FINAL PROJECT REPORT

PROJECT TERM: JULY 1, 2011 – MARCH 31, 2015
PROJECT TITLE: UNDERSTANDING THE SCALE AND MECHANISMS OF CONNECTIVITY BETWEEN SPLITTAIL POPULATIONS AND THE IMPLICATIONS FOR MANAGEMENT
AMOUNT FUNDED: $600,000

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PROJECT SUMMARY

Splittail is the only extant member of its genus and is endemic to the San Francisco Estuary. It is listed as a California Species of Special Concern and there is an urgent need to understand splittail population dynamics and the physiological mechanisms underlying them in order to better manage and protect this native minnow species. Our research compared key characteristics and potential long-term viability of the two known splittail populations by integrating genetic, physiological, and modeling approaches.

The genetic component was used to investigate: 1) the effective population size of each population, and 2) the potential overlap in range distribution of the two populations at two different life stages (age-0 and adult). We found that the Central Valley population has an effective population size that is considerably (~3x) larger than the San Pablo (Petaluma/Napa) population. This finding suggests that more focused monitoring and/or habitat conservation should occur for the San Pablo Bay population since its population size is considerably reduced in comparison to the Central Valley population. When examining age-0 splittail distribution patterns, we found that the two populations are predominantly spatially segregated when water flow rates are average or below average, but substantial geographic overlap may occur during years of high flow rates. Similarly, adults exhibit increased spatial overlap between the populations during spawning seasons with high flow rates. Intermittent spatial intermixing, however, has not weakened the observed genetic distinctions between the two populations during the past decade. The mechanisms enabling continued population differentiation despite flow-dependent spatial overlap are currently unknown.

The physiological component examined responses of each population to different environmental factors (i.e. temperature and salinity) to investigate the potential for locally adapted differences between these populations. Wild-caught splittail of both juvenile and adult life stages were used for preference and tolerance challenges in order to gain insight into each population's environmental preferences and potential selective factors that may influence splittail population structure and long-term viability. We showed that splittail populations varied little in upper thermal tolerance limits (critical thermal maxima ranging from 33.7-34.6°C), but did vary in preferred temperatures. In general, juvenile San Pablo and Central Valley fish preferred 19-21°C, as did San Pablo adults. Central Valley adults selected cooler temperatures of 14-19°C. Salinity tolerance and associated osmoregulatory capacities also differed among splittail populations and lifestages. In hatchery-born and wild caught juvenile San Pablo splittail, we found upper salinity tolerances to be 16 ‰, which was higher than the upper salinity tolerance of 14 ‰ for wild caught juvenile Central Valley splittail. This, in conjunction with differential magnitudes of osmoregulatory disturbances between Central Valley and San Pablo splittail in response to salinity, supported our hypothesis of inter-population variation. In wild-caught adults, we found both populations to tolerate salinities of up to 11 ‰, with 100% survival for 336 hr. Cellular and tissue osmoregulatory disturbances, assessed by measuring plasma osmolality and ions, skeletal and ventricular muscle moisture and Na+-K+-ATPase during a 24 to 336 hr 11 ‰ salinity treatment, showed evidence for impaired osmoregulatory capabilities in adult Central Valley relative to San Pablo splittail. Osmoregulatory disturbances under this salinity treatment corroborated findings for juvenile splittail and further supported our hypothesis of variation in salinity tolerance between Central Valley and San Pablo splittail. Overall, the improved salinity tolerance of San Pablo juvenile and adult splittail is consistent with its higher salinity habitat. Population differences in salinity tolerance and temperature preference support the recommendation of population specific management that acknowleges differential habitat preferences and preserves resiliency to salinity demonstrated in San Pablo fish.

Results from the genetic and physiological studies were used to inform a more comprehensive splittail model, which saught to determine the degree of connectivity between the two splittail populations and assess extinction risk for each population. The modeling effort has resulted in two models, which demonstrate that the two populations are capable of surviving independently. The Central Valley population is essentially invulnerable to extinction. While the San Pablo population is stable, its extinction risk is higher because it is considerably smaller and exists in a very limited environment. However, considerable flow of Central Valley individuals into the San Pablo population results in the distinctive San Pablo genetic composition being swamped by the Central Valley. Since it is in conflict with existing data, considerable reproductive linkage between the two populations is not a realistic alternative.

BUDGET SUMMARY

EXPENSE	AMOUNT INVOICED (1ST YEAR)	AMOUNT INVOICED (2ND YEAR)	AMOUNT INVOICED (3RD YEAR)	AMOUNT INVOICED (4TH YEAR)	AMOUNT INVOICED TO DATE (ALL YEARS)
Salaries and Wages	55,866.01	38,164.76	105,199.32	115,336.59	314,566.68
Employee Benefits	10,912.43	10,790.28	34,597.11	28,113.96	84,413.78
Equipment & Facilities	0	0	0	0	0
Supplies, Materials & Services	11,568.24	6,013.23	26,516.80	18,340.11	62,438.38
Travel	0	78.00	280.84	5,418.38	5,777.22
Subcontracts					
Indirect Costs	17,581.99	12,954.06	38,821.56	41,802.45	111,160.06
Overhead					
Totals	95,928.67	68,000.33	205,415.63	209,011.49	578,356.12

FOURTH YEAR AMOUNTS ARE STILL BEING FINALIZED.

LIST OF TASKS AND ACTIVITIES PERFORMED

Tasks were identified as part of the scope of work, and this section is a cummulative overview of the activities performed for each task. This section serves as the full historical record of all activities performed for this project.

TASK 1:

Task 1 Semi-annual Report 1 (Dec. 2011)

After the completion of genotyping and tests for linkage disequilibrium and Hardy-Weinberg equilibrium, we characterized 36 new microsatellite markers for splittail. Twenty-four markers successfully cross-amplified and were found to be polymorphic in at least one of the five additional California cyprinid species (*Ptychocheilus grandis*, *Siphateles bicolor*, *Lavinia exilicauda*, *Orthodon microlepidotus* & *Mylopharodon conocephalus*) examined in the screening. Moreover, we initiated the DNA extraction for the tissue samples we will be using for the effective population size estimation.

