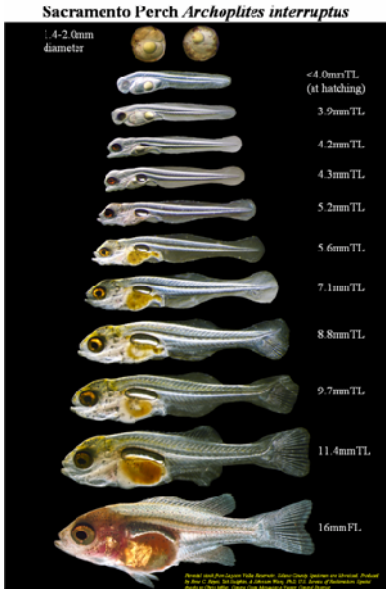
# Final Report ERP-02-P34

# Restoration of Sacramento perch to the San Francisco Estuary

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## Executive Summary

UC Davis proposed to study the basic biology Sacramento perch (*Archoplites interruptus*) in response to CALFED's Ecosystem Restoration Program in 2002. Within the UC Davis research proposal were four objectives. The *first objective* was to summarize existing information on Sacramento perch, emphasizing factors that contributed to survival of introduced populations, collapse of native populations, and persistence of some native populations. The existing information is summarized in a white (review) paper. This paper basically shows that Sacramento perch have apparently been extirpated from their native range and that many populations once established outside their native range have disappeared. There are four populations outside the native range that appear to be secure, at least in the short term. The *second objective* was to study the early life history of Sacramento perch, documenting conditions that contribute to survival in this least-understood life history stage. Three years of light trapping data produced the following generalizations: (1) Sacramento perch like other native California fishes spawn early in the spring when daylight and dark hours are almost equal; (2) they are not dependent on a full moon for stimulation to spawn, as has been thought; (3) they spawn in a temperature range of about 16 to 22°C; and (4) they usually do not spawn in a single event, but spawn several times over a period of time. Dietary studies show that small perch eat primarily zooplankton then switch to larger prey items as they increase in size. The *third objective* was documenting the physiological tolerance limits of both juvenile and adult Sacramento perch. Juvenile fishes showed high tolerance to both high and low temperature, high tolerance to low oxygen, high salinity and alkalinity. Adult Sacramento perch seem to have less tolerance to extreme

temperature and salinity and are much slower swimmers than juveniles. Finally, *objective four* was to document the genetic variation within and among Sacramento perch populations. Genetic analysis of major populations shows founder effects, reflecting both the small initial introduction sizes and long isolation of populations from each other.

It is clear that the plight of Sacramento perch can no longer be ignored, because to do so will allow it to further decline towards extinction. Its conservation and restoration to its native range will require sustained management effort.

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## Introduction

The Sacramento perch (SP, *Archoplites interruptus*) is a native sunfish that once was abundant, but is thought to be extirpated from almost all of its former habitats throughout the Sacramento- San Joaquin watershed (Tharratt and McKechnie 1966, Aceituno and Nicola 1976, Leidy 1984, Gobalet and Jones 1995, Moyle 2002). In the 19th century it was abundant enough in the San Francisco Estuary to support a commercial fishery (Moyle 2002). SP have been listed as a species targeted for recovery in the Delta Native Fishes Recovery Plan (Moyle et al. 1995), are listed by the Department of Fish and Game as a species of special concern (Moyle et al. 1995), and are classified by CALFED as an At-Risk (Priority Group 2) species in the 2001 ERP (Goal 1,objective 2, pp.140). SP would undoubtedly be listed as an endangered species if there were not many populations established outside its native range. Prior to this study, only two native populations seemed to be maintaining themselves, if tenuously: in Clear Lake and in the Alameda Creek drainage. Of the introduced populations, the ones in the upper Klamath watershed, in Pyramid Lake, Nevada, in the lower Walker River, and in the Owens River are somewhat secure because of their abundance and fairly broad distribution within these waters (Crain and Moyle, ("White paper" on SP in prep). However, the history of most populations established outside their native range suggests that long-term persistence is problematical (P. Crain, unpublished data). Extirpations of introduced populations are usually the result of changing conditions in managed waters, but precise causes are often unknown. Although some life history information for California populations is available (e.g., Aceituno and Vanicek 1976, summarized in Moyle, 2002), little is known about the physiological tolerance limits and behavioral preferences of SP.

SP have a reputation for being physiologically very tolerant (i.e., capable of living in water in which most freshwater fishes cannot persist), but physical/chemical environmental factors may be limiting their distribution and abundance, especially at early life history stages.

Recovery strategies for SP in the San Francisco Estuary have been proposed (Moyle et al. 1995), but have not been developed because the general lack of biological knowledge necessary. While generally considered to have declined because of interactions with alien fishes (Aceituno and Nicola 1976, Marchetti 2000, Moyle 2002), opportunities for recovery may still exist if proper strategies are used. The purpose of this study is to develop key missing information needed to develop recovery strategies for SP: early life history, physiological tolerances and preferences, and genetic diversity. The actual study had eight tasks. The first three were administrative so will not be mentioned further. Task 4 was changed from a report format to that of a peer reviewed publication which will be submitted when the manuscript is submitted for review. This article is in preparation and will be submitted to the *San Francisco Estuary and Watershed Science* online journal. Thus this preliminary report covers Task 5 (early life history), Task 6 (physiological ecology), Task 7 (genetics), and Task 8 (restoration strategies).

## TASK 5 Document early life history requirements of SP

Patrick K. Crain and Peter B. Moyle

Although some life history information for California Sacramento perch (SP) populations is available (summarized in Moyle 2002), little is known about the early life history and behavior of SP. Early life history is important because most fishes experience their highest mortality during their first year of life (Werner and Gilliam 1984). Inherited traits offset this trend by allowing them to adapt to the environmental variability that they encounter, which in turn drives mortality in early-life history (Roff 1992). Variance in life history traits may be explained by the trade-offs among offspring survivorship, fecundity, and age of maturity that maximize fitness (Winemiller and Rose 1992). How these traits interact to effect recruitment must be considered when developing conservation strategies (Frank and Leggett 1994). The trade-off between survival of offspring and other life-history traits is very specific to a population and can vary widely within a species, and is a response to site specific variability (Johansson and Rowe 1999). The differences in environmental variation can determine whether survival is more variable during the larval or juvenile stages of life (Houde 1997). Events that happen during early life history stages often regulate events that occur in adult life (Garvey et al. 1998, Post et al. 1998). For many fish, subtle differences in the timing of hatch may determine whether gape limited juveniles grow fast enough to acquire larger prey items, thus increasing their energy uptake and growth, which enables them to attain the even larger size needed to survive winter (Post and Evans 1989, Miranda and Hubbard 1994, Garvey et al. 1998c).

Links between life stages are probably important to fish that have protracted spawning events, because temporal variability can project into variable spawning success. Sunfishes use this opportunistic reproductive strategy, with reproduction often continuing for more than a month in many cases (Fox and Crivelli 1998). Extended reproduction may extend fitness in temporal environments, with early spawning often conveying distinct advantages to fish (Trebiz 1991). Because timing of reproduction is highly important to the survival of offspring, we attempted to answer four questions in the early life history of the Sacramento perch: 1) what is the relationship of temperature to the timing of reproduction? 2) Is there correlation to phase of moon with time of first spawn? 3) How does the length of daylight hours affect the timing of reproduction? 4) What do perch eat during their early life history?

**Study Area**

The original proposed site for this study was Lagoon Valley Reservoir, which had supported a robust population of Sacramento perch until just before this study began. Because of the severe decline and the cessation of spawning by adult Sacramento perch in Lagoon Valley (Scott Cressey, per com.), we decided to move the site of the study to Curve Pond (CP), which is located in Yolo County just west of Pedrick Rd. and a few hundred meters north of Putah Creek within the Putah Creek Riparian Reserve on the University of California, Davis. CP is a small pond (~.5 ha) that averages about 1m deep, but ranges from about .5m to 2m and is graded from shallow at the east end to deeper on the west. It is surrounded on the edges by yellow iris (*Iris* sp.) mixed in with reeds and cattails. There are a few small willow trees on either end of its kidney shape. The substrate is clay and silt deposited by Putah Creek when it flooded this area before it was

levied for flood control. There are beds of macrophytes mainly coontail (*Certaophyllum*), present. The pond contains four species of fish: Sacramento blackfish, *Orthodon microlepidotus*, threespine stickleback, *Gasterosteus aculeatus,* western mosquitofish, *Gambusia affinis*, and Sacramento perch. Sacramento perch were first introduced to CP in 1979; their origin was from the Sacramento River (Lake Greenhaven) via several farm ponds (Sterling Bunnell, pers. com.). A subsequent introduction was made in 1997 with fish left over from a behavioral experiment involving perch and bluegill (Marchetti, 2000); the origin of those fish Lagoon Valley Reservoir, which apparently was also planted with fish from Lake Greenhaven.

**Methods**

Sampling was conducted for three years beginning in 2004, and continuing through 2006. Sampling began in the first or second week of March and was continued until SP larvae were too large to be captured in light traps, usually around the end of May through the beginning of June. Six sampling sites were chosen haphazardly along the shoreline and were maintained throughout the three years of the study. In 2004, sampling began on 15 March and ended on 15 June. In 2005, sampling initiated on 14 March and continued through July 1. In 2006, the first sample was taken on 10 March and continued through July 1. At each site, light traps were used to sample fish larvae following the design of Kissick (1993) with the following modifications (Marchetti and Moyle 2000, Crain et al. 2004): openings leading into the traps had 5-mm wide slots on each side, traps were equipped with extra foam for floatation, and the light source was a waterproof flashlight powered by two D cell batteries. For each sample date in all years, a single light trap was placed at each site at least 1 h after sunset. Traps were placed in succession so that each

trap could be picked up after 60 min of illumination. Samples were preserved in a 5% solution of buffered formalin for 1 month and then transferred to 70% ethanol. Larvae were identified following the keys in Wang (1986). Temperature was recorded using Onset Computer Corporation 8k optic-stowaway temperature data recorders. Daylight hours and percent illumination of the moon data was obtained from the U.S. Naval Observatory website (http://aa.usno.navy.mil/data/docs/MoonFraction).

Analysis of SP postlarval and juvenile diets was carried out under the following protocol. First, the fish from were removed from alcohol and pat dried it on both sides with a paper towel to remove any excess alcohol. The fish were weighed on a Mettler Toledo AT201 balance to the nearest .001 gram, and measured to standard and total length. The belly of the fish were slit (just through the skin) starting at the anus up to the base of the gill, the esophagus was then clipped as close to the throat as possible, with the same process for the colon after the stomach, then the stomach was removed from the fish The fullness of the stomach was estimated using the following categories empty, 1-25% , 26-50%, 51-75%, 76-100%. The stomach wall was then slit to expose the stomach contents, which were weighed and recorded. The gut contents were then separated into piles according to taxa (copepods, cladocerans, chironomids, etc.), each pile of taxa was weighed and recorded.

**Statistics**

The relative numbers of SP were analyzed using graphs to look at relative abundance in relation to temperature and moon illumination. Pearson-product-by-moment correlation analysis and linear regression was used to analyze the relation of prolarvae appearance to temperature, moon illumination, and length of daylight hours. Percent moon illumination

was arc-sine transformed before evaluation. Basic descriptive statistics were used to establish means, ranges, and minimum and maximum values. The analyses were performed using Statgraphics v.5.0 package Inc. (Manugistics, MD).

## Results

### *Catch Summary*

We collected 1,984 larval fish in 2004, 2,174 in 2005, and 2,149 in 2006 (Table 1). Sacramento blackfish was the most abundant species, accounting for 40% of the total number of individuals. Other common taxa were western mosquitofish, (33%), Sacramento perch, (24%), and threespine stickleback (3%). The highest overall catch-per-unit effort was in 2004, followed by 2005, and finally 2006 (Table 1). Catch-per-unit effort for SP pro-larvae and post-larvae was highest in 2005, then 2004, and lowest in 2006 (Table 1, Figure 1).

### *Temperature*

Sacramento perch spawning began March 24 in 2004 and continued for 62 days. In 2005 spawning initiated on April 14 and the duration was 78 days. Finally in 2006 the timing of the first spawn was April 7 and subsided after 77 days (Table 2).

Table 1. Average CPUE (number larvae/illumination hour) and annual percent composition of larval fish species caught in 2004-2006. Native species are denoted by (N) and Aliens by (A). CPUE and percentages of all species were rounded to the nearest whole number. Number of species indicates the total number of fish species caught in that year. Prolarvae are larval fish that are still absorbing the egg yolk, Post larvae are larval fish that the egg has been completely absorbed and are actively feeding, and Juveniles are fish that have complete adult characteristics.

| **Variable** | 2004 | 2005 | 2006 | Average |
|---|---|---|---|---|
| Total light trap hours | 14 | 16 | 17 | 16 |
| Average CPUE | 142 | 136 | 195 | 158 |
| Number of species | 4 | 4 | 4 | 4 |
| | | | | |
| **Species** | CPUE (%) | CPUE (%) | CPUE (%) | |
| Sacramento blackfish (**N**) | 61 (43) | 58 (43) | 45 (36) | 55 (41) |
| western mosquitofish (A) | 42 (30) | 39 (29) | 50 (40) | 44 (33) |
| threespine stickleback (**N**) | 6 ( 4) | 4 ( 3) | 3 ( 2) | 4 ( 3) |
| Sacramento perch (**N**) | 33 (24) | 34 (25) | 28 (22) | 32 (24) |
| | | | | |
| **Sacramento perch** | | | | |
| Year | #Pro (CPUE) | #Post (CPUE) | #Juveniles | |
| 2004 | 155 (11) | 244 (17) | 68 | |
| 2005 | 203 (13) | 270 (17) | 74 | |
| 2006 | 161 ( 9) | 230 (14) | 90 | |

The mean temperatures (19.9°C 2004, 20.4°C 2005) during the spawning period are significantly different ($t = -6.1$, $p = 1.2^{-9}$), but the means of the two periods are still only .5°C apart. A closer examination of the time period of 2004 that proceeded the initiation of spawning as compared to 2005 shows a significant difference in the mean temperatures for that period with a mean temperature of 18.3°C in 2004, and 16.6°C in 2005 ($t=16.5$, $p = .0.0$ ).

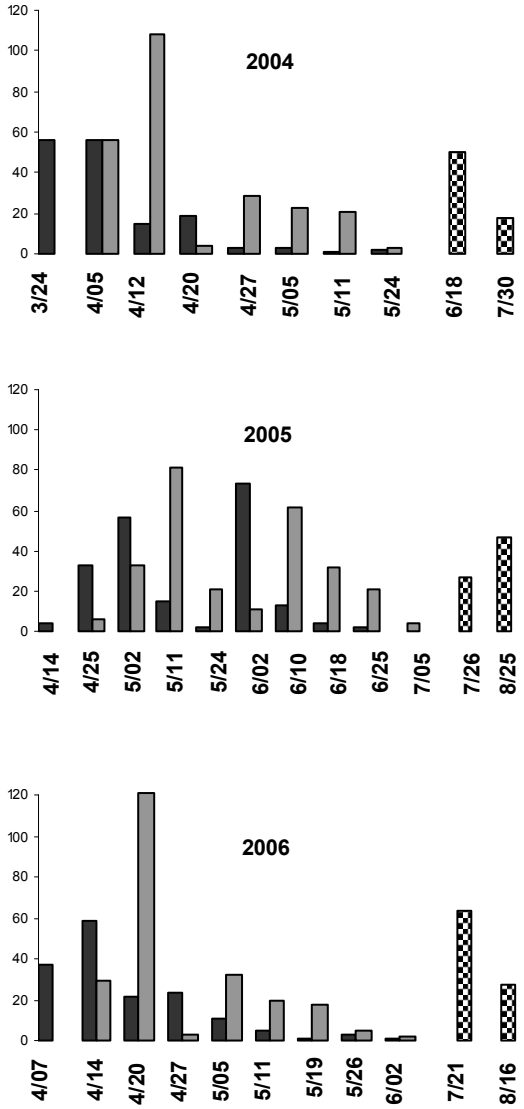


Figure 1. Catch numbers over time of (prolarvae (black), postlarvae (grey), juvenile (checked)) for 2004-2006 from Curved Pond, Yolo County, California.

Table 2. Month/day of the beginning of spawning, the month/day of the termination, and the duration in days.

| Year | Month | Begin | Month | End | Duration |
|---|---|---|---|---|---|
| 2004 | March | 24 | May | 24 | 62 |
| 2005 | April | 14 | June | 25 | 78 |
| 2006 | April | 7 | June | 2 | 77 |

Table 3. Temperatures (°C), with mean, min-max, range, standard error, and confidence intervals for the beginning and ending day temperatures, and throughout the spawning period.

