholinesterase system. *Edited by* M. Parveen and S. Kumar. IOS Press, Amsterdam.
Marty, G. D., R. A. Heintz, et al. (1997). "Histology and teratology of pink salmon larvae near the time of emergence from gravel substrate in the laboratory." Canadian Journal of Zoology **75**(6): 978-988.
Mayer FL, V. D., McKee MJ, Folmar LC, Graney RL, McCume DC, Rattner BA (1992). Physiological and nonspecific biomarkers.
McArdle, M., A. Elskus, et al. (2000). "Estrogenic and CYP1A response of mummichogs and sunshine bass to sewage effluent." Mar Environ Res **50**(1-5): 175-9.
McArdle, M. E., A. E. McElroy, et al. (2004). "Enzymatic and estrogenic responses in fish exposed to organic pollutants in the New York-New Jersey (USA) Harbor Complex." Environ Toxicol Chem **23**(4): 953-9.
McCormick, S. D., F. O'Dea M, et al. (2005). "Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic salmon by 4-nonylphenol and 17beta-estradiol." Gen Comp Endocrinol **142**(3): 280-8.
Meyer, J. N., D. E. Nacci, et al. (2002). "Cytochrome P4501A (CYP1A) in killifish (Fundulus heteroclitus): heritability of altered expression and relationship to survival in contaminated sediments." Toxicol Sci **68**(1): 69-81.
Miller, K. A., R. F. Addison, et al. (2004). "Hepatic CYP1A levels and EROD activity in English sole: biomonitoring of marine contaminants in Vancouver Harbour." Mar Environ Res **57**(1-2): 37-54.
Mortensen, A. S. and A. Arukwe (2007). "Interactions between estrogen- and Ah-receptor signalling pathways in primary culture of salmon hepatocytes exposed to nonylphenol and 3,3',4,4'-tetrachlorobiphenyl (congener 77)." Comp Hepatol **6**: 2.
Mortensen, A. S. and A. Arukwe (2007). "Modulation of xenobiotic biotransformation system and hormonal responses in Atlantic salmon (Salmo salar) after exposure to tributyltin (TBT)." Comp Biochem Physiol C Toxicol Pharmacol **145**(3): 431-41.oI am
Mortensen, A. S., C. C. Tolfsen, et al. (2006). "Gene expression patterns in estrogen (nonylphenol) and aryl hydrocarbon receptor agonists (PCB-77) interaction using rainbow trout (Oncorhynchus Mykiss) primary hepatocyte culture." J Toxicol Environ Health A **69**(1-2): 1-19.
Navas, J. M. and H. Segner (2000). "Antiestrogenicity of beta-naphthoflavone and PAHs in cultured rainbow trout hepatocytes: evidence for a role of the arylhydrocarbon receptor." Aquat Toxicol **51**(1): 79-92.
Okihiro, M. S., D. J. Ostrach and D. E. Hinton (1990). Histologic Evaluation of Larval Striped Bass Exposed to the Rice Herbicides Ordram (molinate) and Bolero (thiobencarb), Final report on research to California Department of Fish and Game Environmental Services Branch, Pesticide Investigations Unit**:** 20p + Appendices.
Osmann, A. G. M., Wuertz, Sven, Mekkaway, Iman A., Hans-Jürgen Exner, Kirschbaum, Frank (2007). "Lead Induced Malformations in Embryos of the African Catfish Clarias gariepinus (Burchell, 1822)." Environmental Toxicology.

Ostrach, D. J. (2006). "Implications of Sublethal Contaminant Exposure on the Early Life Stages of Striped Bass (Morone saxatilis) In the San Francisco Estuary." University of California: 71.

Ostrach, D. J., J. M. Low, K.J. Eder, S.J. Whiteman and J.G. Zinkl. Maternal Transfer of Xenobiotics and Effects on Larval Striped Bass in the San Francisco Estuary (2008). Proceedings of the National Academy of Science, volume 105, (49), p. 19353 – 1935

Ovenell, J., R. McIntosh, et al. (1987). "The enhanced induction of metallothionein by zinc, its half-life in the marine fish Pleuronectes platessa, and the influence of stress factors on metallothionein levels." Experientia **43**(2): 178-81.