Our primer note describing the discovery of additional microsatellite markers for California cyprinidae species was accepted to the journal "Conservation Genetics Resources" and is now published online. Over five hundred age-0 splittail (which include fish from the Petaluma River, Napa River, Sacramento River, San Joaquin River, and the Sacramento-San Joaquin delta) each collected from 2011 have been genotyped for the thirteen original microsatellite loci from Baerwald et al. (2004). We are also currently in the process of optimizing the newly published microsatellite markers for multiplex PCR (Polymerase Chain Reaction), which would help reduce cost and improve the efficiency of any future genotyping of these novel loci. Furthermore, preliminary analysis to estimate effective population size using the software *LD-Ne*, *ONeSAMP*, and *Ne-estimator* is in progress. Information acquired from these tests will be used to direct the next step in our study (whether additional samples, markers, or both are necessary).

Task 1 Semi-annual Report 2 (July 2012)

Age-0 splittail were collected from the field and we have begun isolating genomic DNA from their fin clips for future effective population size estimation. Screening of previously created microsatellite-enriched libraries was initiated to identify new splittail microsatellite markers. These markers will also be tested for cross-species amplification on five other cyprinid species (Sacramento pikeminnow, tui chub, hardhead minnow, hitch, and Sacramento blackfish).

We finished sequencing clones for two microsatellite-enriched libraries. Based on this sequencing, primers were designed for over 200 microsatellite regions to examine amplification and polymorphism level on six cyprinid species (splittail, Sacramento pikeminnow, tui chub, hardhead minnow, hitch, and Sacramento blackfish). A minimum of eight individuals per species were screened with the exception of splittail, which was screened with forty individuals (24 from the Central Valley population and 16 from the Petaluma-Napa population).

A total of 37 polymorphic microsatellite markers were detected for splittail, with allele number per locus ranging from 2 to 10. Microsatellite loci that amplify in the other five species vary from 8 in blackfish to 42 in pikeminnow. The genotyping of every primer set test is still in progress for some species (i.e., blackfish and hitch).

Task 1 Semi-annual Report 3 (Dec. 2012)

Approximately 400 additional age-0 splittails collected from the Napa River in 2012 for the salinity preference study were genotyped and assigned to a population. To date, it appears that the increased influence of Central Valley adult splittails in 2011 did not have a significant effect on the distinctiveness of the Petaluma-Napa splittail population. Out of this 2012 group so far, 86.5% are assigned to the Petaluma-Napa population, 11.5% remains unassigned, while only 2% assigned to the Central Valley population. We are also currently in the process of genotyping additional Central Valley population fish for all 19 microsatellite loci (we previously used 13 for population assignment analysis) for the purpose of improving the accuracy of our genetic population assignment. Updated effective population size estimates and assignment will follow once the genotyping process is completed for all the required individuals.

Task 1 Semi-annual Report 4 (July 2013)

We began our harmonic N_e estimation by using the temporal method as there is sufficient time gap (>1 splittail generation) between our sampling sessions (2002-2003, 2011-2012) to allow for such analysis. The temporal F-statistics method was used to calculate a harmonic mean of N_e over two time points based on the variance of neutral allele frequencies between two temporally segregated samples. Three measurements of variance in allele frequencies were used for this study: F_c, F_k, and F_s. Calculations for F', F estimates corrected for sample size, were conducted in the GONe software package. Additional Structure analyses were also conducted during this period to ensure that no further population substructure exists within the multiple age-0 cohorts.

Task 1 Semi-annual Report 5 (Dec. 2013)

Our Nb and Ne estimates for the Petaluma-Napa splittail population are mainly lower than that of the Central Valley splittail population. However, the previously mentioned Ne estimators all assume that each population is isolated from gene flow/immigration. To further assess the conformity of our harmonic mean Ne estimates and evaluate the effect of gene flow on Ne calculations, we used the MLNe program. MLNe uses a pseudo-maximum-likelihood approach to provide an estimate of temporal Ne with the option of incorporating gene flow into the analysis, assuming a small focal population and an infinitely large immigration source. We conducted our MLNe analyses in a couple of ways: one with immigrants included and gene flow considered, another without gene flow and immigrants excluded.

As expected based on the larger region of suitable spawning habitat within the Central Valley and apparent higher genetic diversity (allelic richness) within the Central Valley population, it is then somewhat unsurprising that annual N_b and harmonic N_e would be larger for the Central Valley splittail population in comparison to the Petaluma-Napa population. Although harmonic N_e estimates for both populations were reduced when immigration is considered, Central Valley splittail N_e numbers remain higher than the proposed minimum threshold for long-term evolutionary potential. Due to the observed lower effective size for the Petaluma-Napa splittail in our study, we conducted the M-ratio bottleneck test for both populations. This method of detecting bottleneck is based on the concept that a bottlenecked population should experience a more rapid decline in number of microsatellite alleles than the total range in fragment size (M-ratio). We used the program ARLEQUIN v 3.5 to calculate M-ratio for each cohort and M-CRIT to determine the critical M-ratio. Recent simulation study showed that results from this test should be interpreted with caution however, as the proportion of multistep mutations is often underestimated and may bias our results.

Task 1 Semi-annual Report 6 (July 2014)

DNA has been extracted from fin clip samples received from Dr. Fangue's lab (see Task 2) and amplified using our microsatellite panel. Nineteen markers are typically used for the panel, but we conducted simulations to determine if one of these markers can be removed from the panel without impacting genetic assignment accuracy. This marker is the only one not included in a multiplex with other loci so its removal allows for greater lab efficiency. We successfully determined that its removal did not affect assignment accuracy. We have extracted and genotyped 645 samples for 6 multiplexes and genotyped one full plate of repeats. Individual assignment back to population is on-going. The manuscript that was previously submitted to the journal "Conservation Genetics" has been returned to us with revisions requested. It is predicted that these revisions will be submitted back to the journal in the next few weeks.

Task 1 Semi-annual Report 7 (Dec. 2014)

Completed genotyping to assign 642 individuals to their respective populations for the physiology experiments (see Task 2). Additionally, an oral presentation was given at the 2014 Bay-Delta Science Conference and a manuscript was published in the journal *Conservation Genetics* (see deliverables).

Task 1 Final Update (March 2015)

We had a meeting with project participants (PIs and their students/technicians) to discuss synthesis of results, conclusions, and components of the final report. A manuscript was accepted by the journal *Canadian Journal of Fisheries and Aquatic Sciences* (see deliverables).