**2004**

| | N | Mean | Min | Max | Range | SE | 95%CI |
|---|---|---|---|---|---|---|---|
| Begin | 24 | 19.8 | 18.4 | 21.8 | 3.4 | .2 | 19.8± .4 |
| Ending | 24 | 21.6 | 20.0 | 23.5 | 3.5 | .2 | 21.6± .5 |
| Mean | 1487 | 19.9 | 13.7 | 25.8 | 12.1 | .1 | 19.9± .1 |

**2005**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Begin | 24 | 16.1 | 14.9 | 17.3 | 2.4 | .2 | 16.1± .3 |
| Ending | 24 | 22.3 | 21.3 | 23.3 | 2.0 | .1 | 22.3± .3 |
| Mean | 1752 | 20.4 | 14.9 | 25.1 | 10.2 | .05 | 20.4± .1 |

**2006 No Data**

*Moon Phase*

Graphs of Sacramento perch larvae appearance in relation to lunar illumination show some strong spawning events in relation to illumination, although there is high variation in numbers (Figure 2). Moon phase as a predictor of spawning, as indicated by number of Sacramento perch prolarvae caught in traps on specific dates, was not significant (ANOVA F = .75, P = .55 ) although, the Model (linear) as fitted explained 42.9 % of the variability in the prolarvae numbers. The relationship between prolarvae and percent moon illumination was moderately strong, but in a negative direction with a correlation coefficient of -0.65.

***Photoperiod***

Daylight hours at the initiation of spawning in 2004 were 12 hours and 20 minutes and continued until the daylight period had lengthened to 14 hours and 32 minutes. In 2005 the beginning was at 12 hours and 52 minutes and ended at 14 hours and 32 minutes. Finally, in 2006 spawning began at 12 hours 54 minutes and ended at 14 hours and 32 minutes (Table 4).

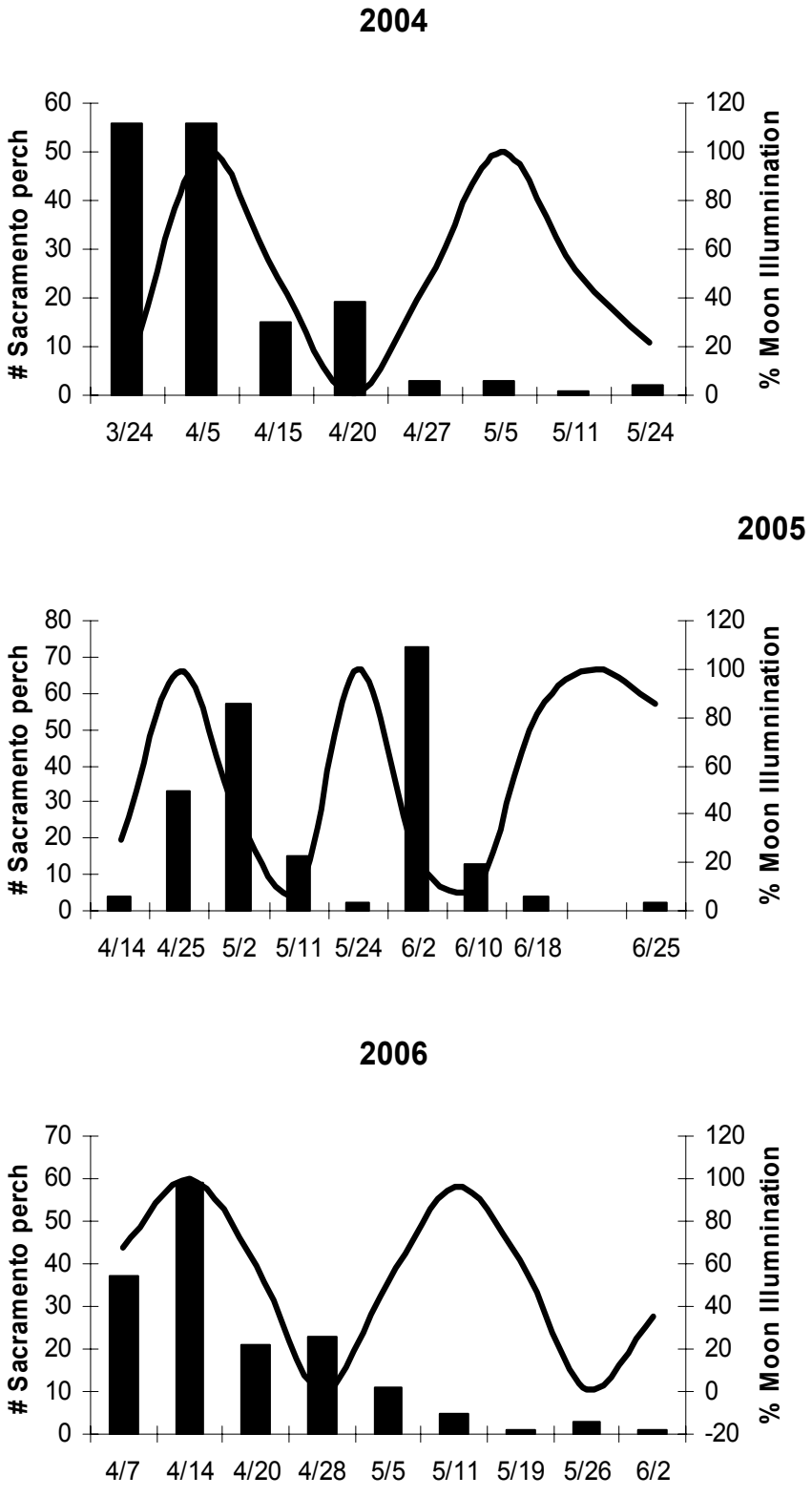


Figure 2. Moon illumination (line) in relation to numbers of prolarvae ( bar) captured

Table 4. Shows the number of hours/minutes of daylight at the initiation of spawning and the termination of spawning for 2004-2006.

| Year | Beginning | Ending |
|---|---|---|
| 2004 | 12:20 | 14:32 |
| 2005 | 12:52 | 14:51 |
| 2006 | 12:54 | 14:32 |

**Diet**

The diets of Sacramento perch were examined during the larval stage and the juvenile stage. The two groups were split, with larva and post larva being considered any fish below 16 millimeters in notochord length. This length was determined to be the average size at which larval fish have developed all adult characters, so are considered to be juvenile fish (Wang, 1986). An index of relative importance was calculated for both groups. In the larval fish, cladocerans were the most important prey item followed by copepods. Every other category can be considered rare in the diet (Figure 3). Juvenile fish switched from eating cladocerans to eating primarily amphipods and chironomids, although amphipods were three times as likely to show in the diet then chironomids. Insects, cladocerans and copepods were also found to lesser extent in the diets (Figure 3). In a plot of the percent zooplankton versus the notochord length of the perch, there is clearly a negative relationship between fish size and percentage of zooplankton in the diet, although there is some scatter through the different sizes of larvae and juveniles (Figure 4). The pattern of the IRI and the zooplankton plot is away from water column feeding towards more benthic feeding as the perch become older. Intermixed with this is some feeding in or on macrophytes as indicated by other larval insects consumed, predominately odonates.

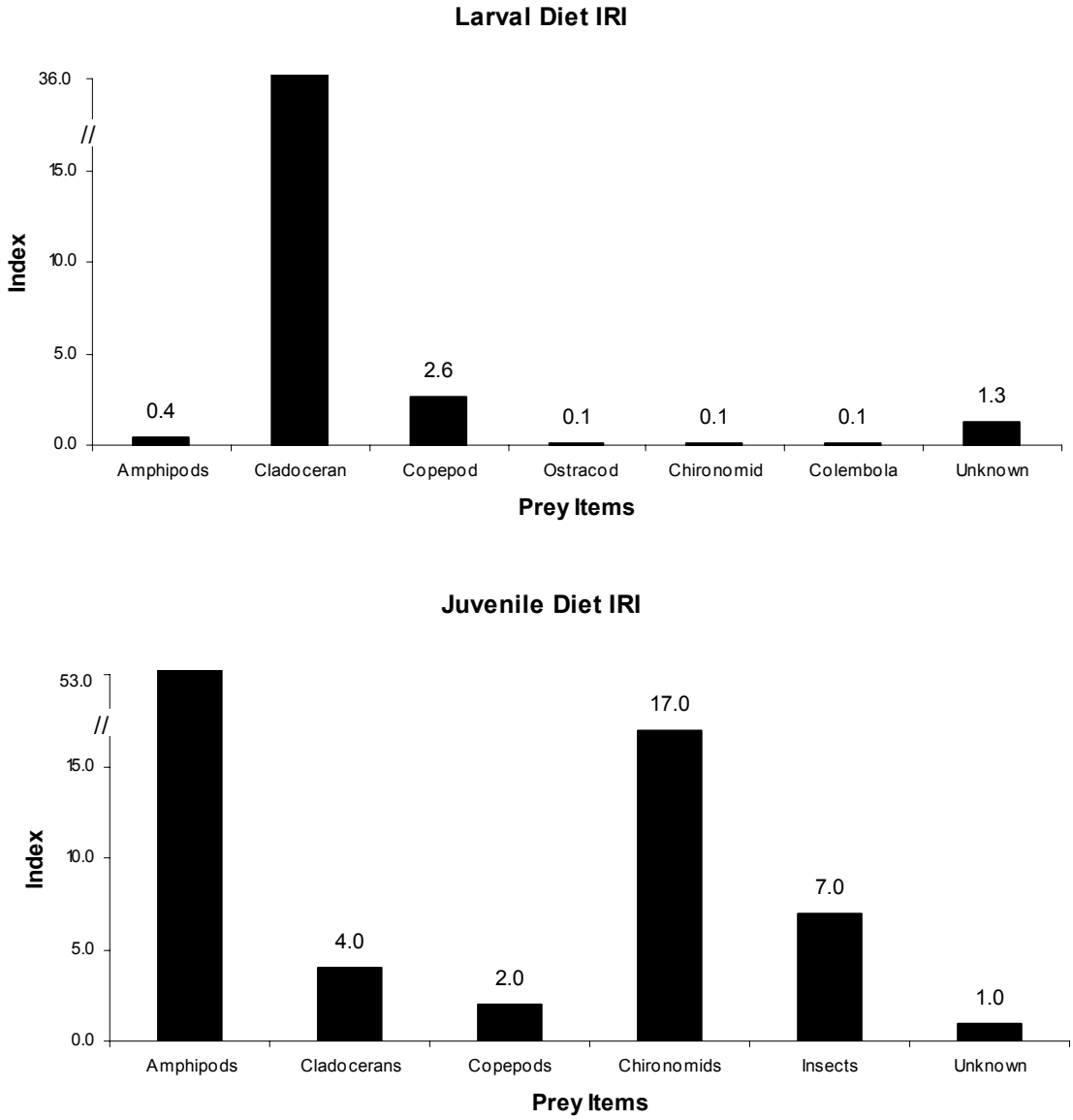


Figure 3 Index of relative importance for prey items (Amphipods, Cladocerans, Copepods, Chironomids, Insects, Unkown) consumed by larval and juvenile Sacramento perch.

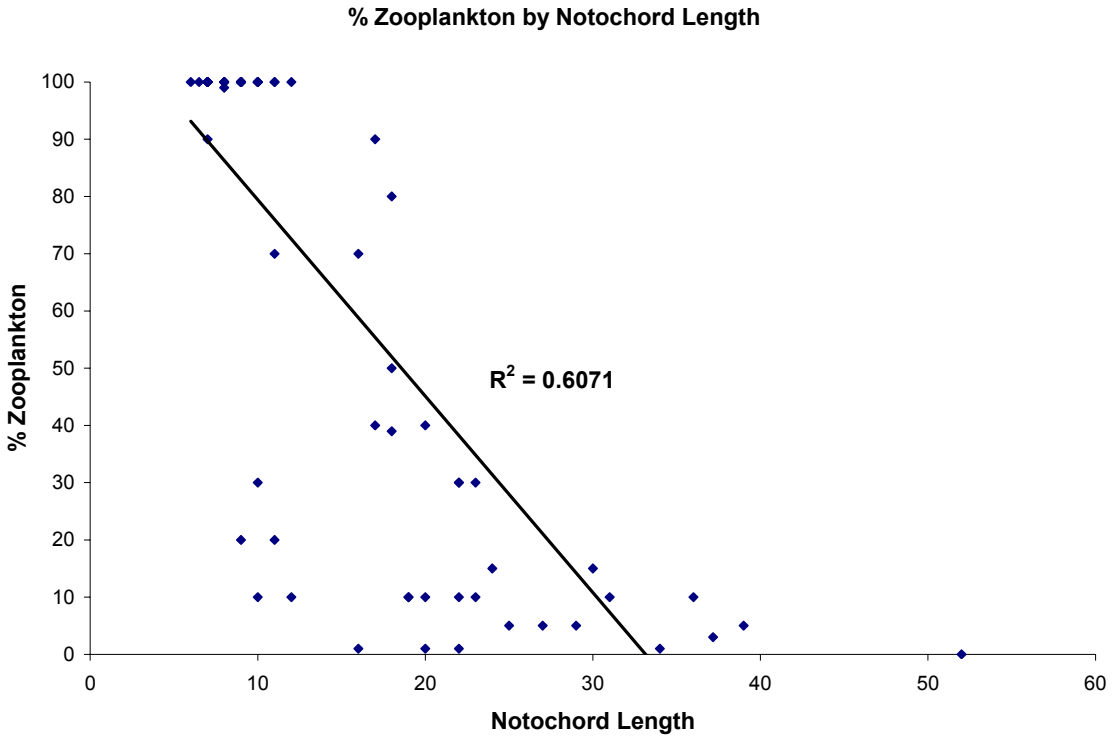


Figure 4 Plot of notochord length versus the amount of zooplankton consumed by larval and juvenile Sacramento perch.

## Discussion

It is clear that Sacramento perch young-of-the-year had a variable response to temperature, moon illumination and photoperiod in curved pond, as indicated by comparisons among years, changes in timing of first spawn, and duration of spawning after initial spawn. Monthly larval perch catch data show variable patterns related to the temperature, photoperiod and moon illumination, and finer scale examination of the data provided little clarity as to the causal effect of the high variability. Part of the variability results from Sacramento perch being fractional spawners, spawning several times over a period of time. In larger bodies of water (Crowley Lake) Sacramento perch may spawn intermittently over a 4 month period (Christa Woodley, pers. com.). In Curve Pond the

duration was much shorter (Table 2) presumably due to bioenergetic costs of spawning during the higher temperatures later in the season.

***Temperature***

The relationship of temperature to spawning events is similar to other California native fish, in that initial spawning takes place earlier at cooler temperatures than that of most alien fishes. Temperatures that Sacramento perch seem to cue in on are very similar to those of splittail spawning on floodplains (Crain et al. 2004) (Table 3). In both years the mean temperatures during spawning duration were very close (19.9°C, 20.4°C), although statistically different. The difference in the date of spawning initiation between years could be attributed to difference in temperatures, 19.3°C in 2004 versus 16.6°C in 2005 for the same time period that led up to spawning in 2004. The spawning initiation temperature cue in this study seems to be around 18°C.

***Moon Phase***

There doesn't seem to be a clear pattern of cueing on a specific illumination of the moon. There were some strong spawning events coupled with maximum illumination, but there seemed to be strong events without illumination also. Generally fishes that cue on moon illumination are doing so because of the tides associated with lunar events. Perch may be able to find each other better during these events, or circadian rhythms may be enhanced. From the data that we have presented it seems likely that moon illumination is at best a weak interaction with temperature and photoperiod as a spawning cue for perch.

***Photoperiod***

In each of the spawning periods in this study the beginning photoperiod was over 12 hours (Table 4). In culture Sacramento perch can be induced to spawn using a 15/9 L: D

photoperiod and temperature of 27°C (Bolnick and Miller,2006). At UC Davis, perch have been induced to spawn at lower temperatures and shorter day length, using 22°C and a 12/12 L: D photo period. Perch captured from Crowley Lake for physiological experiments spawned in tanks where the water was 15°C (Christa Woodley, pers. com.). Because tradeoffs between offspring survival and other life-history traits differ broadly among populations it seems likely that perch in Curved Pond are adapted to the environmental variability within this small system.

***Diet***

The diet of Sacramento perch is typical of larval fish, primarily containing small crustaceans and dipteran larvae. Juvenile Sacramento perch become more benthic in their feeding habits and take larger prey as their gape increased. The variety of prey items including cladocerans, copepods, insects, amphipods, and chironomids indicates a very opportunistic feeding pattern. Juvenile perch did show a pronounced increase in parasitic worms in the gut cavity during the warmer months of the summer. Whether this phenomenon is a problem associated with the size and high summer temperatures of small ponds is unanswered.