Radenac, G., G. Coteir, et al. (2004). "Measurement of EROD Activity: Caution on Spectral Properties of Standards Used." Mar. Biotechnology **6**: 307-311.

Rose, W. L., R. M. Nisbet, et al. (2006). "Using an integrated approach to link biomarker responses and physiological stress to growth impairment of cadmium-exposed larval topsmelt." Aquat Toxicol **80**(3): 298-308.

Sadzikowski, M. R., Wallace, Dale C (1974). "The Incidence of Lironeca ovalis (Say) (Crustacea, Isopoda) and Its Effects on the Growth of White Perch, Morone americana (Gmelin), in the Delaware River near Artificial Island." Chesapeake Science **15**(3): 163-165.

Sakamoto, T. and S. D. McCormick (2006). "Prolactin and growth hormone in fish osmoregulation." Gen Comp Endocrinol **147**(1): 24-30.

Sandahl JF, B. D., Jenkins JJ, Scholz NL (2005). "Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos." Environ Toxicol Chem: 24:136-45.

Sarkar, A., D. Ray, et al. (2006). "Molecular Biomarkers: their significance and application in marine pollution monitoring." Ecotoxicology **15**(4): 333-40.

Schlenk, D., Y. S. Zhang, et al. (1995). "Expression of hepatic metallothionein messenger RNA in feral and caged fish species correlates with muscle mercury levels." Ecotoxicol Environ Saf **31**(3): 282-6.

Secor, D. H. and J. M. Dean (1992). "Comparison of Otolith-based Back-calculation Methods to Determine Individual Growth Histories of Larval Striped Bass, Morone-saxatilis." Canadian Journal of Fisheries and Aquatic Sciences **49**(7): 1439-1454.

Secor, D. H. and E. D. Houde (1995). "Temperature Effects on the Timing of Striped Bass Egg Production, Larval Viability, and Recruitment Potential in the patuxent River (Chesapeake Bay)." Estuaries **18**(3): 527-544.

Secor, D. H., J.M. Dean & E.H. Laban (1991). Manual for otolith removal and preparation for microstructural examination. Columbia, South Carolina, U.S.A., University of South Carolina, Baruch Institute for Marine Biology and Coastal Research.

Secor, D. H. and P. M. Piccoli (1996). "Age- and Sex-dependent Migrations of Striped Bass in the Hudson River as Determined by Chemical Microanalysis of Otoliths." Estuaries **19**(4): 778-793.

Sergerie, M., S. Ouhilal, et al. (2000). "Lack of association between smoking and DNA fragmentation in the spermatozoa of normal men." Hum Reprod **15**(6): 1314-21.

Spearow, J. L., P. Doemeny, et al. (1999). "Genetic Variation in Susceptibility to Endocrine Disruption by Estrogen in Mice." Science **285**(5431): 1259-1261.

Springman, K. R., G. Kurath, J.J. Anderson, J.M. Emlen (2005). "Contaminants as Viral Co-Factors: Assessing Indirect Population Effects with the Vitality Model." Aquatic Toxicology: 71: 13-23.

Stresser, D. M., S. D. Turner, et al. (2002). "Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4." Drug Metab Dispos **30**(7): 845-52.

Suzuki, K. T., M. Rui, et al. (1996). "Production of hydroxyl radicals by copper-containing metallothionein: roles as prooxidant." Toxicol Appl Pharmacol **141**(1): 231-7.

Thompson, J. A. and A. E. Sutherland (1992). "A comparison of methods for sample clean-up prior to quantification of metal-binding proteins." Comp Biochem Physiol B **102**(4): 769-72.

Thorpe, K. L., T. H. Hutchinson, et al. (2001). "Assessing the biological potency of binary mixtures of environmental estrogens using vitellogenin induction in juvenile rainbow trout (Oncorhynchus mykiss)." Environ Sci Technol **35**(12): 2476-81.