TASK 2:

Task 2 Semi-annual Report 1 (Dec. 2011)

We have submitted, with subsequent approval by UC Davis' Institutional Animal Care and Use Committee, an amendment to our approved protocol to allow artificial spawning as a source of larval and juvenile splittail. We utilized ultrasound to assess reproductive condition in our 2011 collected adults from both the Central valley and Napa/Petaluma populations. Ten adults from each population were separated into large 7-foot tanks that were nearing the final stages of their reproductive cycles. We attempted natural spawning with no success, and refrained from injecting fish with gonodotropin releasing hormones when reabsorption of eggs unexpectedly began. Unfortunately artificially spawning fish captured last winter was unsuccessful but we will retry these methods during the next spawning season.

Young-of-the-year splittail were collected from the Tracy Fish Collection Facility (US BOR, n=500) and transported to UC Davis' CABA laboratory for experimental acclimations. We also began sampling for 2012 young-of-the-year splittail from both the Sacramento and Petaluma/Napa Rivers. Sampling consisted of focal seine netting and deploying minnow traps overnight. All fish transported to the laboratory received prophylactic treatments to maintain good health and were acclimated to a stable temperature and salinity. Adult splittail from both Central Valley and Petaluma-Napa populations were implanted with VI Alpha Tags™ for individual identification during laboratory experiments. Fin clips were taken from tagged adults to confirm their genetic population of origin. We

received genetic assignment results for these fish and shared corresponding field data (fish weight, length, and collection location) with our BOR collaborators. We have tested several fish in 24-hour temperature preference experiments, including adults and juveniles, using both our large and small annular temperature preference devices. Preliminary results indicate that the selection behavior of splittail in a horizontal temperature gradient of 13-28˚C is affected by the presence of another fish in the chamber. Whereas individual splittail in our preference experiments swam through the entire gradient almost continuously, an unusual observation for these types of studies, experiments with multiple fish of the same cohort exhibited less active behavior. We submitted an abstract for the upcoming Bay-Delta meeting (October 2012) to present our temperature preference findings. Construction is currently underway to remodel our wet lab for conducting chronic salinity trials on splittail.

Task 2 Semi-annual Report 2 (July 2012)

We initiated sampling for young-of-the-year splittail from both the Central Valley and Napa/Petaluma populations. Sampling consisted of focal seine netting to capture healthy individuals for transport to the laboratory for experimental acclimations. Experimental acclimations consist of feed conversion to commercial pelleted diet, acclimation to stable water temperature and salinity, and prophylactic treatment to maintain good animal health. Laboratory equipment was inventoried, calibrated, and lab space was remodeled for conducting specific trials on splittail. New supplies were purchased in preparations for live animal experiments.

We began sampling for adults from both the Central Valley and Napa/Petaluma populations. Adult sampling consisted of electro-fishing and gill netting to capture individuals for transport to the laboratory for experimental acclimations. All fish were converted to commercial feed and received veterinarian-advised treatments for disease prevention and parasitic organism removal. We are modifying one of our animal holding facilities before separating fish into different temperature and salinity groups for acclimation. We have also evaluated several fish identification techniques and settled on VI Alpha Tags as the best method to track individual fish from each sub-population during laboratory experiments. Individually tagged fish will allow us to track an individual through time and allow for genetic confirmation of their population of origin in later stages for our project. We have submitted a Protocol for Institutional Animal Care and Use (IACUC) approval, which is currently under review by the IACUC at UC Davis. Some supplies were purchased in preparations for live animal experiments.

Task 2 Semi-annual Report 3 (Dec. 2012)

We continued field collection for young-of-the-year (YoY) splittail (2012). We were successful using beach seines to collect 425 fish from the Napa River, 12 from the Sacramento River, and 29 from Suisun Marsh. The 2012 YoY fish were transported to the laboratory and treated with prophylactics to minimize disease outbreaks. Once fish were acclimated to a stable temperature and salinity, we began salinity tolerance and temperature preference experiments on them.

We have performed studies to identify long-term salinity tolerance for freshwater acclimated YoY splittail. Preliminary salinity challenge studies involved a 6 hour increase to a “high” salinity (15, 18, or 20 ppt), after which the salinity remained constant for two weeks. Fish at 15 ppt salinity survived for the duration of the experiment whereas the two higher salinities resulted in loss of equilibrium or mortality within a couple of days. A follow up study involved a gradual increase from

acclimation salinity (0 ppt) to treatment salinity (12 or 16 ppt) over 6 hours and then a 14-day chronic exposure to the treatment salinity. Blood, gill, kidney, and muscle samples were collected at 0, 6, 12, 24, 96, 168, and 336 h post salinity increase in order to assess osmo- and ionoregulatory capabilities. Tissue processing and data analysis from this experiment is now in progress for the latter study.

A pilot study investigating temperature preference of Central Valley and Petaluma/Napa adult splittail was performed using an annular preference chamber with a gradient of 12-28˚C and results were presented as a poster at the 7th Biennial Bay-Delta Science Conference. Furthermore, we showed population differences in temperature preference, with Central Valley adult splittail selecting much cooler water temperatures (16.7 ˚C + 0.1 SE) compared with juvenile and adult Petaluma/Napa splittail (20.9 ˚C + 0.1 SE and 19.9 ˚C + 0.1 SE, respectively). Based on these preliminary results, further temperature preference experiments are currently underway comparing temperature preference adults and juveniles of both Central Valley and Petaluma/Napa splittail populations. These experiments are expected to be completed in March.

In order to secure juvenile splittail populations for future experiments, we have begun monitoring our adult populations in preparation for spawning in mid-February. Ultrasound examination for assessment of gonad development status was performed in December 2012 on adult splittail from both populations (captured in 2011). As a result, more than a dozen mature fish from each of three collection locations (Petaluma, Napa, and Sacramento Rivers) were successfully identified as likely spawners. These fish are now being held for anticipated spawning in mid-February 2013.

UC Davis' Institutional Animal Care and Use Committee renewed our protocol and are reviewing an amendment to this protocol to permit the use of passive integrated transponder (PIT) tags for individual identification of fish, since our Visible Implant (VI) Alpha tagged splittail showed poor retention rates.