## Conclusions

The spawning of Sacramento perch is apparently cued by temperature and length of daylight hours (18°C, 12/12), and possibly to a much lesser extent lunar illumination. The diet of perch at this life stage is varied and correlates with gape of the mouth, but larval perch seemed to prefer cladocerans, while juveniles prefer amphipods. Early life history does not seem to be limiting under these conditions (no other sunfish present), so the use of ponds as a source of perch to move into the larger environment may be a

compatible strategy with restoration goals as long as temperatures within the pond are compatible.

## Task 6 Ecological Physiology of Sacramento perch

Christa M. Woodley and Joseph J. Cech, Jr.

### Introduction

The basic environmental tolerances of Sacramento Perch are poorly defined, although Sacramento Perch have a reputation for being physiologically very tolerant, capable of living in water in which other centrarchids cannot persist. These limits and tendencies need to be better defined in order to determine what factors (i.e., water temperature, salinity, pH, dissolved oxygen, and velocity) may be limiting the distribution and abundance of both juvenile and adult Sacramento Perch. The objective of this study therefore was to document physiological tolerance limits and preferences of juvenile and adult Sacramento Perch, specifically regarding upper and lower temperature limits, upper salinity limits, upper and lower pH limits, lower dissolved oxygen limits, and upper velocity limits. The basic hypotheses tested were: (1) Sacramento Perch are not limited by present conditions in SF Estuary; (2) Sacramento Perch are physiologically capable of living and reproducing in parts of estuary that may be too saline for potential competitors, especially other centrarchids (e.g., Suisun Marsh) and (3) re-established Sacramento Perch populations may be limited by hydrological factors, such as transport to undesirable habitats or entrainment.

**Sacramento perch hatchery**

Due to the difficulties of obtaining Sacramento perch of similar size and environmental conditions, we built a Sacramento perch hatchery in the spring of 2004. Prior attempts to rear Sacramento perch for commercial aquaculture typically ended in failure, with one exception. At the Contra Costa Mosquito Vector and Control District (MVCD-CC) facility, Chris Miller, operates a hatchery for Sacramento perch. The MVCD-CC facility production rates vary from 0 – 12.2 % survival at 104 days post-hatch. Factors that seem to influence the larval success rates are: substrate and tank size, pairing of mates, age and size, time of year, number of spawns that year by the female, temperature, infections and water quality. In attempt to avoid some of these confounding factors, we choose to design a system that would offer substrate acclimation, flow through conditions, mimic ambient photoperiods and water temperatures, and offer live feed for 30 - 40 d. There were three successful spawns (in 2004) with larvae estimates of 8000 individuals. The first and third spawns were very small and separated by three weeks. During the first month, we suffered an estimated 90 % loss (80 % related to procedural accident). By 104 days post-hatch, we successfully raised 250 individuals (3.5 % survival). In 2006, to verify some initial data collected, we spawned 10 pairs of Sacramento perch. After culling 80% of the larvae, by 104 days post-hatch, we had an 80 % survival rate.

Large adults were collected from Abbott's Lagoon (located in Point Reyes National Seashore) and Crowley Reservoir (located south of Mammoth Lakes in the eastern Sierra Nevada mountain range) during 2004 through 2006. During 2004-2005, 40 adults and 15 juveniles from Crowley Reservoir were transported to the Center for Aquatic Biology and Aquaculture (CABA), University of California-Davis, Davis, CA. Wild adult

Sacramento perch proved to be quite sensitive to transportation and the holding facilities. The fish seemed stressed even though a variety of techniques were used to ease chemical and visual stimuli. Because of this, the adults were constantly contracting diseases from their natural flora and the surrounding environment. Disease rates and intensity of the illnesses were greatly reduced by holding the fishes at 12 ºC, and feeding them a diet that varied between live fish (juvenile goldfish, blackfish and mosquitofish) and invertebrates (worms, grubs, and chironomid larvae). In 2004 and 2005, we experienced a 95 % and 60% mortality rate of the adults and juveniles, respectively. Changes to the procedure in 2005 were noted and further altered in 2006 when wild adults were captured and transported to the Center for Aquatic Biology and Aquaculture. The transportation in 2006 included Crowley Lake water at 14 ºC, 7 ppt salts, and two 50% water changes using available hatchery water in the Sierras and foothills. A variety of immediate low dose preventative treatments to minimize disease contraction rates were also used, malachite-formalin (10 days), followed by a low dose of nitrofurazone (10 days), to the holding tanks when the fish were brought to the Center for Aquatic Biology and Aquaculture. After these treatments, the fish were held for 3 weeks while monitoring their behavior and health daily. The live fish fed to the Sacramento perch were maintained on a commercial diet of Salmon Silver cup which was treated with oxytetracycline. In 2005 and 2006, we experienced a morality rate of 5 % after capturing and transporting 120 individuals to the Center for Aquatic Biology and Aquaculture.

When exposing acclimated healthy wild adult Sacramento perch held at 12 ºC to greater temperatures for experiments, the fish often became stressed and contracted diseases. After many attempts and a moderate rate of mortalities (45 %), we chose only to use only

one elevated temperature at 26 ºC (compared to the three temperatures used with the juveniles), a slower increase in acclimation temperature (0.65 ºC/day; thus transition time was twice as long), and a typical acclimation period (2 weeks) for the 18 and 26 ºC temperature regimes.

**Environmental tolerance tests**

***Methods***:

Fish were exposed to their lethal limits, through frequent water changes. Water changes occurred every minute until the fish exhibited a loss of equilibrium or opercular movement. When the endpoint was reached, the time and water condition were noted, the chamber was immediately flushed with fresh, acclimation-condition water to recover the fish. Live fish wet weights (ww; g) were collected along with standard and total lengths (SL, TL; mm) to detect tolerance relationships with fish size. This approach allowed us to document fine-scale physiological changes, yet we could reverse the experimental process before the fish entered an unrecoverable state (no mortalities occurred with 14 days of the experiment). The parameters measured were critical temperature maxima ($CT_{max}$), critical temperature minima ($CT_{min}$), critical dissolved oxygen minima ($CDO_{min}$), critical salinity maxima ($CSal_{max}$) and critical pH maxima ($CpH_{max}$).

Because of the unexpected difficulties capturing and maintaining adult Sacramento perch, we were limited in the abundance of fish available for experiments. Once a fish was exposed to a tolerance test, it could not used in other experiments (unlike the metabolism and swimming experiments). Thus, the adult environmental tolerance tests were not conducted as thoroughly as the juvenile environmental tolerance tests. These tests were

only conducted at 12 ºC for the adults. Based upon our field data, large adult Sacramento perch are not found in water over 15 ºC. The exception was during spawning when some shallows (i.e., 6 ft or less) would experience diurnal temperature changes that ranged from 12 to 18 ºC.

***Results***

Please refer to Table 1 for the juvenile data and Table 2 for the adult data. Overall, the results indicate that Sacramento perch are quite tolerant to adverse conditions at the temperatures tested. For the juvenile life stage, their ability to tolerate extreme dissolved oxygen, salinity and pH conditions lessens with increased acclimation temperature. Adults have a lower dissolved oxygen tolerance and salinity tolerance at 12 ºC than juveniles. There is little information available on the tolerance limits of juvenile confamilial species to compare to our results. The temperature tolerances of adult Sacramento perch are within the ranges of other centrarchids (i.e., bluegill and largemouth bass); however they do have lower dissolved oxygen limits and greater salinity tolerances than that of their confamilial species.

**Table 1.** The environmental tolerances (preliminary) for juvenile Sacramento perch. The symbol "---" indicates parameters that were not tested.

| Acclimation temperature (ºC ) | Replicates (n) | Total length (mm) | Wet weight (g) | $CT_{max}$ (ºC ) | $CT_{min}$ (ºC ) | $CDO_{min}$ (mmHg) | $CSal_{max}$ (ppt) | $CpH_{max}$ |
|---|---|---|---|---|---|---|---|---|
| 19 | 32 | 99 | 20.6 | 36.1 | --- | --- | --- | --- |
| 19 | 32 | 99 | 21.5 | --- | 9.0 | --- | --- | --- |
| 27 | 10 | 120 | 39.8 | --- | --- | 59.0 | 24.3 | 10.72 |
| 24 | 10 | 128 | 45.3 | --- | --- | 50.4 | 26.1 | 10.84 |
| 19 | 16 | 98 | 20.1 | 36.1 | 9.0 | 42.7 | 27.6 | 10.93 |
| 12 | 16 | 88 | 13.1 | --- | --- | 22.2 | 28.0 | 10.98 |

**Table 2.** The environmental tolerance results for adult Sacramento perch. The symbol "^" indicates the experiment ended before the final endpoint was achieved. For example during the 12 ºC experiment, behavioral and physiological changes were observed, such as an increase in movement within the chambers and an increase in ventilation frequency. These changes initiated at a 46.55 ± 1.29 mmHg and began to decrease at 23.50 ± 0.77 mmHg. However, we were limited by the cold water and altitude and could not strip the oxygen from the water below 15 mmHg.

| Acclimation temperature (ºC ) | Replicates (n) | Total length (mm) | Wet weight (g) | $CT_{max}$ (°C ) | $CT_{min}$ (°C ) | $CDO_{min}$ (mmHg) | $CSalinity_{max}$ (ppt) |
|---|---|---|---|---|---|---|---|
| 12 | 16 | 196 | 130.4 | 32.05 | | | |
| 12 | 16 | 180 | 94.0 | | 10.0^ | | |
| 12 | 16 | 191 | 124.7 | | | 15.0^ | 26.3 |

**Water velocity tests**

***Methods***

We measured ontogenetic changes in swimming performance or the critical swimming velocity, *U*crit, with Sacramento perch increasing in age and total length (TL). Fish in all *U*crit experiments were used only once. A Brett-type swim chamber (Brett 1964) that generated rectilinear flow was used to measure *U*crit. Each fish was acclimated for 60 min at a low water velocity to orient to the flow and chamber. After acclimation, water velocity was increased in 30 min intervals. *U*crit, as a measure of the maximum aerobic swimming ability (Beamish 1978), was calculated from Brett (1964): *U*crit = Vf + (Vi*(Tf/Ti)) where *V*f is the final velocity at which the fish swam for the entire 30 min period, *V*i is the increment of water velocity increase (4.5 cm/s for juveniles and 10.1 cm/s for adults), *T*f is the time swum at the water velocity of fatigue, and *T*i is the time increment for each water velocity. The swimming chamber was maintained at a constant temperature by a thermostat-controlled water bath, and its water was partially exchanged

with fresh, air-equilibrated well water between experiments to ensure dissolved oxygen levels near air saturation. No solid blocking corrections were used, because the maximum cross-sectional area of the fish was always <10% of the cross-sectional area of the swimming chamber (Bell and Terhune 1970). *U*crit swimming tests were concluded when fish impinged three times at the same water velocity. Impingement was determined as either impingement (one-third or greater of body in contact with the rear screen) or fatigue (sustained contact with the rear screen for 60 s). After the initial impingement, the water velocity was decreased for <60 s until the fish came off the rear screen. If the fish did not come off the rear screen within 60 s, the experiment was ended. If the fish did come off the rear screen, the water velocity was increased until it was at the same velocity of impingement. Usually, the second and third impingements occurred in rapid succession at the water velocity of the first impingement. Fish were not touched, prodded, or shocked during the experiment. During the experiments, tail beat frequency (TBF) and ventilation frequency (VF) was also counted. Both measurements were means from watching the fish for 15-s periods three times at every water velocity interval: 5, 10, and 15 min after water velocity increases. After the experiment, fish were measured for wet weight (g), standard and total lengths (mm; TL).

***Results***

Our results show a positive correlation between increasing temperature and final swimming velocity for the juvenile stages. The adults' swimming performance, however, exceeds that of the juveniles by only a small margin, which we attributed to the morphological difference in the body depth and caudal peduncle length relationships.

Conversely, adult swim performance does not increase significantly with temperature; and the calculated body length per second actually decreases with temperature.

**Table 3:** Water velocity tolerances for juvenile and adult Sacramento perch.

| Age (replicate) | Acclimation temperature (ºC) | Total length (mm) | Wet weight (g) | Ucrit (cm/s) | Ucrit (bl/s) |
|---|---|---|---|---|---|
| Juvenile (16) | 12 | 88.50 ± 3.22 | 14.11 ± 1.73 | 23.61 ± 1.67 | 3.76 ± 0.23 |
| Juvenile (16) | 18 | 101.85 ± 8.17 | 22.68 ± 6.01 | 31.42 ± 2.66 | 3.30 ± 0.28 |
| Juvenile (16) | 24 | 130.6 ± 10.0 | 45.39 ± 8.79 | 32.82 ± 5.79 | 4.26 ± 1.86 |
| Juvenile (16) | 27 | 124.63 ± 13.3 | 41.83 ± 14.7 | 36.05 ± 5.71 | 3.55 ± 0.71 |
| Adult (16) | 12 | 257.73 ± 22.1 | 440.97 ± 92.7 | 34.87 ± 5.91 | 0.74 ± 0.12 |
| Adult (16) | 18 | 272.93 ± 29.5 | 368.14 ± 134.0 | 38.38 ± 4.72 | 0.71 ± 0.15 |
| Adult (16) | 26 | 293.25 ± 18.6 | 449.69 ± 66.0 | 42.78 ± 7.53 | 0.69 ± 0.14 |

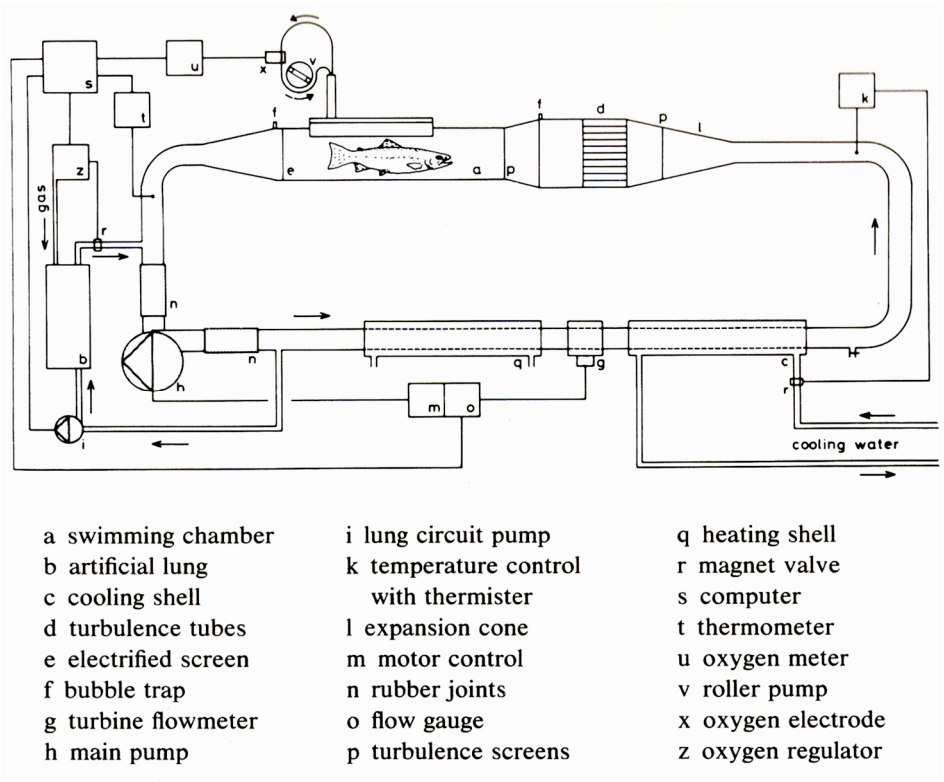


**Figure 1.** The diagram represents a closed swimming tunnel in which water is propelled, forcing the fish to swim. The flume is temperature controlled by the water bath and works to maintain constant temperature within the flume (where propeller and water friction would create heat).