Tiedeken JA, Ramsdell JS, Ramsdell AF, (2005). Developmental toxicity of domoic acid in Zebrafish (Danio rerio). Neurtoxicol. Teratol. 2005 July 29.

Todorov, J. R., A. A. Elskus, et al. (2002). "Estrogenic responses of larval sunshine bass (Morone saxatilis x M. Chrysops) exposed to New York City sewage effluent." Mar Environ Res **54**(3-5): 691-5.

Tom, M., C. R. Myers, et al. (2002). "Evaluating molar CYP1A level in fish hepatic microsomes by competitive ELISA using recombinant membrane-free CYP1A standard protein." Aquatic Toxicology (Amsterdam) **59**(1-2): 101-114.

Vaccaro, E., V. Meucci, et al. (2005). "Effects of 17beta-estradiol, 4-nonylphenol and PCB 126 on the estrogenic activity and phase 1 and 2 biotransformation enzymes in male sea bass (Dicentrarchus labrax)." Aquat Toxicol **75**(4): 293-305.

van Aerle, R., N. Pounds, et al. (2002). "Window of sensitivity for the estrogenic effects of ethinylestradiol in early life-stages of fathead minnow, Pimephales promelas." Ecotoxicology **11**(6): 423-34.

Van Campenhout, K., L. Bervoets, et al. (2003). "Metallothionein concentrations in natural populations of gudgeon (Gobio gobio): relationship with metal concentrations in tissues and environment." Environ Toxicol Chem **22**(7): 1548-55.

Viarengo, A., B. Burlando, et al. (1999). "Role of metallothionein against oxidative stress in the mussel Mytilus galloprovincialis." Am J Physiol **277**(6 Pt 2): R1612-9.

Voelker, D., C. Vess, et al. (2007). "Differential gene expression as a toxicant-sensitive endpoint in zebrafish embryos and larvae." Aquat Toxicol.

Wassenberg, D. M. and R. T. Di Giulio (2004). "Synergistic embryotoxicity of polycyclic aromatic hydrocarbon aryl hydrocarbon receptor agonists with cytochrome P4501A inhibitors in Fundulus heteroclitus." Environ Health Perspect **112**(17): 1658-64.

Wassenberg, D. M., A. L. Nerlinger, et al. (2005). "Effects of the polycyclic aromatic hydrocarbon heterocycles, carbazole and dibenzothiophene, on in vivo and in vitro CYP1A activity and polycyclic aromatic hydrocarbon-derived embryonic deformities." Environ Toxicol Chem **24**(10): 2526-32.

Weber, L. P., G. C. Balch, et al. (2004). "Increased kidney, liver, and testicular cell death after chronic exposure to 17alpha-ethinylestradiol in medaka (Oryzias latipes)." Environ Toxicol Chem **23**(3): 792-7.

Weber, L. P., Y. Kiparissis, et al. (2002). "Increased cellular apoptosis after chronic aqueous exposure to nonylphenol and quercetin in adult medaka (Oryzias latipes)." Comp Biochem Physiol C Toxicol Pharmacol **131**(1): 51-9.

Westernhagen, H., Ed. (1988). Sublethal effects of pollutants on fish eggs and larvae. Fish Physiology The Physiology of Developing Fish. San Diego, CA, Academic Press.

Wheelock, C. E., Kai J. Ederb, c, Inge Wernerb, Huazhang Huanga, Paul D. Jonesa, Benjamin F. Brammelld, Adria A. Elskusd, 1 and Bruce D. Hammock (2005). "Individual variability in esterase activity and CYP1A levels in Chinook salmon (Oncorhynchus tshawytscha) exposed to esfenvalerate and chlorpyrifos." Aquatic Toxicology **74**(2): 172-192.

Whyte, J. J., R. E. Jung, et al. (2000). "Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure." Crit Rev Toxicol **30**(4): 347-570.

Zafarullah, M., P. E. Olsson, et al. (1989). "Endogenous and heavy-metal-ion-induced metallothionein gene expression in salmonid tissues and cell lines." Gene **83**(1): 85-93.s