Task 2 Semi-annual Report 4 (July 2013)

During this reporting period we made several attempts to artificially spawn mature splittail by injecting [D-Ala6, Des-Gly10]-Luteinizing Hormone-Releasing Hormone analog at a dose of 10-20µg/kg of body weight. We obtained fertilized eggs in February from fish collected from the Petaluma and Napa Rivers (2011). We estimate 60% of the total eggs were hatched, and that 90% of these larvae were successfully weaned. These juveniles are now approximately 150 days post-hatch (dph). We also tried to spawn the Central Valley broodstock (Sacramento River), however these eggs were not fertilized. Our second spawn attempt occurred in March for both populations but this time we were only successful producing larva from the Petaluma River adults. These juveniles are now approximately 115 dph. The Napa River splittail did not spawn on the second attempt and our Central Valley fish were no longer reproductive by this time. Our third attempt to spawn splittail focused solely on the Central Valley population, using reproductive fish that were captured from the wild, early April 2013. We tried several times to artificially spawn these fish up to a week after their arrival at UC Davis, but no eggs were produced. We will begin assessing the reproductive condition of our captive adults again in Fall 2013 for potential spawning the following spring.

We performed critical thermal maximum (CTmax) experiments with 40 and 80 dph Petaluma/Napa larvae. These tests were also conducted with both populations using Age 1+ and 2+ splittail collected from the wild. Our preliminary data suggests that splittail have a CTmax of 34 ppt with no apparent differences among age or population. Out of 112 total fish, we recorded one mortality at 24-h posttest.

We performed critical salinity maximum experiments (CSmax) with 40 and 80 dph Petaluma/Napa larvae. Preliminary data from these two groups show a decrease in CSmax between 40 and 80 dph (32.2 and 29.4 ppt, respectively). We also conducted CSmax experiments with both populations using Age 1+ splittail captured from the wild. Preliminary data from this age class shows no difference between populations and a higher CSmax (35 ppt) compared with both 40 and 80 dph. Mortality was 4% at 24-h posttest for 40 dph fish and 50% for 80 dph. Differences in mortality between populations were observed for Age 1+ splittail at 24-h posttest, with Central Valley fish exhibiting a higher incidence of mortality than Napa River fish (8% vs. 33% respectively).

To assess the effect of salinity on survival, we performed chronic salinity experiments with Petaluma/Napa larvae. Beginning at 60 dph we increased the salinity at a rate of 2 ppt per day. Results from this challenge show 100% survival up to 19 ppt and 0% survival at 23 ppt.

We investigated the effect of salinity on growth in larval splittail. Beginning at 13 dph, when fish had just completed yolk absorption and started to feed on their own, Petaluma larvae were exposed to a 6 h increase in salinity at a rate of 2 ppt per hour. At the target salinity of 12ppt, the salinity increase was ceased and the fish were held constant for the duration of the experiment. A control group of 13 dph fish were held concurrently for comparisons. Weights and lengths of the fish were recorded every two weeks until 69 dph. No mortalities occurred during the experiment. This data demonstrates a remarkable salinity tolerance for Petaluma/Napa splittail at very early life stage. No difference in growth was observed until 41-55 dph when fish at 12 ppt salinity weighed approximately 32% less than the controls. The difference in mass increased further between 55 and 69 dph, from 32% to 44%.

Tissue processing and data analysis are in progress for a study assessing osmo- and ionoregulatory capabilities of Age-0 splittail. We have nearly completed measurements of Na-K-ATPase activity in gill samples collected at 0, 6, 12, 24, 96, 168, and 336 h post salinity increase. Preliminary results suggest that juvenile Petaluma/Napa splittail held in 12 or 16 ppt salinity may be less effective at maintaining ion balance than freshwater controls. Gill enzyme activity began to decrease 24 h after transfer to 12 or 16 ppt and persisted for 14 days in both salinity treatment groups.

We completed all of our 22-hour temperature preference experiments for Central Valley and Petaluma/Napa adult and juvenile splittail. Video analysis is currently underway to compare adult and juvenile temperature preferences of both Central Valley and Petaluma/Napa splittail populations.

We began field collection for YoY splittail (2013) to use in salinity tolerance experiments. Through many coordinated efforts (e.g., DWR, FWS) we have attempted to collect fish using beach seines, minnow traps, light traps, fyke traps, screw traps, and trawls. We currently have 41 Central Valley age-0 splittail; 28 were collected from Suisun Marsh, 13 were collected from the North and Central Delta regions (i.e., Clarksburg, Isleton, and Discovery Park). The 2013 YoY fish were transported to the laboratory and treated with prophylactics to minimize disease outbreaks.

UC Davis' Institutional Animal Care and Use Committee has approved our amendment to use passive integrated transponder (PIT) tags for individual identification of adult fish in chronic salinity experiments, and has approved our more recent amendment which permits us to perform studies on larval splittail in addition to the juvenile and adult life stages we have been experimenting with.

Task 2 Semi-annual Report 5 (Dec. 2013)

We have focused on establishing our salinity challenge methodologies on adult splittail from both populations. This has involved salinity range-finding experiments to determine upper salinity

limits (both in magnitude and duration) necessary to develop the final study design. These studies involve salinity challenges, paired with physiological measures (active and resting metabolic rates, metabolites, and enzyme activities) as well as tissues sampled for gene expression.

Tissue processing and data analysis continued for a study assessing osmo- and ionoregulatory capabilities of Age-0 splittail. Preliminary results suggest that juvenile Petaluma/Napa splittail held in 12 or 16 ppt salinity may be less effective at maintaining ion balance than freshwater controls. Gill enzyme activity began to decrease 24 h after transfer to 12 or 16 ppt and persisted for 14 days in both salinity treatment groups. Thermal preference video data continues to be analyzed. The UC Davis' Institutional Animal Care and Use Committee approved our amendment to exercise fish to exhaustion paired with salinity exposures and metabolism experiments.

Additionally, we have focused on performing the adult splittail salinity challenge experiments. We completed a comparative study on wild-caught Napa/Petaluma vs Central Valley adult fish that assessed both their osmoregulatory capacity when exposed to elevated salinities as well as their metabolic responses at rest as well as following an exhaustive exercise challenge designed to elicit maximum metabolic rates in these fish. These data allow for the calculation of aerobic scope. Tissue processing and data analysis are in progress to assess osmo- and ionoregulatory capabilities of adult splittail. We have nearly completed measurements of muscle moisture and plasma osmolality, and other biochemical indices such as Na-K-ATPase activity in gill samples are in progress.