**Behavioral preference tests**

***Methods***

A horizontal, annular environmental gradient tank of 1 or 3 m diameter (Figure 2) was used to determine the behavioral preferences of Sacramento perch acclimated to 12, 18 or 26 ºC to temperature gradients or acclimated to freshwater (0 ppt) and brackish water (15 ppt) to determine the behavioral salinity preference. The annular preference tanks were divided into equal visual segments (not physical segments) that could be observed by the above camera. Environmental gradients were produced by the simultaneous introductions of water (of different temperatures or salinities) from plastic reservoirs into

mixing chambers outside the annulus. In the temperature experiments, cold (12.0 ºC), ambient (18.0 ºC), and hot (24.0 ºC) water was pumped into the mixing chambers which fed a swimming channel forming a temperature gradient. For the salinity experiments, freshwater (0 ppt), brackish water (15 ppt), and saltwater (33 ppt) were pumped into the mixing chambers to produce a salinity gradient from 0 to 33 ppt. YSI telethermometers and thermistor arrays, and water sampling cannulae were used to measure mid-depth water temperatures and salinity against the inner and outer walls of the swimming channel in each segment. Four measurements per segment were monitored. The water and their mixtures flowed from the swimming channel towards the center drain in the apparatus, via holes and v-notches. Thus, individual fish were exposed to the resulting gradient as they swim through the annular path.

Sacramento perch were transferred to the annular preference chamber from the rearing tanks at acclimated temperatures and allowed 30 min to recover from handling before the gradient was established. Fish location was monitored remotely via a charge-coupled device (CCD) camera connected to a video monitor, and recorded. Fish locations were noted every 2 min for the acclimation period and for the 60-min experiment (Table 4).

The preference chamber construction and calibration was completed in September 2006. Due to the unavoidable 6-month delay in the delivery of the preference chamber, the unexpected difficulties associated with capturing and maintaining adult Sacramento perch (i.e., time of year, illness), and large quantities of water needed, only juveniles were tested for a salinity preference at 12 ºC.

***Results***

The juvenile Sacramento perch were tested for a salinity preference using the 1 m annular ring and showed no preference. The mean final salinity preference would be 15.6 ppt. The adults were tested for temperature preference using the 3m annular ring and an acclimation period of 2 weeks prior to testing their temperature preference. All three temperature groups (12, 18, and 26 ºC) choose the water temperature closest to that of the ambient conditions between 17.6 and 18.9 ºC. This indicates that the adults are similar to other native Central Valley species in temperature preference, and cataloging them as a cool water centrarchid..

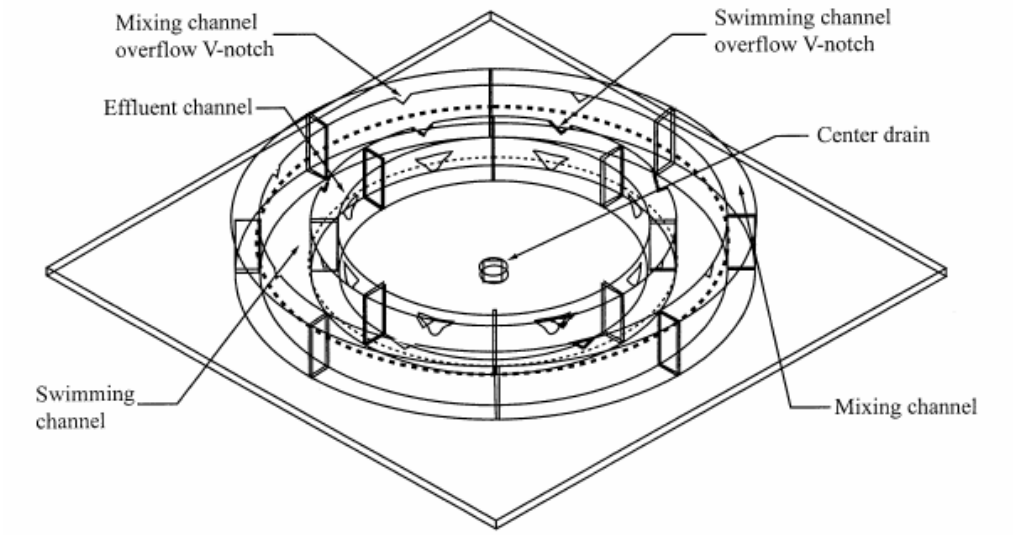


**Figure 2:** Isometric view of the annular preference chamber. Water flows radically from the outer mixing channel through the small holes (dashed lines) into the swimming channel and from there into the effluent channel. Water leaves the effluent channel through the large triangular openings and drains through the center drain.

**Table 4:** Behavioral preferences of the juvenile and adult Sacramento perch using annular preference chambers. The Sacramento perch were offered temperature of 12-24 ºC and a salinity range of 0-33 ppt. "NC" is a treatment not complete due to the lack of appropriate size for the test or difficulties with attaining the needed amount of temperature regulated saltwater. The juvenile temperature preference test could be conducted at a later date. However, the adult salinity preference experiments can not be conducted due to the larger quality of saltwater needed for the experiments, which is not available on the UC Davis campus nor physically could the UC Davis campus hold and regulate that much saltwater at the campus for each temperature needed without major investments in holding tanks, chillers and heaters.

| Age (n) | Acclimation temperature (ºC ) | Total length (mm) | Wet weight (g) | Temperature (ºC) | Salinity (ppt) |
|---|---|---|---|---|---|
| Juvenile (24) | 12 | 62.0 ± 1.24 | 12.1 ± 0.21 | NC | No preference (15 ppt) |
| Adult (16) | 12 | 264.4 ± 1.86 | 407.4 ± 85.5 | 17.61 ± 3.24 | NC |
| Adult (16) | 18 | 300.0 ± 1.21 | 544.2 ± 75.8 | 18.90 ± 2.94 | NC |
| Adult (16) | 26 | 296.8 ± 1.10 | 501.4 ± 102.7 | 18.96 ± 3.23 | NC |

## Task 7 Genetics

**Rachel Schwartz and Bernie May**

Prior to this study, little was known about the genetics of Sacramento perch, aside from studies of their relationships with other centrarchids. However, understanding the genetic structure of a rare species is crucial for developing conservation strategies. In this study we first developed genetic markers to enable the use of mitochondrial DNA to study population structure. This was followed by the genetic characterization of eleven Sacramento perch populations from California and Nevada. which was most of the remaining large populations.

**Sample Collection**

Sacramento perch were sampled from eleven populations in California and Nevada by electrofishing, hook and line, gill nets, or seining (Table 1). A small section was clipped from the anal fin and stored in 95% ethanol or dry in a paper envelope, or a few scales were removed and stored dry. At least 29 samples were collected from ten of the populations, although only eight samples were collected from Hume Lake. For three populations, samples were collected for more than one year to ensure that less genetic variability occurred between years than between populations (Table 1). DNA was extracted from fin clips using the Promega Genomic DNA purification system, the Qiagen DNeasy Tissue kit, or the Gentra PureGene kit.

Historical samples of extant and extirpated populations were obtained from collections of Sacramento perch held by the California Academy of Sciences and UC Davis Department of Anthropology (Table 2). These samples include the Lake Greenhaven population, which is believed to be the source of many extant populations (Moyle 2002). DNA was

extracted from formalin preserved fin clips using a Gentra PureGene kit, and from dried bone using a phenol-chloroform protocol followed by a silica column extraction (Smith et al. unpublished). Extractions were conducted in a clean room free of PCR-amplified DNA.

## Development of Microsatellite DNA Loci

### *Methods*

In order to analyze genetic variation it was first necessary to develop suitable markers. Microsatellite DNA loci are the best marker type available for assessing genetic variation within and among populations. Microsatellite loci are noncoding regions of DNA containing repeating DNA units (such as CAGA or CATA). These loci show high levels of length variation due to different numbers of repeats. Microsatellite loci are codominant makers; individuals may have either two different or two identical alleles at a locus, a feature that makes them useful for determining whether populations have experienced bottlenecks.

Microsatellite DNA loci developed for other centrarchid species failed to amplify consistently in Sacramento perch, making it necessary to develop new loci for this species. Four libraries enriched for tetranucleotide repeat motifs $(TAGA)_n$, $(CAGA)_n$, $(TACA)_n$, and $(CATC)_n$ were constructed from Sacramento perch DNA by Genetic Identification Services (Chatsworth, CA) (protocol described in Meredith and May 2002). These libraries were screened for DNA fragments between 300 and 700 base pairs (bp) by bacterial cloning, amplification with Polymerase Chain Reactions (PCR), and agarose gel electrophoresis. Bacterial colonies containing fragments of the correct sizes were

sequenced (also by Genetic Identification Services) and primers were developed to amplify these sequences.

Primers were initially tested for amplification using PCR on six individuals from three populations to determine whether loci contained more than one allele. Amplification products were mixed with formamide loading dye, denatured at 95C, and run on a denaturing acrylamide gel. Products were visualized using SYBR Green nucleic acid stain (protocol described in Rodzen et al. 1998). Polymorphic loci were screened following the same protocol using 34 individuals: 27 from Stillwater Refuge, two from Pyramid Lake, two from Clear Lake Reservoir, and one from Abbotts Lagoon.

***Results***

Twenty three polymorphic microsatellite DNA loci were selected based their ability to be amplified reliably in the initial panel of 34 individuals. The number of alleles in the Stillwater Refuge population ranged from one to eight, while the number of alleles in all sampled individuals ranged from two to eleven. At least 20 individuals amplified in all loci. Primer sequences and results are summarized in Table 3. Results were published as a paper in the journal Molecular Ecology Notes in 2004 (Schwartz and May 2004).

**Analysis of Populations Using Microsatellite DNA Loci**

***Methods: Data Collection***

10µl PCR consisted of 5-10ng of DNA, 1x buffer, 2mM $MgCl_2$, 0.8mM dNTPs, 0.5µM each primer, and 0.375U Promega Taq polymerase. Each forward primer was fluorescently labeled with one of three fluorescent dyes (fluorophores). Loci were amplified using standard thermalcyclers with the following program: 94C for 150s, followed by 25 cycles of 94C for 30s, 60C for 30s, and 72C for 30s, with a final

extension at 60C for 30m. Two or three PCR products with different florescent labels (1µl each) were mixed with 2µL water, 1.45 µl de-ionized formamide, 0.5µl loading dye and 0.05µl internal size standard. Samples were denatured at 95C for 210s and chilled on ice. One to two µl of PCR product mixture were loaded on a 51 lane denaturing polyacrylamide gel in 1x TBE buffer in a Basestation DNA Fragment Analyzer (MJ Research). Gels were prerun at 1900 volts for two minutes, followed by loading the samples for 30s at 4000 volts and 5600 scans at 2600 volts. Samples were analyzed using Cartographer fragment analysis software (MJ Research).

Of the loci developed and published (Schwartz and May 2004), 12 were selected for analyzing genetic variation in extant populations of Sacramento perch: *AinA117, AinA2, AinA203, AinD119, AinA218, AinD106, AinA120, AinA216, AinA108, AinA6, AinA212, and AinD101*. This decision was based on the number of alleles found in the initial screening process and the ability to visualize the PCR products consistently using the Basestation protocol described previously.

***Methods: Data Analysis***

***General statistics***

Populations were tested for deviation from Hardy-Weinberg equilibrium and linkage disequilibrium. Allele frequencies, observed and expected heterozygosities, and the inbreeding coefficient ($F_{IS}$) were calculated for each locus and population.

***Relationships between populations***

Distances between populations were calculated using two measures. Cavalli-Sforza and Edwards's (1967) chord distance ($D_{CSE}$) assumes no mutation (changes in gene frequency

depend solely on genetic drift) and allows for fluctuating effective population size; this model is most compatible with the known history of fish introductions in which only a few individuals were introduced to current locations and recent introductions are responsible for differentiation between populations. Pairwise $F_{ST}$ (Wright 1965) assumes that new mutations will always produce new alleles; we chose to use this measure based on this model due to the disjunct distribution of alleles in most loci and populations (Table 4). $F_{ST}$ is a measure of reduced heterozygosity in subpopulations as compared with the overall population, indicating population structure. An overall measure of $F_{ST}$ (Weir and Cockerham's 1984) was also computed

***Tests for population bottlenecks***

Because populations were most likely established from a small number of individuals (P. Moyle, personal communication), all populations were tested for genetic bottlenecks. The M ratio test (Garza and Williamson 2001) indicates genetic bottlenecks based on the proportion of "missing" alleles in an allele frequency distribution; an M value below 0.67 indicates a bottleneck. This test has the power to detect historical population bottlenecks, but can be influenced by the mutation model of the loci. A mode-shifted distribution occurs when an allele frequency distribution has fewer alleles in the lowest frequency class than in one or more intermediate frequency classes (Luikart et al. 1998). This test has the greatest power to detect extremely small bottlenecks and populations that have remained small over time. Sign and Wilcoxen's signed rank test for excess heterozygosity use the coalescent process to simulate the distribution of heterozygosity at mutation-drift equilibrium, given the specified sample and number of alleles (Cornuet and Luikart 1996). Data were analyzed using the two phase model (TPM) with two sets

of parameters. Differences in results among the four tests for bottlenecks may result from the power of these tests to detect bottlenecks that occurred over multiple generations or historically. Simulations by Williamson-Natesan (2005) indicate that methods using excess heterozygosity, including the sign and Wilcoxen tests, have the highest likelihood of detecting a population bottleneck if the bottleneck was very recent, less severe, and the pre-bottleneck value of theta ($4N_eu$, where $N_e$ is the effective population size and u is the mutation rate) was small; bottlenecks are more likely to be detected by the M test when they lasted for multiple generations, the pre-bottleneck value of theta ($4N_eu$) was large, or populations have recovered.

***Results***

***General statistics***

Alleles per locus ranged from 6 to 24 with the overall number of alleles per population ranging from 34 (Curve Pond) to 95 (Abbotts Lagoon) out of a total of 153 alleles (Table 4), 39 of which were unique to a single population; Sindicich Lagoon was the only population not to contain unique alleles. Observed heterozygosity ranged from 0.40 to 0.74, while expected heterozygosity ranged from 0.41 to 0.75 (Table 4). Estimates of the inbreeding coefficient were significantly positive in Abbotts Lagoon, Pyramid Lake, and Jewel Lake, although very small (Table 4).

***Relationships between populations***

Significant differentiation was observed between all populations; no significant differentiation was observed between years within a single population, except for the 2004 sample of Jewel Lake, which consisted of only 14 individuals. $F_{ST}$ values ranged from non-significant (between years from the same location) to 0.39 (Table 5). Chord

distances ($D_{CSE}$) and $F_{ST}$ values produced similar relationships between populations (Figure 1; Table 5; Table 6). Overall $F_{ST}$ for all populations was 0.22; indicating 22% of the genetic variation in the species is due to variation among populations.

***Tests for population bottlenecks***

Significant population bottlenecks were detected in six of the eight populations: Sindicich Lagoon, Stillwater Refuge, Crowley Lake, Pyramid Lake, Jewel Lake, and Curved Pond (Table 7; Table 8, Schwartz and May submitted). Bottlenecks were detected in five populations by the M test: Sindicich Lagoon, Stillwater Refuge, Crowley Lake, Pyramid Lake, and Curved Pond (Table 4; Table 7). Bottlenecks were detected in four populations by tests for excess heterozygosity: Crowley Lake, Pyramid Lake, Jewel Lake, and Curved Pond. Only one bottleneck was detected using the test for a mode shifted distribution: Curved Pond. (Table 7; Table 8) Although results differed between tests, indications of population bottlenecks were consistent with the ability of each test to determine bottlenecks based on their timing, severity, duration, or current and pre-bottleneck population size (Williamson-Natesan 2005).

**Determination of a Variable Mitochondrial DNA Locus**

***Methods***

Primers were selected from Yanagimoto et al. (2004) for four regions of the mitochondrial genome. DNA was amplified with PCR using six individuals and run on an agarose gel. Successful amplifications were purified using a Qiagen gel extraction kit and sequenced with both forward and reverse primers. Sequences were aligned with Sequencher and checked for variation.

***Results***

Approximately 1450 base pairs of the mitochondrial control region were selected for analysis based on the discovery of three haplotypes in the six initial sequences. Primers used to amplify this region were 15998PRO(TACCCCAAACTCCCAAAGCTA) and 12SH1067 (ATAATAGGGTATCTAATCCTAGTTT).

**Analysis of Populations Using Mitochondrial DNA**

***Methods:***

***Data Collection***

Approximately 1450 bases of the control region were amplified with PCR using 6-12 individuals each from eleven extant populations. Amplifications were run on an agarose gel; successful amplifications were purified using a Qiagen gel extraction kit, PCR purification kit, or AMPure PCR purification kit, and sequenced with both forward and reverse primers. A third primer (CR972R: GCTACGCTAGCATACGCATT) was designed in a conserved region in the middle of the long sequence and used to obtain clean sequence in the middle of the region.

For historical samples, primers were redesigned to amplify a 350 base pair region in two overlapping regions of less than 200 bases at the beginning of the control region. Primers for the first fragment were CR80F (AGGATTTCCCCATTCATTCA) and CR280R (CAACTGATGGTAGGCTCTTA); primers for the second fragment were CR260F (ACCTCAAAATATTAATGTAG) and CR420R (TATGCAAGCGTCGATGAAAG). DNA extraction and PCR were attempted on 22 samples from Lake Greenhaven, six samples from Tulare Lake, two samples from a Native American midden site south of Sacramento, one sample from the Pajaro River, 15 samples from Clear Lake, four

samples from Alameda Creek, and 10 samples from the Salinas River. DNA was successfully amplified and sequenced in 10 samples from Lake Greenhaven and four samples from Tulare Lake. The region was partially amplified for 12 additional Lake Greenhaven samples.

***Analyses***

Three or four sequences (from either extant or historical samples) were aligned, proofread, and assembled with Sequencher software. Final sequences were assembled in Sequencher to determine nucleotide polymorphism. Haplotypes and frequencies were determined by exporting the sequence alignment from Sequencher in nexus format and drawing a minimum spanning network using TCS.

***Results***

Twelve variable sites were found in 1450 base pairs of the control region. 13 haplotypes were found in the eleven extant populations. Each population contains just one to five haplotypes, supporting the results of the microsatellite DNA study of reduced genetic diversity and genetic differentiation. (Figure 2; Figure 3)

Fourteen individuals from two extirpated populations were sequenced, resulting in eight variable sites and six haplotypes, including two found only in historical samples (Figure 4; Figure 5). A minimum spanning network including both historical and extant populations indicates that northern and southern regions of the Central Valley contained distinct lineages of Sacramento perch, indicating limited migration of individuals in the Sacramento and San Joaquin rivers (Figure 4). Based on the distribution of haplotypes, it appears that the Clear Lake Reservoir population may have its origin in the Lake

Greenhaven stock. Additionally, Jewel Lake, Hume Lake, and Abbotts Lagoon all appear to share "northern" haplotypes (i.e. from Lake Greenhaven or a nearby locality). In contrast, Crowley Lake, Bridgeport Reservoir, and Curve Pond all appear to share "southern" haplotypes (i.e. for Tulare Lake or a nearby locality), while Sindicich Lagoon seems to share haplotypes from both regions. Stillwater Refuge, Pyramid Lake, and Little Washoe Lake all contain haplotypes not found in the sampled historical populations; however, in an evolutionary sense these haplotypes group with the southern samples (Figure 4; Figure 5).

**Discussion**

Genetic concerns associated with conservation and reintroductions of Sacramento perch include loss of adaptability or maladaptation (due to adaptation to extant locations or to genetic drift), inbreeding depression, and outbreeding depression (Keller et al. 1994; Frankham et al. 2000; Amos and Balmford 2001). The potential for local adaptation has been increased by a high probability that founder populations were small, allowing for rapid selection on particular beneficial traits, and by a complete absence of gene flow between populations. Alternatively, genetic drift in populations with small founding sizes may lead to an increase in the frequency of poorly adapted phenotypes merely by chance. The potential for inbreeding depression has been increased by founding extant populations with few individuals. Translocations of a few individuals of other species have also resulted in reduced heterozygosity and genetic diversity and differentiation between populations (e.g. Williams et al. 2002; Stephen et al. 2005). Our results indicate that inbreeding depression and effects of local adaptation and genetic drift could be

minimized in reintroductions by drawing from multiple sources. Continued stocking from multiple sources over multiple years would ensure a large number of parents contributing to future generations at reintroduction sites.

However, the genetic differentiation indicated by our results suggests a potential threat from outbreeding depression if multiple source populations are used as stocks for reintroductions. Outbreeding depression is reduced fitness due to interbreeding of individuals from two different source populations that have diverged genetically; it is usually attributed to the disruption of coadapted gene complexes and is therefore often seen only in the $F_2$ generation following recombination of chromosomes from different individuals of different stocks (e.g. Edmands 1999).

With the exception of the genetically similar group including Crowley Reservoir, Sindicich Lagoon, and Curve Pond, all the populations appear genetically unique and efforts should be made to protect this genetic diversity. However, six of the eight populations analyzed showed signs of founder effects, increasing the risk of reducing genetic diversity and limiting future adaptation if a single source is used for reintroductions. The genetic markers used in this study may be used to identify the number of parents contributing by genetically assigning larval fish to their parents. Genetic monitoring of reproductive success can also be used to identify whether individuals from some source populations are better adapted to their new environment due to the environmental differentiation between locations of each current population, founder effects, and genetic drift.

**Figure 1:** Dendrogram of genetic relationships among populations using $D_{CSE}$ and the neighbor joining method. $p<0.05$ for all distances except between samples collected in different years from Abbotts Lagoon and Crowley Lake.
(from Schwartz and May, submitted)

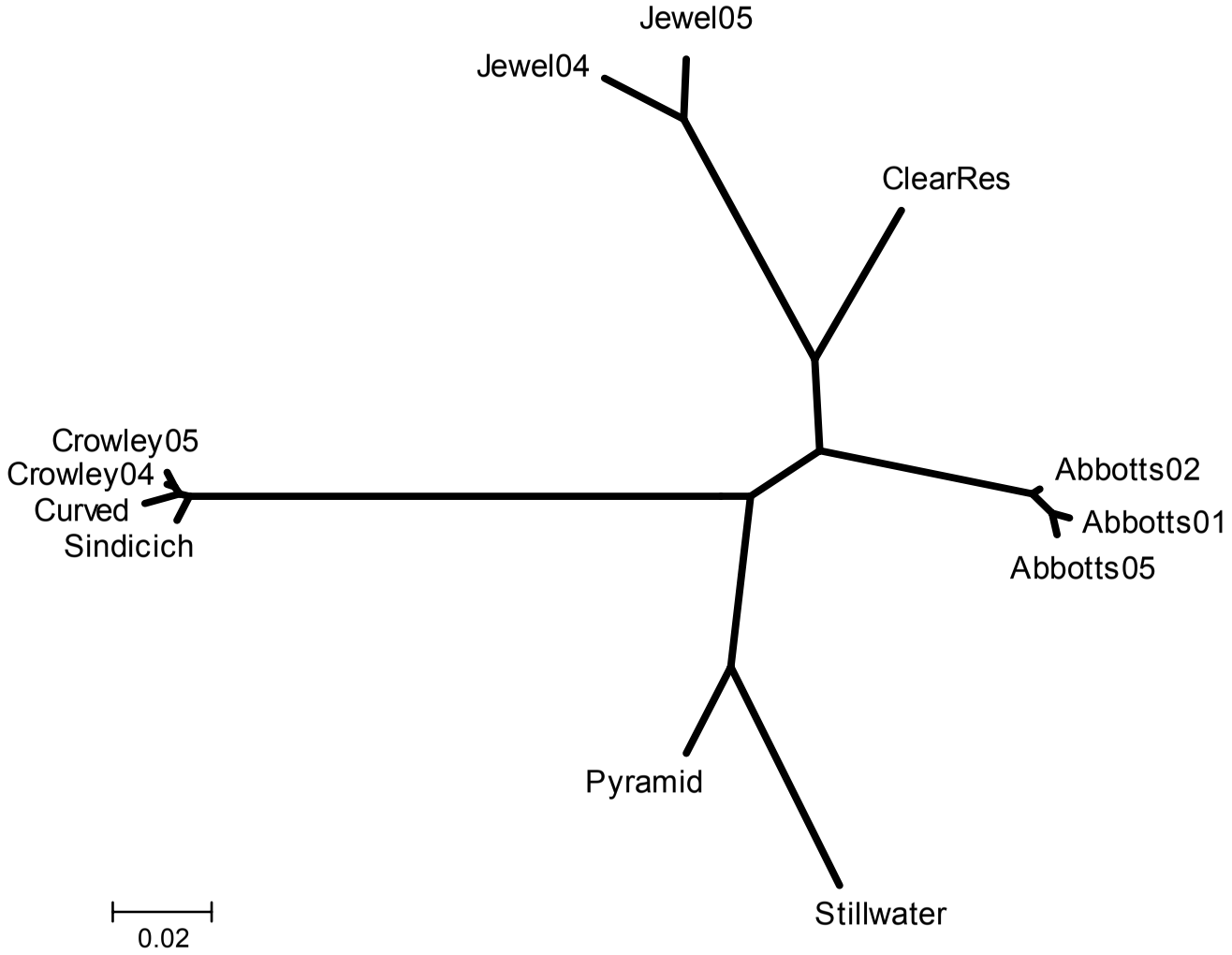

**Figure 2:** Minimum spanning network for mtDNA haplotypes from extant populations using 1450 base pairs of DNA sequence. Circle size represents overall frequency of the haplotype. Haplotypes are one base pair different from the neighboring haplotype unless separated by a node.
(Schwartz and May, unpublished data)

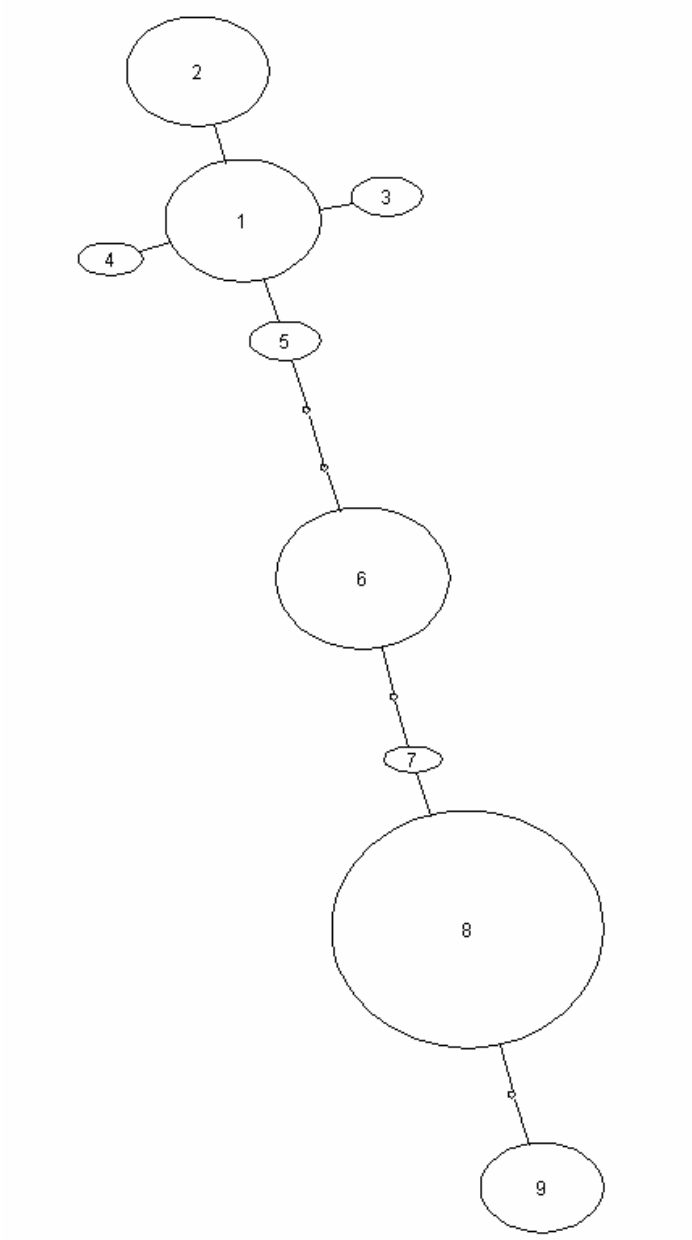

**Figure 3:** Proportion of each haplotype in a sample of each extant population. (Schwartz and May, unpublished data)

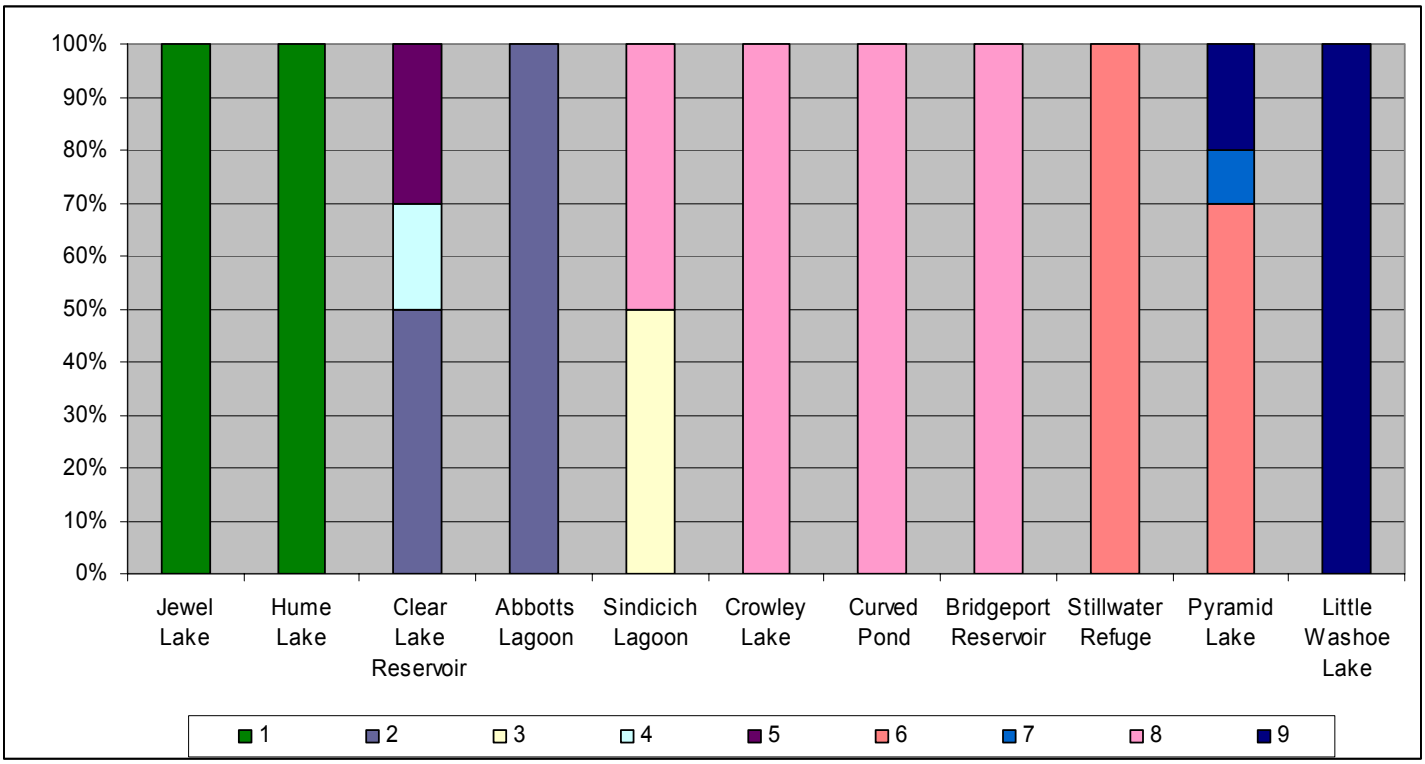

**Figure 4:** Minimum spanning network for mtDNA haplotypes from extant and historical populations using 120 base pairs of DNA sequence. Circle size represents overall frequency of the haplotype. Haplotypes are one base pair different from the neighboring haplotype unless separated by a node.
(Schwartz and May, unpublished data)