Task 2 Semi-annual Report 6 (July 2014)

During this reporting period, we analyzed biochemical samples from our adult splittail salinity challenge experiments completed last reporting period. That experiment compared wild-caught Napa/Petaluma vs Central Valley adult fish that assessed both their osmoregulatory capacity when exposed to elevated salinities as well as their metabolic responses at rest as well as following an exhaustive exercise challenge designed to elicit maximum metabolic rates in these fish.

The other major task this period has been the completion of a salinity challenge experiment performed on juvenile splittail. We successfully assessed the osmoregulatory capacity of wild-caught Napa/Petaluma vs Central Valley juvenile fish when exposed to elevated salinities. We have exchanged relevant fish fin clips with Dr. Baerwald so that parentage can be assigned. Analysis of biochemical samples is ongoing.

Task 2 Semi-annual Report 7 (Dec. 2014)

During this reporting period, we completed biochemical measurements and data analysis for the adult and juvenile salinity exposure experiments. For the 2013 adult splittail salinity experiment, metabolic rate data was analyzed, and sodium potassium ATPase activity was measured on gill tissues and analyzed. These data in addition to plasma osmolality, sodium, potassium and chloride levels were related to population and salinity. Osmolality and plasma ion levels show that the high salinity treatment (11 ppt) results in an osmotic disturbance in both populations. The osmolality response of the Central Valley population was greater than for the San Pablo population, suggesting the San Pablo fish were better able to adjust to a salinity of 11 ppt.

For the 2014 juvenile splittail salinity experiment, osmolality and sodium potassium ATPase activity were measured for plasma and gill tissues, respectively, and this data as well as muscle

moisture and haematocrit were analyzed and related to population and salinity exposure. Exposure to both 11 and 14 ppt salinity caused changes in plasma osmolality, muscle moisture and gill sodium potassium ATPase activity, and these changes were greater in magnitude for the fish at 14 ppt than at 11 ppt. There were no clear differences between Central Valley and San Pablo populations in physiological response to salinity exposures.

An oral presentation was given at the 2014 American Physiological Society Intersociety Meeting (see deliverables). Further analysis of data and compilation into manuscripts for both juvenile and adult salinity experiments will continue over the next few months.

Task 2 Final Update (March 2015)

We had a meeting with project participants (PIs and their students/technicians) to discuss synthesis of results, conclusions, and components of the final report. The final report was completed during this period. Data are presented in manuscript form, and three manuscripts will be submitted for peer review following this report.

TASK 3:

Task 3 Semi-annual Report 1 (Dec. 2011)

The San Pablo Splittail Model(s). In our judgment, the development of the San Pablo models cannot proceed productively until more information becomes available, particularly on splittail tolerance of salinity and movement through saline waters. Emphasis was placed instead on a thorough examination of the behavior of the existing Central Valley splittail model, particularly on the coupled questions of population variability and extinction risk.

Experimental testing of the viability of the Sacramento Splittail population in the Central Valley model is nearly complete. The Central Valley splittail Simulation Model demonstrates that Sacramento Splittail in the Central Valley region are essentially invulnerable to the risk of extinction. Invulnerability is the result of very high fertility, more than adequate to sustain population size, even under the worst possible circumstances, such as a 100 year drought. Sensitivity testing on adult mortality rates, fecundity differences, recruitment of yearlings and the nature of population regulation do not alter the result.

Note that low risk of extinction is not the same as limited variability in population size. The Central Valley Sacramento Splittail population is well known for pronounced variability in population size, in response to the fluctuation in water flow downriver from the Sierras. The model reproduces this trend well, responding with heavy reproduction in wet years and limited production in drier years. The relative stability of adult numbers contributes greatly to population stability, even while the current young of the year is subject to great variability in size.

These results demonstrate that the Central Valley Splittail population is secure from extinction. It remains to be seen what models of the San Pablo splittail yield, but since these populations are smaller and live in restricted habitats, we suspect that the San Pablo population is far more at risk than the Central Valley one. To examine this proposition, a separate model for the San Pablo population will be required; this is the next step in the process once the empirical parts of this program begin to yield data.

Task 3 Semi-annual Report 2 (July 2012)

Model definition. The splittail modeling effort was launched in the first year of the program. The initial activity was model design. The result was three series of projected models, all together designed to answer the principal model objective of determining how much individual exchange among populations was consistent with maintaining genetic distinction between the San Pablo and Central Valley splittail populations.

The first series of models explores the effects of specified levels of exchange between splittail spawning in the two San Pablo locations (Petaluma and Napa drainages). Genetic evidence suggests that splittail existing in the two locations represent a single panmictic population, but manipulating the rate of exchange and rate of mutation should permit a conclusive test of the hypothesis.

The second series of models assumes that there is a single San Pablo splittail population, open to immigration from the Central Valley population by specified age groups of splittail and specified environmental conditions. This will be a direct test of the hypothesis that the San Pablo population cannot tolerate even moderate levels of Central Valley immigrants.

The final series of models investigates the opposite assumption that splittail spawning in the two San Pablo locations are largely isolated from one another and differentially open to Central Valley population immigrants at specified levels.

The three series together should reveal how much exchange among the San Pablo and Central Valley populations is feasible without destroying genetic distinction. Secondarily, they also will distinguish between feasible and infeasible population dynamic patterns in the populations, which in turn will define growth potential, minimum viable population size and extinction risk.

Connections between empirical research and modeling. As part of the next phase of development, we have begun the process of coordinating empirical research and modeling information requirements. The process of empirical research is much slower and in cases more difficult to execute satisfactorily than the process of modeling. As a result, it is expected that there will be elements where reliable data will not be available, at least initially. In these cases, assumptions will substitute for data, at least temporarily. This is the primary reason why alternative models are used rather than a single version.

The next phase. The plan is to revise the existing Central Valley splittail population model to a form consistent with the objectives of this program. After that task is completed, we can begin the programming of the alternative models in the series specified above.