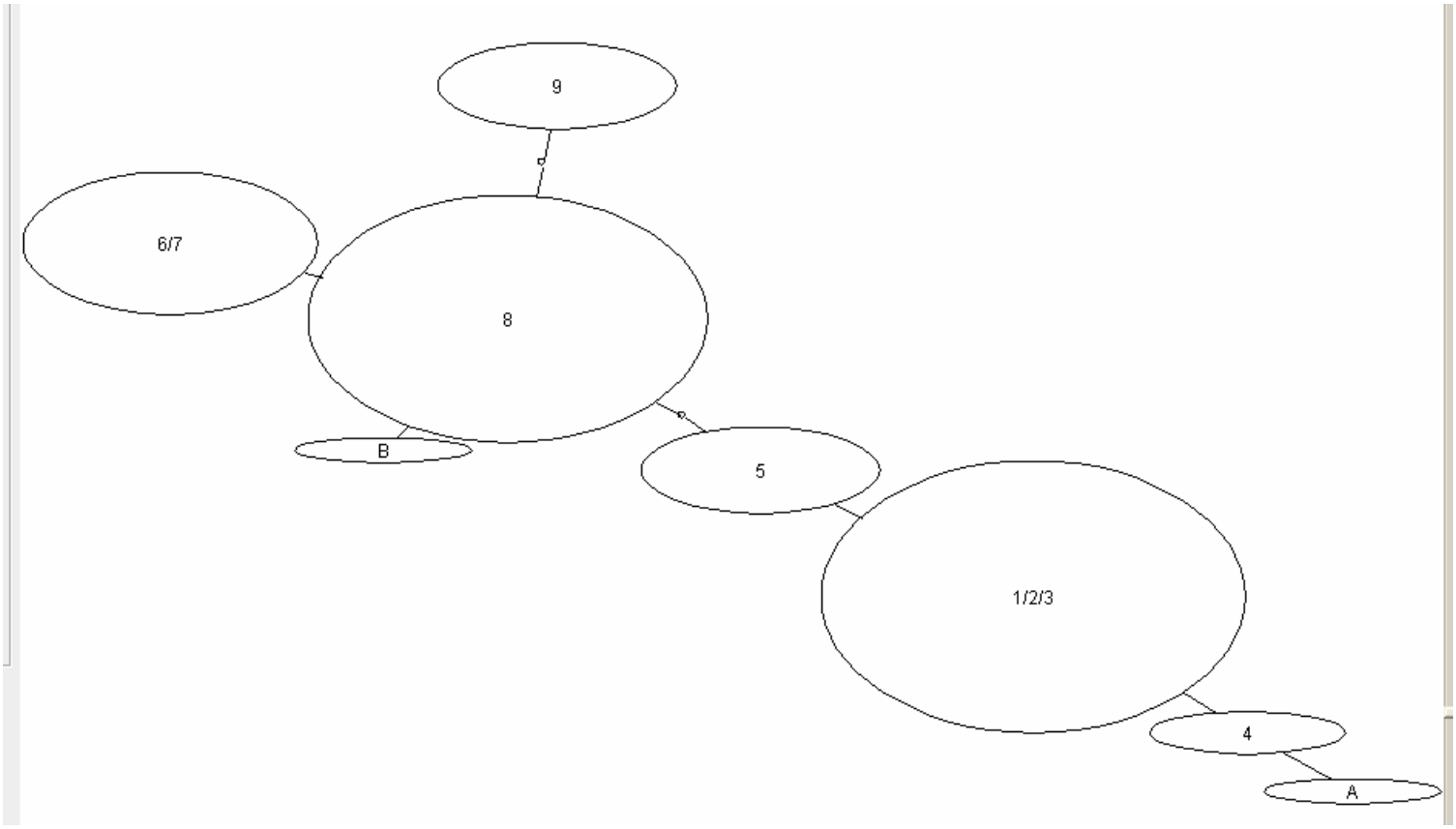

**Figure 5:** Proportion of each haplotype using 120bp in a sample of each extant and two historical populations. (Schwartz and May, unpublished data)

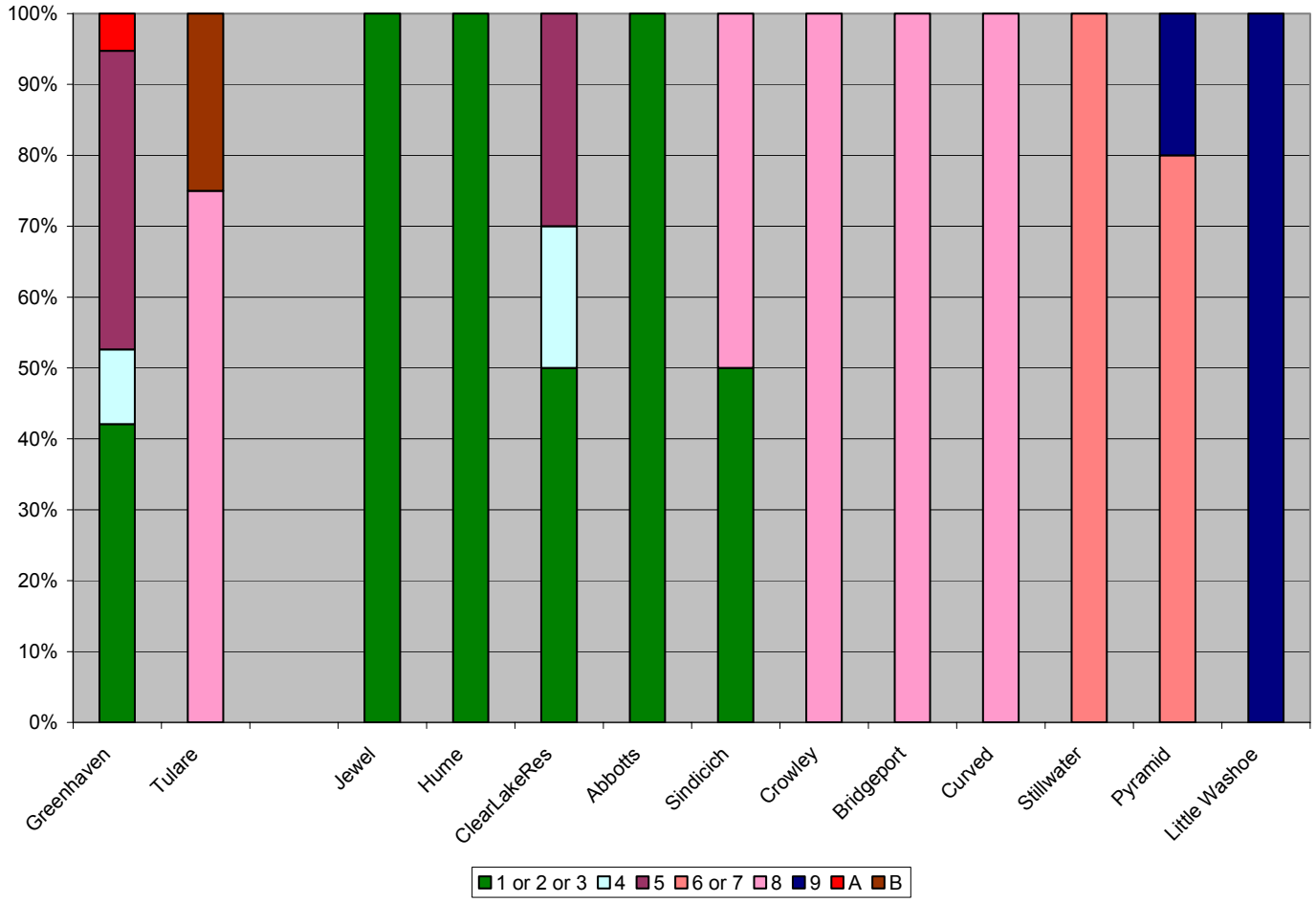

**Table 1:** Locations, numbers, and dates of samples

| Name | Location | # Samples | Collector | Date |
|---|---|---|---|---|
| Sindicich Lagoon | Martinez, CA | 33 | Pete Alexander; Manfred Kittel | 2003 |
| Stillwater National Wildlife Refuge | Fallon, NV | 35 | Misty Johnson | 2003 |
| Clear Lake Reservoir | Modoc Co., CA | 29 | Rich Piaskowski; Dan Bennetts | 2003 |
| Abbotts Lagoon | Pt. Reyes, CA | 48, 51, 40 | Kasey Grubb; Christa Woodley; Rachel Schwartz | 2001, 2002, 2005 |
| Pyramid Lake | Reno, NV | 39 | Sudeep Chandra | 2004 |
| Crowley Lake | Mono Co., CA | 19, 53, 46 | Christa Woodley | 2004, 2005, 2006 |
| Jewel Lake | Berkeley, CA | 14, 24 | Pete Alexander; Manfred Kittel | 2004, 2005 |
| Little Washoe Lake | Reno, NV | 38 | Kim Tisdale, Rachel Schwartz | 2005 |
| Bridgeport Reservoir | Mono Co., CA | 36 | Christa Woodley; Rachel Schwartz | 2005 |
| Hume Lake | Fresno Co., CA | 8 | Jim Houk; Rachel Schwartz | 2005 |
| Curved Pond | Davis, CA | 41 | Pat Crain | 2004 |

**Table 2:** Locations, numbers, and dates of historical samples

| Name | Location | # Samples | Date | Collection |
|---|---|---|---|---|
| Tulare Lake | Tulare Co., CA | 4 | 1916 | California Academy of Sciences |
| Lake Greenhaven | Sacramento, CA | 22 | 1969 | UC Davis Zooarchaeology |

Table 3: Summary data for microsatellites developed for Sacramento perch (*Archoplites interruptus*), including the repeat motif, size range of the alleles, number of alleles, expected and observed heterozygosities ($H_e$ and $H_o$), number of individuals (out of 27) that successfully amplified, and probability of Hardy-Weinberg equilibrium for each locus in the population in Stillwater National Wildlife Refuge. (from Schwartz and May 2004)

| Locus | GenBank accession no. | Primer Sequence | Repeat motif | Allele size range[1] | Allele size range[2] | # of alleles[1] | # of alleles[2] | He[1] | Ho[1] | # individuals amplified | p (HWE) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AinA2 | AY643770 | F: ATACCGGCAGATTGTGT<br>R: TTAGGGTGGGAAGAAAG | $(TCTA)_{24}$ | 188-244 | 188-244 | 4 | 5 | 0.75 | 0.77 | 26 | 0.563 |
| AinA6 | AY643771 | F: GTCAAAGCCTTACTCACT<br>R: ACACATAGATGCCCAGAC | $(ATAG)_{26}$ | 170-202 | 158-202 | 6 | 11 | 0.56 | 0.67 | 24 | 0.833 |
| AinA11 | AY643772 | F: AGAATACACTGCAATAAAAA<br>R: GTCCTGATCCTGAACA | $(TAGA)_{24}$ | 196-272 | 186-272 | 6 | 10 | 0.76 | 0.70 | 23 | 0.004 |
| AinA106 | AY643773 | F: CCTACGGCTAATGTGAA<br>R: TACTGAATATGAGAAATGTC | $(ATCT)_{17}$ | 276-296 | 276-320 | 3 | 6 | 0.64 | 0.85 | 20 | 0.067 |
| AinA108 | AY643774 | F: TGCTGCATTAACCAAAACTGT<br>R: GGCGATGAAGACCGTGTG | $(ATCT)_{14}$ | 204-240 | 204-252 | 6 | 9 | 0.73 | 0.58 | 24 | 0.173 |
| AinA117 | AY643775 | F: TGTTCCATTTAGCTGTTTTACCTG<br>R: CACTGATGCTCCTGATTTCTATGA | $(GATA)_{22}$ | 156-196 | 152-196 | 4 | 10 | 0.67 | 0.60 | 25 | 0.631 |
| AinA218 | AY643776 | F: CTCTGCCCAATCTACCAACAC<br>R: AAAACAGAGCAGCAGACTATGAAT | $(TAGA)_9$ | 270 | 262-278 | 1 | 3 | 0.00 | 0.00 | 27 | 1.000 |
| AinA120 | AY643777 | F: GTGCAACTTAAGACAAAACAA<br>R: GTAAGAGCGCACGACAAA | $(TCTA)_{12}(TCCA)$<br>$(TCTA)_9(TA)(CTA)_5$ | 276-400 | 276-400 | 5 | 8 | 0.56 | 0.22 | 27 | 0.000* |
| AinA203 | AY643778 | F: CTGCCTTTCACCCAATA<br>R: CTCAGTTCAGCTCAGTTCC | $(TC)_3(TATC)_4$<br>$(TATA)(TATC)_{26}$ | 280-316 | 280-316 | 5 | 6 | 0.70 | 0.65 | 26 | 0.699 |
| AinA207b | AY643779 | F: CTCTGTGCTGTGACGGGACTGA<br>R: ATGGCTTTTTATTGGGGGTTTTCT | $(TAGA)_{20}$ | 336-380 | 336-380 | 5 | 5 | 0.74 | 0.54 | 24 | 0.224 |
| AinA212 | AY643780 | F: AGGCGAGCTTGACATTTTACC<br>R: TCAGAAGGATTTGTTGGACTAT | $(TCTA)_{11}\ldots(TGTC)_3$ | 296-340 | 296-340 | 8 | 10 | 0.73 | 0.81 | 26 | 0.318 |
| AinA216 | AY643781 | F: ATCAAAGCAGACTCAAGACAG<br>R: GTGCAGTAAAGGAAAAATAGAC | $(ATCT)_{14}$ | 154-190 | 154-190 | 4 | 5 | 0.59 | 0.56 | 27 | 0.862 |
| AinC11 | AY643782 | F: GATGGGGCGACCTCAAAT<br>R: CTAGTCCTCCCCCTCATCAGTCT | $(ATAC)_7(CATC)_9$ | 280-300 | 280-308 | 3 | 7 | 0.54 | 0.48 | 27 | 0.715 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AinC202 | AY643783 | F: TGAGGGGACACAGTTTAC<br>R: GATTCAGGTTTCTCGTTCAT | $(ATAC)_{14}$ | 228-316 | 220-316 | 3 | 8 | 0.63 | 0.65 | 26 | 0.403 |
| AinD101 | AY643784 | F: CCCCCGCGACCCTGTATG<br>R: CACTGTTGCCCTGATGATGAAATG | $(GATG)_{15}$ | 122-134 | 122-162 | 4 | 8 | 0.67 | 0.81 | 27 | 0.472 |
| AinD106 | AY643785 | F: TTAAACAAACCCCCTGAAGAAACC<br>R: TGCGATGGACTGGCGACCTG | $(CATC)_{12}$ | 226-238 | 226-242 | 3 | 4 | 0.45 | 0.41 | 27 | 0.548 |
| AinD119 | AY643786 | F: TGTACACAGGATAAGCGGTTGAC<br>R: CCCCTCTGGCCTGTGGAATC | $(GATG)_{11}$ | 178-194 | 178-202 | 4 | 6 | 0.56 | 0.48 | 27 | 0.500 |
| AinD212 | AY643787 | F: GTGAGGGGCATTTTGGACA<br>R: CATTTTGCACAGGCTACATT | $(TCCA)_{16}$ | 296-318 | 276-370 | 4 | 8 | 0.68 | 0.65 | 26 | 0.522 |
| AinC105 | AY643788 | F: ACGCTAGACGGCTGTTTTCAC<br>R: CATAGGGGAGATTTCGGTCAA | $(CATA)_{7}$ | 272-280 | 272-280 | 3 | 3 | 0.60 | 0.38 | 26 | 0.018 |
| AinC212 | AY643789 | F: CTCGCTTTCATCTTCTGCTCTG<br>R: ACAACCACGCTCCATTTCACT | $(GTAT)_{8}$ | 130-146 | 130-150 | 2 | 3 | 0.33 | 0.33 | 26 | 1.000 |
| AinD202 | AY643790 | F: TGTATGGAGTGGAGTGGTTTATTG<br>R: TAGGTTGATGAGTGGTTGTTTGTC | $(CATC)_{8}$ | 248-252 | 248-252 | 2 | 2 | 0.28 | 0.33 | 27 | 1.000 |
| AinC203 | AY643791 | F: GTGCTGGATTTTACCTGTGTCTGT<br>R: TAGGGTGATGATGGATGGATGAAG | $(ATGT)_{10}$ | 172-180 | 172-180 | 3 | 3 | 0.48 | 0.41 | 27 | 0.539 |
| AinB202 | AY643792 | F: GACACCTGCCCGCCTCCTC<br>R: GACTCCGCCCACCACATCCT | $(CTGT)_{6}$ | 216-220 | 216-224 | 2 | 3 | 0.18 | 0.19 | 26 | 1.000 |

[1]Stillwater population only.
[2]Total for the Stillwater population and selected individuals from other populations.
*This locus shows departure from Hardy-Weinberg equilibrium after Bonferroni correction.

Table 4: Allele frequencies, observed and expected heterozygosities ($H_O$ and $H_E$), and the inbreeding coefficient ($F_{IS}$). Allele sizes are given in bases. (from Schwartz and May, submitted)

| Locus / Allele | Sindicich | Stillwater | Clear Lake Res. | Abbotts | Pyramid | Crowley | Jewel | Curved | Overall |
|---|---|---|---|---|---|---|---|---|---|
| AinA117 | | | | | | | | | |
| 148 | -- | -- | 0.017 | 0.058 | -- | -- | -- | -- | 0.020 |
| 152 | 0.015 | -- | 0.172 | 0.018 | -- | -- | -- | -- | 0.019 |
| 156 | 0.167 | 0.086 | 0.017 | 0.079 | 0.218 | 0.191 | -- | 0.110 | 0.110 |
| 160 | -- | -- | 0.069 | 0.011 | -- | -- | 0.083 | 0.012 | 0.017 |
| 164 | -- | 0.114 | -- | 0.004 | 0.308 | -- | -- | -- | 0.039 |
| 168 | 0.046 | -- | 0.017 | 0.453 | 0.013 | -- | -- | -- | 0.156 |
| 172 | 0.546 | -- | 0.103 | 0.198 | 0.077 | 0.566 | 0.153 | 0.549 | 0.281 |
| 176 | -- | -- | -- | -- | -- | -- | 0.181 | 0.061 | 0.021 |
| 180 | -- | 0.300 | -- | -- | 0.167 | -- | -- | 0.024 | 0.043 |
| 184 | -- | -- | -- | -- | 0.064 | -- | -- | -- | 0.006 |
| 192 | 0.121 | 0.014 | -- | -- | 0.103 | 0.169 | -- | 0.098 | 0.057 |
| 196 | -- | 0.443 | 0.190 | 0.162 | -- | -- | -- | -- | 0.104 |
| 200 | -- | 0.029 | 0.017 | 0.014 | 0.051 | -- | -- | -- | 0.013 |
| 204 | -- | -- | -- | 0.004 | -- | -- | 0.111 | -- | 0.011 |
| 208 | -- | -- | 0.345 | -- | -- | -- | 0.306 | -- | 0.050 |
| 212 | -- | -- | 0.052 | -- | -- | -- | 0.167 | -- | 0.018 |
| 274 | 0.106 | 0.014 | -- | -- | -- | 0.074 | -- | 0.146 | 0.036 |
| $H_O$ | 0.70 | 0.57 | 0.76 | 0.69 | 0.74 | 0.54 | 0.78 | 0.59 | |
| $H_E$ | 0.66 | 0.70 | 0.81 | 0.72 | 0.82 | 0.61 | 0.81 | 0.66 | |
| $F_{IS}$ | -0.06 | 0.19 | 0.06 | 0.04 | 0.09 | 0.11 | 0.05 | 0.11 | |
| AinA2 | | | | | | | | | |
| 190 | 0.091 | 0.257 | 0.121 | 0.305 | 0.308 | 0.293 | 0.158 | 0.171 | 0.244 |
| 194 | -- | 0.214 | 0.190 | 0.265 | 0.397 | 0.021 | -- | -- | 0.157 |
| 198 | -- | -- | 0.103 | 0.232 | -- | -- | -- | -- | 0.082 |
| 202 | -- | -- | 0.207 | 0.011 | -- | -- | 0.697 | -- | 0.081 |
| 206 | -- | -- | -- | 0.114 | -- | -- | -- | -- | 0.037 |
| 222 | -- | 0.186 | 0.155 | 0.033 | 0.103 | -- | -- | -- | 0.046 |
| 226 | 0.258 | -- | -- | -- | -- | 0.293 | -- | 0.585 | 0.126 |
| 234 | -- | -- | -- | 0.037 | -- | -- | -- | -- | 0.012 |
| 240 | -- | -- | -- | 0.004 | -- | -- | -- | -- | 0.001 |
| 248 | 0.030 | 0.343 | -- | -- | 0.064 | -- | 0.092 | -- | 0.045 |
| 252 | 0.621 | -- | -- | -- | 0.128 | 0.379 | -- | 0.244 | 0.147 |
| 256 | -- | -- | -- | -- | -- | 0.014 | -- | -- | 0.002 |
| 276 | -- | -- | 0.190 | -- | -- | -- | 0.053 | -- | 0.018 |
| 280 | -- | -- | 0.017 | -- | -- | -- | -- | -- | 0.001 |
| 284 | -- | -- | 0.017 | -- | -- | -- | -- | -- | 0.001 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $H_O$ | | 0.52 | 0.89 | 1.00 | 0.73 | 0.62 | 0.71 | 0.47 | 0.73 | |
| $H_E$ | | 0.55 | 0.75 | 0.85 | 0.77 | 0.73 | 0.69 | 0.48 | 0.58 | |
| $F_{IS}$ | | 0.06 | -0.19 | -0.18 | 0.06 | 0.15 | -0.04 | 0.02 | -0.28 | |
| AinA203 | | | | | | | | | | |
| | 278 | -- | -- | -- | 0.014 | -- | -- | -- | -- | 0.005 |
| | 282 | -- | -- | 0.069 | 0.022 | -- | -- | -- | -- | 0.012 |
| | 286 | -- | -- | -- | -- | -- | -- | 0.194 | -- | 0.017 |
| | 290 | 0.121 | 0.371 | 0.431 | 0.306 | 0.136 | 0.201 | 0.278 | 0.256 | 0.267 |
| | 294 | -- | -- | 0.103 | 0.345 | 0.015 | -- | 0.153 | -- | 0.136 |
| | 298 | 0.121 | -- | 0.086 | 0.086 | -- | 0.132 | -- | 0.220 | 0.089 |
| | 302 | -- | 0.243 | 0.103 | 0.076 | 0.409 | -- | -- | -- | 0.085 |
| | 306 | 0.758 | -- | 0.017 | 0.101 | -- | 0.667 | -- | 0.524 | 0.261 |
| | 314 | -- | 0.343 | 0.103 | 0.007 | 0.227 | -- | 0.292 | -- | 0.081 |
| | 318 | -- | -- | 0.086 | 0.043 | 0.197 | -- | 0.083 | -- | 0.043 |
| | 322 | -- | 0.043 | -- | -- | 0.015 | -- | -- | -- | 0.005 |
| $H_O$ | | 0.39 | 0.57 | 0.72 | 0.75 | 0.58 | 0.50 | 0.75 | 0.59 | |
| $H_E$ | | 0.40 | 0.69 | 0.78 | 0.76 | 0.73 | 0.50 | 0.78 | 0.62 | |
| $F_{IS}$ | | 0.02 | 0.18 | 0.07 | 0.02 | 0.22 | 0.00 | 0.04 | 0.05 | |
| AinD119 | | | | | | | | | | |
| | 179 | -- | 0.614 | 0.069 | 0.184 | 0.359 | -- | 0.105 | -- | 0.157 |
| | 187 | 0.030 | 0.314 | 0.121 | 0.025 | 0.090 | -- | -- | -- | 0.053 |
| | 191 | -- | 0.029 | 0.035 | 0.047 | 0.064 | -- | -- | -- | 0.026 |
| | 199 | 0.970 | 0.043 | 0.293 | 0.482 | 0.487 | 1.000 | 0.526 | 1.000 | 0.613 |
| | 203 | -- | -- | 0.483 | 0.263 | -- | -- | 0.224 | -- | 0.139 |
| | 207 | -- | -- | -- | -- | -- | -- | 0.105 | -- | 0.009 |
| | 211 | -- | -- | -- | -- | -- | -- | 0.040 | -- | 0.004 |
| $H_O$ | | 0.00 | 0.46 | 0.59 | 0.67 | 0.56 | 0.00 | 0.53 | 0.00 | |
| $H_E$ | | 0.06 | 0.53 | 0.67 | 0.66 | 0.63 | 0.00 | 0.66 | 0.00 | |
| $F_{IS}$ | | 1.00 | 0.14 | 0.13 | -0.01 | 0.11 | 0.00 | 0.20 | 0.00 | |
| AinA218 | | | | | | | | | | |
| | 265 | -- | -- | 0.052 | 0.007 | -- | -- | -- | -- | 0.006 |
| | 273 | 0.985 | 1.000 | 0.328 | 0.899 | 0.962 | 1.000 | 0.276 | 1.000 | 0.852 |
| | 277 | 0.015 | -- | 0.121 | 0.004 | 0.039 | -- | 0.118 | -- | 0.025 |
| | 281 | -- | -- | 0.207 | -- | -- | -- | 0.250 | -- | 0.036 |
| | 285 | -- | -- | 0.155 | 0.083 | -- | -- | 0.303 | -- | 0.065 |
| | 293 | -- | -- | 0.103 | 0.007 | -- | -- | 0.053 | -- | 0.014 |
| | 317 | -- | -- | 0.035 | -- | -- | -- | -- | -- | 0.002 |
| $H_O$ | | 0.03 | 0.00 | 0.79 | 0.19 | 0.08 | 0.00 | 0.68 | 0.00 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $H_E$ | | 0.03 | 0.00 | 0.81 | 0.18 | 0.07 | 0.00 | 0.76 | 0.00 | |
| $F_{IS}$ | | 0.00 | 0.00 | 0.02 | -0.05 | -0.03 | 0.00 | 0.10 | 0.00 | |
| AinD106 | | | | | | | | | | |
| | 222 | 0.258 | -- | -- | -- | -- | 0.444 | -- | 0.500 | 0.137 |
| | 226 | -- | 0.100 | -- | 0.210 | 0.013 | -- | 0.027 | -- | 0.082 |
| | 230 | -- | -- | 0.086 | 0.116 | -- | -- | 0.216 | -- | 0.064 |
| | 234 | 0.015 | 0.157 | 0.086 | 0.225 | 0.013 | -- | -- | -- | 0.096 |
| | 238 | 0.727 | 0.743 | 0.724 | 0.442 | 0.590 | 0.556 | 0.608 | 0.500 | 0.563 |
| | 242 | -- | -- | 0.103 | 0.007 | 0.385 | -- | 0.149 | -- | 0.059 |
| $H_O$ | | 0.42 | 0.40 | 0.38 | 0.71 | 0.44 | 0.36 | 0.57 | 0.45 | |
| $H_E$ | | 0.41 | 0.42 | 0.46 | 0.70 | 0.51 | 0.50 | 0.57 | 0.51 | |
| $F_{IS}$ | | -0.03 | 0.05 | 0.17 | -0.02 | 0.15 | 0.28 | 0.00 | 0.11 | |
| AinA120 | | | | | | | | | | |
| | 275 | 0.030 | 0.014 | -- | 0.007 | -- | -- | -- | -- | 0.006 |
| | 279 | 0.561 | 0.500 | 0.517 | 0.225 | 0.449 | 0.507 | 0.361 | 0.573 | 0.408 |
| | 283 | 0.409 | 0.457 | 0.121 | 0.011 | 0.154 | 0.493 | -- | 0.427 | 0.221 |
| | 287 | -- | -- | -- | -- | 0.039 | -- | -- | -- | 0.004 |
| | 291 | -- | -- | 0.052 | -- | -- | -- | -- | -- | 0.004 |
| | 295 | -- | -- | 0.017 | -- | -- | -- | -- | -- | 0.001 |
| | 311 | -- | -- | -- | 0.051 | 0.090 | -- | -- | -- | 0.025 |
| | 315 | -- | -- | 0.069 | -- | 0.141 | -- | -- | -- | 0.018 |
| | 319 | -- | -- | -- | 0.149 | -- | -- | -- | -- | 0.049 |
| | 323 | -- | 0.014 | -- | 0.007 | -- | -- | -- | -- | 0.004 |
| | 327 | -- | -- | 0.086 | -- | -- | -- | -- | -- | 0.006 |
| | 331 | -- | -- | 0.103 | 0.199 | -- | -- | -- | -- | 0.072 |
| | 335 | -- | -- | -- | 0.007 | -- | -- | -- | -- | 0.002 |
| | 347 | -- | -- | 0.035 | 0.312 | 0.039 | -- | 0.181 | -- | 0.123 |
| | 351 | -- | -- | -- | 0.004 | -- | -- | 0.042 | -- | 0.005 |
| | 363 | -- | -- | -- | -- | -- | -- | 0.069 | -- | 0.006 |
| | 401 | -- | -- | -- | -- | 0.064 | -- | -- | -- | 0.006 |
| | 405 | -- | 0.014 | -- | 0.007 | 0.026 | -- | -- | -- | 0.006 |
| | 409 | -- | -- | -- | 0.018 | -- | -- | -- | -- | 0.006 |
| | 413 | -- | -- | -- | 0.004 | -- | -- | -- | -- | 0.001 |
| | 417 | -- | -- | -- | -- | -- | -- | 0.111 | -- | 0.010 |
| | 445 | -- | -- | -- | -- | -- | -- | 0.069 | -- | 0.006 |
| | 449 | -- | -- | -- | -- | -- | -- | 0.139 | -- | 0.012 |
| | 453 | -- | -- | -- | -- | -- | -- | 0.028 | -- | 0.002 |
| $H_O$ | | 0.52 | 0.29 | 0.62 | 0.76 | 0.64 | 0.32 | 0.81 | 0.22 | |
| $H_E$ | | 0.53 | 0.55 | 0.70 | 0.79 | 0.75 | 0.50 | 0.80 | 0.50 | |
| $F_{IS}$ | | 0.02 | 0.48 | 0.12 | 0.04 | 0.15 | 0.37 | 0.00 | 0.56 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| AinA216 | | | | | | | | | | |
| | 147 | -- | 0.014 | -- | -- | -- | -- | -- | -- | 0.001 |
| | 151 | -- | -- | 0.035 | -- | -- | -- | -- | -- | 0.002 |
| | 155 | -- | 0.071 | 0.190 | 0.192 | 0.064 | 0.139 | 0.053 | -- | 0.115 |
| | 159 | -- | 0.471 | 0.035 | 0.330 | 0.128 | -- | 0.145 | 0.012 | 0.174 |
| | 163 | 0.576 | -- | 0.552 | 0.145 | 0.346 | 0.465 | 0.145 | 0.439 | 0.295 |
| | 167 | 0.121 | 0.014 | -- | 0.047 | -- | 0.201 | 0.013 | 0.317 | 0.092 |
| | 171 | 0.303 | 0.400 | 0.138 | 0.058 | 0.410 | 0.194 | 0.263 | 0.232 | 0.201 |
| | 175 | -- | -- | 0.017 | -- | -- | -- | 0.066 | -- | 0.007 |
| | 179 | -- | -- | 0.035 | -- | -- | -- | 0.079 | -- | 0.009 |
| | 187 | -- | -- | -- | 0.022 | -- | -- | -- | -- | 0.007 |
| | 191 | -- | 0.029 | -- | 0.207 | 0.051 | -- | 0.237 | -- | 0.095 |
| $H_O$ | | 0.45 | 0.51 | 0.66 | 0.79 | 0.77 | 0.69 | 0.82 | 0.51 | |
| $H_E$ | | 0.57 | 0.62 | 0.65 | 0.79 | 0.70 | 0.69 | 0.83 | 0.66 | |
| $F_{IS}$ | | 0.21 | 0.17 | -0.01 | 0.00 | -0.10 | -0.01 | 0.02 | 0.23 | |
| AinA108 | | | | | | | | | | |
| | 181 | -- | -- | -- | 0.015 | -- | -- | -- | -- | 0.005 |
| | 193 | -- | -- | -- | 0.015 | -- | -- | -- | -- | 0.005 |
| | 201 | -- | 0.271 | -- | -- | 0.103 | -- | -- | -- | 0.032 |
| | 209 | 0.015 | 0.357 | -- | 0.055 | 0.090 | -- | 0.042 | -- | 0.061 |
| | 213 | -- | 0.114 | 0.035 | 0.026 | 0.180 | -- | 0.042 | -- | 0.040 |
| | 217 | -- | -- | 0.172 | 0.386 | -- | -- | 0.375 | -- | 0.169 |
| | 221 | -- | -- | 0.207 | 0.026 | -- | -- | -- | -- | 0.023 |
| | 225 | -- | -- | -- | 0.004 | -- | -- | 0.194 | -- | 0.018 |
| | 229 | -- | -- | 0.035 | 0.147 | 0.115 | -- | 0.167 | -- | 0.075 |
| | 233 | 0.227 | -- | 0.086 | -- | -- | 0.271 | -- | 0.073 | 0.077 |
| | 237 | -- | 0.257 | 0.052 | 0.320 | 0.474 | -- | 0.014 | -- | 0.173 |
| | 241 | 0.015 | -- | -- | 0.007 | 0.026 | -- | -- | -- | 0.006 |
| | 245 | -- | -- | 0.052 | -- | -- | -- | -- | -- | 0.004 |
| | 249 | -- | -- | 0.052 | -- | 0.013 | -- | 0.167 | -- | 0.019 |
| | 253 | -- | -- | -- | -- | -- | -- | -- | 0.012 | 0.001 |
| | 257 | -- | -- | 0.241 | -- | -- | 0.014 | -- | -- | 0.019 |
| | 261 | 0.485 | -- | 0.069 | -- | -- | 0.313 | -- | 0.427 | 0.138 |
| | 265 | 0.258 | -- | -- | -- | -- | 0.396 | -- | 0.488 | 0.135 |
| | 269 | -- | -- | -- | -- | -- | 0.007 | -- | -- | 0.001 |
| $H_O$ | | 0.64 | 0.69 | 0.93 | 0.71 | 0.74 | 0.78 | 0.67 | 0.56 | |
| $H_E$ | | 0.66 | 0.73 | 0.86 | 0.72 | 0.72 | 0.68 | 0.77 | 0.58 | |
| $F_{IS}$ | | 0.03 | 0.06 | -0.08 | 0.02 | -0.03 | -0.15 | 0.14 | 0.04 | |
| AinA6 | | | | | | | | | | |
| | 157 | -- | -- | -- | 0.036 | -- | -- | -- | -- | 0.012 |
| | 165 | -- | -- | -- | 0.004 | -- | -- | -- | -- | 0.001 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 169 | 0.078 | -- | -- | -- | -- | 0.049 | -- | 0.122 | 0.026 |
| | 173 | -- | 0.029 | -- | 0.112 | 0.230 | -- | 0.290 | -- | 0.085 |
| | 177 | -- | -- | 0.121 | -- | -- | -- | 0.066 | -- | 0.014 |
| | 181 | -- | -- | 0.328 | 0.004 | -- | -- | 0.040 | -- | 0.027 |
| | 185 | -- | -- | 0.035 | 0.259 | 0.068 | -- | 0.013 | -- | 0.095 |
| | 189 | -- | 0.200 | 0.224 | -- | 0.257 | -- | -- | -- | 0.054 |
| | 193 | -- | 0.014 | 0.017 | -- | 0.095 | -- | -- | -- | 0.011 |
| | 197 | -- | 0.700 | 0.121 | 0.011 | 0.135 | -- | -- | -- | 0.082 |
| | 201 | 0.922 | 0.014 | 0.103 | 0.576 | 0.135 | 0.951 | 0.079 | 0.878 | 0.533 |
| | 205 | -- | 0.043 | 0.052 | -- | 0.041 | -- | 0.342 | -- | 0.041 |
| | 213 | -- | -- | -- | -- | -- | -- | 0.171 | -- | 0.015 |
| | 217 | -- | -- | -- | -- | 0.041 | -- | -- | -- | 0.004 |
| $H_O$ | | 0.16 | 0.51 | 0.79 | 0.55 | 0.84 | 0.10 | 0.68 | 0.24 | |
| $H_E$ | | 0.15 | 0.47 | 0.81 | 0.59 | 0.84 | 0.09 | 0.77 | 0.22 | |
| $F_{IS}$ | | -0.07 | -0.09 | 0.02 | 0.06 | 0.00 | -0.04 | 0.11 | -0.13 | |
| AinA212 | | | | | | | | | | |
| | 298 | 0.677 | -- | -- | -- | -- | 0.679 | -- | 0.390 | 0.206 |
| | 310 | -- | -- | 0.224 | -- | 0.014 | -- | 0.092 | -- | 0.026 |
| | 318 | -- | 0.029 | 0.103 | 0.027 | 0.186 | -- | 0.040 | -- | 0.038 |
| | 322 | -- | 0.014 | -- | 0.027 | 0.200 | -- | 0.013 | -- | 0.028 |
| | 326 | 0.177 | 0.343 | -- | 0.015 | 0.229 | 0.279 | 0.053 | 0.500 | 0.169 |
| | 330 | -- | -- | 0.293 | 0.648 | 0.029 | -- | 0.079 | -- | 0.238 |
| | 334 | -- | 0.029 | 0.103 | 0.159 | 0.029 | -- | 0.316 | -- | 0.093 |
| | 338 | 0.145 | 0.143 | 0.103 | 0.004 | 0.071 | 0.043 | 0.316 | 0.110 | 0.085 |
| | 342 | -- | -- | 0.017 | 0.121 | -- | -- | 0.092 | -- | 0.049 |
| | 346 | -- | 0.443 | 0.155 | -- | 0.243 | -- | -- | -- | 0.069 |
| $H_O$ | | 0.42 | 0.77 | 0.86 | 0.52 | 0.83 | 0.57 | 0.76 | 0.56 | |
| $H_E$ | | 0.50 | 0.67 | 0.82 | 0.54 | 0.82 | 0.46 | 0.78 | 0.59 | |
| $F_{IS}$ | | 0.16 | -0.15 | -0.05 | 0.05 | -0.01 | -0.24 | 0.03 | 0.05 | |
| AinD101 | | | | | | | | | | |
| | 119 | -- | -- | -- | 0.029 | -- | -- | -- | -- | 0.010 |
| | 123 | -- | 0.357 | 0.035 | 0.270 | 0.128 | -- | -- | -- | 0.133 |
| | 127 | 0.016 | 0.414 | -- | 0.022 | 0.128 | 0.007 | -- | -- | 0.056 |
| | 131 | -- | 0.071 | 0.035 | 0.037 | 0.218 | -- | 0.122 | -- | 0.051 |
| | 135 | -- | 0.157 | 0.414 | 0.438 | 0.077 | -- | 0.338 | -- | 0.222 |
| | 139 | 0.339 | -- | 0.103 | 0.011 | 0.013 | 0.707 | -- | 0.573 | 0.211 |
| | 143 | -- | -- | 0.103 | 0.080 | -- | -- | 0.405 | -- | 0.069 |
| | 147 | 0.645 | -- | -- | -- | -- | 0.279 | -- | 0.427 | 0.136 |
| | 151 | -- | -- | 0.276 | 0.110 | 0.115 | -- | 0.122 | -- | 0.076 |
| | 155 | -- | -- | 0.035 | 0.004 | 0.026 | -- | 0.014 | -- | 0.007 |
| | 163 | -- | -- | -- | -- | 0.295 | 0.007 | -- | -- | 0.029 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $H_O$ | 0.52 | 0.69 | 0.72 | 0.68 | 0.72 | 0.37 | 0.54 | 0.61 |
| $H_E$ | 0.48 | 0.68 | 0.74 | 0.72 | 0.82 | 0.43 | 0.70 | 0.50 |
| $F_{IS}$ | -0.08 | -0.01 | 0.02 | 0.05 | 0.13 | 0.13 | 0.23 | -0.23 |
| | | | | | | | | |
| Overall $H_O$ | 0.40 | 0.53 | 0.74 | 0.65 | 0.63 | 0.41 | 0.67 | 0.42 |
| Overall $H_E$ | 0.41 | 0.57 | 0.75 | 0.66 | 0.68 | 0.43 | 0.73 | 0.45 |
| | 0.11 | 0.19 | 0.07 | 0.04 | 0.13 | 0.16 | 0.12 | 0.20 |
| Overall $F_{IS}$ 95% CI | -0.02 | -0.04 | -0.04 | 0.01 | 0.02 | -0.06 | 0.04 | -0.08 |
| | | | | | | | | |
| M value | 0.50 | 0.65 | 0.83 | 0.80 | 0.67 | 0.52 | 0.70 | 0.58 |