Task 3 Semi-annual Report 3 (Dec. 2012)

The San Pablo Splittail Model. Progress towards an understanding about how splittail in San Pablo rivers and splittail in the Central Valley are related dynamically increased significantly during this reporting period. The conundrum was that the San Pablo and Central Valley splittail populations were genetically distinct from each other,which was incompompatible with migratory exchange since the San Pablo genotype would be swamped out. Thus the two populations needed to be isolated, even though it is known that Central Valley splittail reach San Pablo and even San Francisco Bay during high flood years, and some San Pablo fish move upriver into the Suisun area. seen as incompatible with migratory exchange between populations, may be possible without complete isolation of the populations. What was missing was a mechanism that accounted for migratory exchange while still preserving the unique genotypes.

The current hypothesis is that migration exchange occurs routinely, but Central Valley genotypes do not survive to reproduce or do not remain in the San Pablo rivers, while San Pablo fish

in the Delta spawn only in the lower reaches (if at all), whereas Central Valley splittail spawn further upriver. This implies that genetic isolation is not maintained by lack of migratory exchange, but rather by strong selection against the Central Valley genotype in the San Pablo environment. But since the San Pablo genotype would be swamped out numerically by the Central Valley genotype if migration regularly permits interbreeeding between populations, a mechanism for limiting reproductive exchange must be present. We have long suspected that the salinity gradient provides an explanation for selection against the Central Valley genotype, but practical difficulties have limited our ability to investigate this relationship.

It now seems likely that splittail responses to salinity gradients can be measured successfully in the laboratory,so the development of the San Pablo part of the splittail model has been reinitiated. Conceptual planning has been completed and model layout has begun in anticipated that salinity responses will be obtained to support model development. The model being developed has options to control the timing and magnitude of migration exchange, placeholders for survival in relation to salinity, avoidance behavior of saline water, and reproductive effort in relations to size and condition of the river spawning environment. Responses to salinity are the key to this model in anticipating that experimental results will eventually bear this expectation out. As results from the experimental research of Task 2 become available, the corresponding placeholders will be replaced with specific routines. Granted there is no certainty that we will find a strong relationship of survival and/or behavior of splittail to salinity, the current modeling format seems likely to remain useful, even if other causal factors prove to be importantl,

This approach avoids the problem of wasted effort devoted to multiple models. While empirical results may still indicate the need for additional models, the current version contains enough options to simulate multiple dynamic alternatives and in particular avoids the problem of wasted effort. As long as salinity remains a key factor, this model should be a major part of Task 3.

Task 3 Semi-annual Report 4 (July 2013)

The effort devoted to extend the Central Valley splittail model to the San Pablo Bay population has been limited mostly to literature research for this reporting period. This was a decision made during the last reporting period, due to the lack of information needed on two critical aspects of the population dynamics and physiological responses of the San Pablo splittail:

the physiology of splittail in low to moderate salinities insofar as these affect survival, avoidance behavior and reproductive activity
the impact of restricted basin sizes in the Petaluma and Napa Rivers and their impact on population dynamics.

Both of these critical needs have been subject to limited research in what amounts to a new problem, although both are currently under development. For the modeling effort, this has meant that critical aspects of the population dynamics remain too poorly known in order to proceed with useful model development. This actually represents progress as the original conceptual model expected to be developed in this project is quite probably inadequate.

Sufficient results are expected from the current experiments by Nann Fangue and her students to at least develop a defensible model of salinity effects on local movement and survival in combination with field observations. Early results suggest the effort will bear fruit. Salinity avoidance by movement will likely remain the least-known aspect.

Reproduction and growth of fish remain uncertain issues, since both the Napa or Petaluma River basin quality for splittail growth and reproduction are likely to remain poorly known. Current data on water quality and flows are nearly absent at present. It remains to be seen how these problems are to be dealt with in the future, but obtaining sufficient data for unambiguous model development now seems doubtful.

Task 3 Semi-annual Report 5 (Dec. 2013)

Decisions regarding the appropriate model structure still depend on the physiology experiments of Task 2, whose outcome remains stalled by the lack of sufficient fish from both locations with which to conduct the experimental trials. As noted previously, information regarding movements, juvenile survival and reproductive effort in relation to salinity in both splitail populations are all critical elements in the model. Although model design has been completed (assuming salinity is a critical element in San Pablo splittail life history) there is little reason to move to the programming stage until there is more evidence to support model structure. If salinity relationships are not as important as we suspect, the effort invested in the model could be wasted if the model needed to be revised completely.

Modeling San Pablo splittail has been delayed while Dr. Fangue completed current experiments on salinity effects. While the experiments are not yet complete or fully analyzed, it is becoming increasingly clear that the draft San Pablo model does not represent the population dynamics of the San Pablo population. Most likely, the model will have to be redesigned to 1) permit episodic movements between the two populations, at least in high flow years, while at the same time minimizing or eliminating cross-population breeding; and 2) develop a model with shorter time steps to take average growth of fish in a salinity gradient into account in order to capture maturation asynchrony that can prevent population exchange and preserve genetic identity.

Task 3 Semi-annual Report 6 (July 2014)

The splittail model has been totally redesigned. The working model proved to be inadequate because the leading question was not: how are the San Pablo and Central Valley splittail populations linked functionally? The more interesting and appropriate question is: what factors exclude Central Valley splittail for influencing the San Pablo population? Several alternative models postulating mechanisms to permit the latter outcome have been developed under the assumption that salinity is the key factor. Experiments with adults support this assumption.

Now that the salinity experiments have been completed and the results fairly clear, the basic framework of the San Pablo submodel has been created and programmed into STELLA. The submodel includes separate populations for the San Pablo genotype and the Central Valley genotype, which permits independent population dynamics and the calculation of genotypic frequencies. The submodel can be easily expanded to depict independent dynamics for the Petaluma and Napa Rivers if so desired.

In order to complete the model, two major tasks remain: The first is to work with the research team to translate the results of the salinity experiments into causal relationships linking San Pablo salinity to flood events coming from the Central Valley and the subsequent rise in salinity as upstream flows wane, and then to translate salinity changes to movement, growth and survival of splittail in the San Pablo environment. The other is to link the San Pablo submodel to the existing Central Valley submodel to create the linked model. Doing so depends on the nature of the causal linkages in the preceding task. There remains sufficient uncertainty, particularly about salinity-driven movement, that several versions of the model will be required instead of a single one.

The development of multiple models determines the basic experimental design for model manipulation. For each relationship specifying movement in relation to salinity, there will be a set of specified relationships governing growth, survival and reproduction for each genotype. Each variable can be held constant over a range of values for the other variables This design permits comparison across conditions that helps isolate the impact of single assumptions. The current design calls for at least six combinations, but additional experiments are likely.

Much work remains to be done in Task 3, but we are finally well underway.

Task 3 Semi-annual Report 7 (Dec. 2014)

Modeling splittail requires a sequence of models to deal with uncertain information and missing data. The first Coupled Splittail Model has been completed as an uncoupled model. The model simulates the Central Valley (CV) population and one in the Petaluma and Napa (PN) Rivers. The model assumes that the two populations do not affect one another dynamically (i.e, exchange of individuals does not alter the population dynamics of the other as there is no cross-population recruitment).

The coupled model performs as expected. Both populations maintain a variable steady-state population size, with the CV population around a million females and the PN one about a fifth of that. These results confirm that the population dynamics of each is realistic, although hardly unexpected. This version of the model serves to confirm that the two populations are stable when independent of one another. This result does not mean that the two populations are necessarily independent of one another, even though they can be.

The second version of the model is in progress. This model relaxes the assumption of no net interaction; it assumes there is bidirectional periodic exchange driven by river flow. Although it is too early to tell conclusively, initial runs of the coupled model appear to be very sensitive to infusion of CV young-of-the-year (YOY). Where survival of PN YOY into the lower Central Valley can be successful, the much larger size of the CV population serves to swamp out the influence of PN fish. To the contrary, in the model the PN population readily recruits CV YOY. However, this is inconsistent with the known dynamics of the two populations. To maintain genetic divergence in the PN population, the CV population cannot have much more than a small and irregular effect. The actual extent of exchange is not currently known, but this result fits prevailing opinion that invasion from the CV must be quite limited.

Unfortunately, the combination of factors limiting exchange is not clear and unlikely to be revealed by modeling. This is especially true for the limitation of survivorship and recruitment of immigrant YOY. However, it should be possible to determine the relative efficacy of different mechanisms, which may not be decisive but will provide important clues for future research.

Task 3 Final Update (March 2015)

The modeling component of the splittail project has two completed model versions, with two more in construction (will be completed after the termination date of this proposal).

The first (PNCV1) consists of population simulations for the Central Valley (CV) splittail population and that found in the Petaluma-Napa (PN) Rivers. The key factor is that they remain functionally decoupled; even if CV fish reach PN, they do not join the SP population. The CV population is much larger than the PN one by a ratio of 500:1 at the start of the simulation. Both go to a variable steady-state, although the variation in CV is considerably greater. They both progress to

their own steady-state size and hold it indefinitely over the entire range of climatic variation. The population size ratio at steady state is approximately 400:1 (CV:PN).

The second model (PNCV2) permits potential contributions from CV to PN via emigration of young-of-the-year (YOY). The reverse trend (from PN to CV) has happened in the past was not considered here because of the extensive programming required and the lack of evidence of any chance of reality or significance. In this version, the free emigration of CV YOY into SPB leads to swamping of the SP genotype, even with reduced emigration. The appropriate conclusion is that successful emigration cannot happen if the PN genotype is to be maintained. However, the mechanism is not clear. One problem is that the SP population is small and has limited reproductive effort, further constrained by the small size of the population. This means that CV YOY from a much larger and more reproductive CV population, combined with a PN population with severely limited reproduction, will swamp out the SP genotype with a few wet/high river flow years. The majority of the CV YOY must therefore be excluded from joining the SP population.

Two versions are being constructed using mechanisms that offer the possibility of limiting recruitment of CV to SP. One of these (PNCV3) considers the loss of CV YOY to salinity mortality as the SP Bay waters become increasing saline in the summer. This takes advantage of the difference in salinity tolerance of PN (up to 19 ppt) vs. CV (up to 11 ppt). However, current runs with PNCV3 lead us to doubt the efficacy of this mechanism, especially that it cannot eliminate virtually all CV YOY and prevent their recruitment to SP, thereby swamping the PN genotype.

The second version (PNCV4) considers the impact of those few fish in the CV population that emigrate successfully, have the PN genotype, and subsequently are no worse at salinity tolerance than SP fish. These fish would be virtually undetectable, but have the advantage of contributing to PN as a limited source population.

Overall, while the simulation modeling phase of the research has progressed more slowly and in different directions than anticipated, the results have lead to robust conclusions that justify the effort directed towards modeling, All work to date suggests that the two splittail populations are functionally independent to a large extent. If the CV population is to be a potential source population to supplement the PN population, it can only be under very restrictive circumstances that remain to be fully elaborated.

LIST THE ACHIEVED OBJECTIVES, FINDINGS, AND MAJOR CONTRIBUTIONS

1) Microsatellite marker development: 37 new microsatellite markers have been designed for splittail, with 24 cross-amplifying in at least one other CA cyprinid species,
2) Potentially increased migration associated with increased water flows: Central Valley splittail were found in the Petaluma and Napa rivers in high proportion during 2011 (a high flow year) but comprised a low minority (~2%) of the 2012 genetically assigned young-of-year cohort in these rivers.
3) Central Valley population effective population size is considerably (~3x) larger than the San Pablo population.
4) Juvenile salinity tolerance: In hatchery-born and wild caught juvenile San Pablo splittail, upper salinity tolerances was 16 ‰, which was higher than the upper salinity tolerance of 14 ‰ for wild caught juvenile Central Valley splittail. Cellular, and tissue osmoregulatory disturbances, assessed by measuring plasma osmolality, skeletal muscle moisture and gill Na+-K+-APTase during salinity treatments of 12 to 16 ‰ for up to 336 hr showed evidence for improved osmoregulatory capabilities in juvenile San Pablo relative to Central Valley splittail.
5) Adult salinity tolerance: In wild-caught adult splittail, we found both populations to tolerate salinities of up to 11 ‰, and potentially higher for San Pablo splittail, with 100% survival for 336 hr. Cellular, and tissue osmoregulatory disturbances, assessed by measuring plasma osmolality and ions, skeletal and ventricular muscle moisture and gill Na+-K+-APTase during a 24 to 336 hr 11 ‰ salinity treatment, showed evidence for improved osmoregulatory capabilities in adult San Pablo relative to Central Valley splittail.
6) Temperature preference: Central Valley adult splittail prefer cooler temperatures (16.7 ˚C) compared with juvenile (20.9 ˚C) and adult (19.9 ˚C) San Pablo splittail. Additionally, the selection behavior of splittail in a horizontal temperature gradient of 13-28˚C is affected by the presence of another fish in the chamber, with single fish in almost constant motion versus multiple fish showing temperature preferences.
7) Modeling: splittail in the Central Valley region are essentially invulnerable to the risk of extinction. The coupled model performs as expected. Both populations maintain a variable steady-state population size, with the Central Valley population around a million females and the San Pablo population about a fifth of that.