Table 5: Pairwise genetic differentiation between populations (Weir and Cockerham's (1984) estimate of $F_{ST}$).
(from Schwartz and May, submitted)

| | Sindicich | Stillwater | ClearLakeRes | Abbotts | Pyramid | Crowley | Jewel |
|---|---|---|---|---|---|---|---|
| Stillwater | 0.39 | | | | | | |
| | (0.28-0.49) | | | | | | |
| ClearLakeRes | 0.29 | 0.21 | | | | | |
| | (0.21-0.36) | (0.15-0.26) | | | | | |
| Abbotts | 0.27 | 0.22 | 0.14 | | | | |
| | (0.22-0.33) | (0.16-0.29) | (0.10-0.19) | | | | |
| Pyramid | 0.28 | 0.13 | 0.14 | 0.16 | | | |
| | (0.20-0.34) | (0.10-0.16) | (0.10-0.19) | (0.12-0.20) | | | |
| Crowley | 0.05 | 0.39 | 0.32 | 0.28 | 0.29 | | |
| | (0.01-0.10) | (0.27-0.53) | (0.22-0.42) | (0.22-0.34) | (0.21-0.37) | | |
| Jewel | 0.32 | 0.25 | 0.10 | 0.17 | 0.17 | 0.34 | |
| | (0.25-0.39) | (0.19-0.31) | (0.06-0.13) | (0.11-0.24) | (0.12-0.24) | (0.27-0.42) | |
| Curved | 0.07 | 0.37 | 0.29 | 0.26 | 0.27 | 0.03 | 0.31 |
| | (0.03-0.11) | (026-0.48) | (0.22-0.36) | (0.21-0.31) | (0.21-0.32) | (0.01-0.06) | (0.25-0.38) |

Bootstrapped 95% confidence intervals are shown in parentheses.

Table 6: $D_{CSE}$ values for Figure 1
(from Schwartz and May, submitted)

| | Sindicich | Stillwater | ClearLakeRes | Abbotts01 | Abbotts02 | Abbotts05 | Pyramid | Crowley04 | Crowley05 | Jewel04 | Jewel05 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sindicich | | | | | | | | | | | |
| Stillwater | 0.21 | | | | | | | | | | |
| ClearLakeRes | 0.19 | 0.16 | | | | | | | | | |
| Abbotts01 | 0.19 | 0.16 | 0.11 | | | | | | | | |
| Abbotts02 | 0.18 | 0.14 | 0.10 | 0.01 | | | | | | | |
| Abbotts05 | 0.19 | 0.16 | 0.11 | 0.01 | 0.01 | | | | | | |
| Pyramid | 0.17 | 0.07 | 0.13 | 0.13 | 0.12 | 0.13 | | | | | |
| Crowley04 | 0.02 | 0.21 | 0.19 | 0.19 | 0.18 | 0.19 | 0.17 | | | | |
| Crowley05 | 0.01 | 0.21 | 0.19 | 0.19 | 0.19 | 0.19 | 0.18 | 0.01 | | | |
| Jewel04 | 0.23 | 0.20 | 0.11 | 0.14 | 0.14 | 0.15 | 0.17 | 0.24 | 0.24 | | |
| Jewel05 | 0.22 | 0.20 | 0.11 | 0.14 | 0.14 | 0.14 | 0.16 | 0.23 | 0.22 | 0.03 | |
| Curved | 0.01 | 0.21 | 0.20 | 0.20 | 0.19 | 0.20 | 0.18 | 0.01 | 0.01 | 0.23 | 0.22 |

Table 7: Demographic parameters of population bottlenecks detectable by genetic bottleneck tests

| criteria / test | M-rato | Mode shift | Sign |
|---|---|---|---|
| size pre-bottleneck | large | | small |
| severity | severe | severe | less |
| time since bottleneck | long | | recent |

Table 8: Results of tests for population bottlenecks

| population / criteria met by known population history | M-ratio | Mode shift | Sign | Wilcoxen |
|---|---|---|---|---|
| Sindicich Lagoon | severe | | | |
| Stillwater Refuge | long | | | |
| Clear Lake Reservoir | | | | |
| Abbotts Lagoon | | | | |
| Pyramid Lake | large; severe; long | | | recent* |
| Crowley Lake | severe | | | recent |
| Jewel Lake | | | recent; less | recent; less |
| Curved Pond | severe | severe | | recent |

When a test indicated a population bottleneck the box is filled in with any known demographic history of the population that would provide a reason for the indication of a bottleneck.

* The identification of a bottleneck in the Pyramid Lake population using a heterozygosity test suggests that a recent genetic bottleneck occurred in this population, in addition to an older bottleneck, which would be suggested by the M-ratio test.

## TASK 8. Restoration Strategy

The key findings in this report that can assist in the development of restoration strategies for Sacramento perch (SP) are the following:

1. SP have been extirpated from natural habitats in their native range and many populations established outside the native range have disappeared.
2. The optimal environment for SP is cool riverine habitat, with flooded areas available for spawning. They can survive in extreme environments (high temperatures, alkalinities, etc) but they eventually die out through a combination of poor growth, survival, and reproduction if such habitat is permanent rather than seasonal.
3. Most Sacramento perch today live in artificial habitats, mainly reservoirs and ponds.
4. All existing populations of SP show signs of genetic bottlenecking (limited genetic diversity) although diversity among some populations is reasonably high.
5. Presence of non-native centrarchids, especially sunfish (*Lepomis*) and crappie (*Pomoxis*), in SP habitat is usually associated with their eventual extirpation, although the exact mechanism of displacement is still not fully understood.
6. The long-term trajectory for Sacramento perch in all its scattered populations combined is towards increasingly low genetic diversity, the gradual disappearance of populations in isolated ponds and reservoirs, and species extinction.
7. Many of the problems encountered during experiments on Sacramento perch demonstrated an exceptionally high susceptibility to a variety of diseases. This could be a product of their loss of heterogeneity due to inbreeding and founder

effects or low resistance to non-native diseases. Disease susceptibility needs to be studied and incorporated into their overall restoration plan.

Any strategy for re-establishing Sacramento perch must take these factors into account. We propose the following as a 10 point conservation strategy, in no particular order of priority.

1. Insure the future of all existing populations by establishing backup populations from each source, including those not in California. Ideally, these would be habitats within the native range of SP but ponds or lakes under controlled conditions may be necessary.

2. Establish a breeding program that brings the genotypes together from isolated populations to re-establish a genetically diverse source population for future planting programs. This would have to be done in a carefully controlled program with genetic monitoring of the fish produced as a source stock.

3. Establish a Sacramento perch rearing facility in the Central Valley, with facilities for selective breeding and ponds for large-scale rearing of fish for planting where populations should be established. Realistically, it may be necessary to maintain this facility indefinitely as a source of SP for recreational ponds and reservoirs and as an insurance policy for wild populations.

4**.** Reintroduce fish into habitats that seem to be suitable in terms of other species present and environmental conditions. Our physiological and ecological studies suggest that there are habitats from which Sacramento perch were extirpated decades ago that

have changed enough so they may once again be suitable for them. Some of these habitats include:

- Suisun Marsh. Sacramento perch have already been introduced (2006) into a pond at the Blacklock restoration site, but the success of this introduction is not known. We think there maybe opportunities for re-introducing perch into some of the more natural tidal sloughs in the Marsh but this will require large numbers of fish and some careful evaluation of the potentially suitable sites (e.g., Mallard Sloughs 1and 2)
- Putah Creek, Solano Reservoir. This is a shallow, weedy run-of-river reservoir into which several hundred perch were introduced in 2005. We have found no sign of their presence since, however, although sampling was limited (1 elecrofishing survey of three hours).
- Woodduck Slough, Cosumnes River Preserve. This slough has a small dam with a tidal gate across it. Sampling in 2004 indicated that other fishes were relatively scarce in the upper slough, so 400+ SP were planted there in 2005. Resampling six months later showed that the slough had been massively invaded by other centrarchids and no SP were found (1 electrofishing survey of 4 hours).
- Barker Slough and Liberty Island region, Solano County. This freshwater tidal area is likely to be the focus of habitat restoration for native fishes, especially Chinook salmon and splittail, for the Delta region. Sacramento perch should be incorporated into restoration plans.

- San Luis Reservoir. This large reservoir apparently contains a small population of SP but it has not been studied. It is not a natural habitat but may contain clues as to what conditions are needed to sustain SP.

5. Build/use floodplain ponds that will allow SP to become distributed into natural environments during periods of flooding. A successful reintroduction will require a fairly large propagule size and this is one way to achieve that. This strategy would be take advantage of our previous studies of restoration of flooded habitat on the McCormick-Williamson Tract (CALFED project #99-B193) and the Cosumnes River Floodplain (CALFED Project #99-N06). There may also be potential for using ponds developed in gravel and sand mining operations for this purpose or in the Sutter and Yolo bypasses.

6. Develop a source-sink strategy by locating rearing ponds next to streams or sloughs so the ponds can 'leak' Sacramento perch on regular basis into natural habitats. We have had success in developing populations of Sacramento perch in ponds on the UC Davis campus and have observed that small numbers have wound up in Putah Creek via drainage canals.

7. Rear Sacramento perch in large numbers in ponds and other artificial situations for large-scale introduction into the wild. This is the least desirable of the options we have been considering but may be necessary if information indicates that a large propagule size is necessary for re-establishment in the

wild. This strategy may be especially important for trying to re-establish or maintain SP populations in Clear Lake, Lake County, historically one of the last hold-outs of wild SP in their native range.

8. Develop and maintain an annual monitoring program for all known SP populations in California. We have observed (e.g. in Lagoon Valley Reservoir) that large SP populations that have existed for long periods of time can be extirpated in 3-4 years. Monitoring will be essential for determining which populations are maintaining themselves and which ones are not, and why. Genetic monitoring of wild populations should also be done on a regular basis.

9. Promote the use of Sacramento perch in recreational fisheries, especially farm ponds and urban fishing programs. Their recreational and culinary properties are currently underappreciated and a program like this would not only acquaint people with an edible native sport fish but increase the likelihood of SP being maintained in private ponds and in escaping to the wild.

10. Give the Sacramento perch special status to emphasize the urgency of its recovery. It is currently a Species of Special Concern in California and it could qualify as a state Threatened Species. It was included as a key component of the Delta Native Fishes Recovery Plan (USFWS 1996) but nothing has been done with this. The most appropriate status for this fish needs to be determined but its need for conservation does not.

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