DISCUSS THE MANAGEMENT IMPLICATIONS OF PROJECT FINDINGS

- **More consistent monitoring for the San Pablo splittail population is warranted.**

The San Pablo population's relative isolation combined with its lower effective population size makes it more vulnerable to extinction. We recommend annual monitoring of the population to establish trends for determining if the population requires state and/or federal protection. This is possible since the U.S. Fish and Wildlife Service has designated the San Pablo population a Distinct Population Segment. With on-going drought conditions, the threat of extinction increases for the San Pablo population.

- **The two splittail populations are largely separate from one another and likely require different management regimes.**

Dynamically, the two populations appear to have different reproductive behavior, growth rates, and migratory behavior. Despite their conspecific status, they are ecologically different species. The Central Valley population is large and invulnerable to extinction risk. Conversely, the San Pablo population is considerably smaller and probably has higher extinction risk. While the Central Valley population needs little enhancement to management already in place, mitigating extinction risk for San Pablo remains largely conjectural.

- **We recommend further research into critical aspects of movement and recruitment of San Pablo splittail.**

The models show that connectivity of the two splittail populations must be limited to protect the unique genetic composition of the San Pablo population. Apparently, natural dynamics already limits recruitment of Central Valley splittail into the San Pablo population, although how and why remains speculative. There is little information on the movement of splittail YOY in San Pablo Bay as salinity increases, or on the factors that limit recruitment of Central Valley YOY into the San Pablo population. Research about movement patterns in relation to environmental characteristics (e.g. salinity, water flow, temperature) is critical if we are to understand splittail dynamics adequately.

- **We recommend further research using common garden approaches to determine the causes of physiological differences.**

The growth and osmoregulatory data presented in this report suggest fundamental differences between populations in osmoregulation capacity. Common garden experiments of lab-reared splittail from both populations are needed to fully determine if these differences are due to environmental factors, genetic variation, or a combination of the two.

PROJECT DELIVERABLES

- List here any presentations given at the Bay-Delta Science Conference and at other events
- List here and provide hardcopies and electronic files of all materials and published papers resulting from this grant

Presentations:

Baerwald, M., N. Fangue, and T. Foin. 2014. Splittail populations and environmental conditions - mechanisms, scale, and connectivity. Delta Stewardship Council's Delta Science Program brown bag seminar series. Oral presentation.

Mahardja, B., F.V. Feyrer, R. Coalter, B. May, N.A. Fangue, and M.R. Baerwald. 2013. Temporal variation in distribution and effective number of breeders among the two distinct populations of splittail (*Pogonichthys macrolepidotus*). American Fisheries Society Cal-Neva Annual Conference, Davis, CA. Oral presentation.

Coalter, R., D. Cocherell, F. Feyrer, J. Cech, and N. Fangue. 2012. Thermal preference of two populations of splittail, *Pogonichthys macrolepidotus*. Bay-Delta Science Conference, Sacramento, CA. Poster presentation.

Mahardja, B., B. May, F. Feyrer, and M. Baerwald. 2012. Comparison of effective population sizes for the two splittail populations in the San Francisco Estuary. Bay-Delta Science Conference, Sacramento, CA. Oral presentation.

Verhille, C., T. Dabruzzi, D. Cocherell, M.R. Baerwald, and N. Fangue. 2014. Adaptive variability in salinity tolerance explains habitat variability between genetically distinct populations of Sacramento splittail. 2014 American Physiological Society Intersociety Meeting: Comparative Approaches to Grand Challenges in Physiology. San Diego, CA. Oral presentation.

Mahardja, B., B. May, F. Feyrer, N. Fangue, T. Foin, and M.R. Baerwald. 2014. Connectivity and effective size of the two genetically distinct splittail populations. Biennial Bay-Delta Science Conference. Sacramento, CA. Oral presentation.

Publications (attached):

Mahardja B, May B, Baerwald MR. 2012. Characterization of 36 additional microsatellite loci in splittail (*Pogonichthys macrolepidotus*) and cross-amplification in five other native Californian cyprinid species. *Conservation Genetics Resources* 4: 917-921.

Mahardja B, May B, Feyrer F, Coalter R, Fangue N, Foin T, Baerwald MR. 2015. Interannual variation in connectivity and comparison of contemporary effective population size between two splittail (*Pogonichthys macrolepidotus*) populations in the San Francisco Estuary. *Conservation Genetics* 16(2):385-398.

Feyrer F, Hobbs J, Acuna S, Mahardja B, Grimaldo L, Baerwald M, Johnson RC, Teh S. In press. Metapopulation structure of a semi-anadromous fish in a dynamic environment. *Canadian Journal of Fisheries and Aquatic Sciences*, DOI: 10.1139/cjfas-2014-0433.

Verhille CE, Dabruzzi T, Cocherell D, Feyrer F, Foin T, Baerwald MR, Fangue NA. 2015a. Inter-population differences in salinity tolerance and osmoregulation of juvenile wild and hatchery-reared Sacramento splittail. Manuscript in preparation.

Verhille, C.E., Dabruzzi, T., Cocherell, D., Feyrer, F., Foin, T., Baerwald, M.R. Fangue, N.A. 2015b. Inter-population differences in salinity tolerance of adult wild Sacramento splittail: osmoregulatory and metabolic responses to salinity. Manuscript in preparation.

Fangue NA, Cocherell D, Verhille CE, Feyrer F, Foin T, Baerwald MR. 2015. Thermal performance of two genetically distinct populations of splittail (*Pogonichthys macrolepidotus*), a native California Cyprinid. Manuscript in preparation.

Modeling Software (attached):
Model 1: Central Valley and San Pablo populations remain functionally decoupled.
Model 2: Permits Central Valley to contribute to San Pablo population via immigration of YOY.