## ENVIRONMENTAL REQUIREMENTS AND TOLERANCES OF THE SACRAMENTO SPLITTAIL, POGONICHTHYS MACROLEPIDOTUS (AYRES)

(November 1993 - February 1994)

**Progress Report** 

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# CONTENTS

INTRODUCTION	1			
Background information and justification	1			
Review of pertinent literature	1			
Anticipated benefits	2			
Objectives				
METHODS	3			
Fish collection and maintenance	3			
Tolerance tests				
Swimming performance	4			
Physiological responses	4			
PROGRESS TO DATE	6			
Fish collection and maintenance	6			
Thermal tolerance tests	7			
ACKNOWLEDGMENT				
LITERATURE CITED				
TABLES	12			
Table 1. Date of collection, number of splittail, place of collection and person/group responsible for collection	12			
Table 2. Body weight, origin, acclimation temperature, upper thermal   tolerance limit and 24-h survival after test of Sacramento splittail   1				

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## **INTRODUCTION**

## Background information and justification

The Sacramento splittail, Pogonichthys macrolepidotus (Ayres), used to be one of the most abundant estuarine species in the Sacramento-San Joaquin estuary to which it is endemic (Moyle 1976) and supported a small, but enthusiastic, hook-and-line fishery (Caywood 1974). It was once widely distributed throughout the California central valley (Rutter 1908) but disappeared from much of its native range because of loss or alteration of lowland habitats following dam construction, water diversion, and agricultural development, and is now restricted to the estuary (Herbold et al. 1992). The last strong recruitment of the population was in 1986. The population is rapidly declining so that a petition has been submitted in November, 1992, to list the Sacramento splittail as an endangered or threatened species (Moyle and Yoshiyama 1992) resulting in the publication by the U.S. Fisheries and Wildlife Services (USFWS) on January 6, 1994 of a proposal ruling to list the Sacramento splittail as a threatened species (Pisces Vol. 24 No. 1, 1994). The future of this species seems bleak, considering the continued loss of freshwater flow through diversion (e.g. for power plant cooling), water pollution (from agricultural run-offs) and the threat of global warming. Global warming and the continued loss of freshwater flow through diversion will eventually increase the amount of seawater entering the estuary, relative to fresh water (Herbold et al. 1992), thereby changing both salinity and temperature levels, possibly decreasing dissolved oxygen content, and altering water flow velocity due to tidal influences. The potential effects of environmental changes on the abundance of Sacramento splittail cannot be fully understood because of the lack of information regarding its environmental tolerances and habitat requirements.

1

The present study will identify environmental requirements and tolerances of Sacramento splittail which will aid in its management restoration. Data on swimming performance at different salinity and temperature combinations will help predict the survival, distribution, and abundance of this species. Factors which impair swimming would modify predator avoidance behavior with consequent decreases in survival (Beamish 1978). Data on physiological responses will show how the fish adjust to changes in abiotic factors and signal when these changes become too stressful. Taken together, splittail tolerance, swimming performance, and physiological responses will elucidate environmental conditions which adversely impact this species. These data will allow informed water/fish/activities decisions to be made regarding splittail management.

## **Review of pertinent literature**

The Sacramento splittail, was first described by Ayres (1854) as *Leuciscus* macrolepidotus. It has now been reassigned to the genus Pogonichthys that is considered to be allied to cyprinids of Asia (Howes 1984). It is considered primitive compared to other endemic cyprinid fishes of the California central valley because of its two rows of pharyngeal teeth in contrast to the more advanced characteristic of one row (Caywood 1974, Moyle 1976). The splittail was once widely distributed in the rivers and marshlands of the Central Valley (Rutter 1908), comprised varying proportions of the diets of the Indians (Caywood 1974), and even supported a small angler fishery in Miller Park on the Sacramento River (Caywood 1974). In 1974 the splittail distribution has been reported to be moderately reduced as a result of the introduction of other fishes and the engineering activities of man (Caywood 1974) and, in 1983 reportedly confined to the lower Delta and the main channel of the Sacramento River, a fraction of its former distribution (Daniels and Moyle 1983). It was speculated that unfavorable conditions for splittail would include high salinity, low water levels, and/or high temperatures. However, little data exist regarding its environmental requirements or tolerances.

In 1986, the Sacramento splittail was reported to be one of the ten most abundant species collected on a regular basis over a 54-month period in Suisun Marsh, a portion of the Sacramento-San Joaquin estuary in central California (Moyle et al 1986). It was also the population's last year of strong recruitment (Moyle and Yoshiyama 1992). Since 1980 splittail numbers in the Delta have declined steadily (although large pulses of young fish were observed in 1982, 1983 and 1986) and in 1992 were probably the lowest in record (Moyle and Yoshiyama 1992). The splittail has become an estuarine species because suitable habitats were no longer present in the central valley (Herbold et al. 1992; Moyle and Yoshiyama 1992).

In November, 1992, a petition was submitted to list the Sacramento splittail as an endangered or threatened species (Moyle and Yoshiyama 1992). On January 6, 1994, the USFWS proposed a rule to list the Sacramento splittail as a threatened species (Pisces Vol. 24, No. 1, 1994). In spite of the studies (Caywood 1974; Daniels and Moyle 1983) made on its life history (age and growth, condition, reproductive biology, and year class strength), little is known of its environmental requirements or tolerances. Recommended future studies on Sacramento splittail include: effects of flow on year class strength, ability to negotiate fishways, and life cycle characteristics (Caywood 1974), along with habitat requirements of young-of-year splittail, and identification of spawning areas (Moyle and Yoshiyama 1992). The present study directly addresses splittail swimming performance (associated with effects of flow and fishway negotiations) along with tolerance limits and physiological responses to environmental conditions (associated with habitat requirements).

#### **Anticipated** benefits

Results of the study will define environmental requirements and tolerances of Sacramento splittail and elucidate conditions which adversely impact this species. Together with existing hydrologic models of Bay-Delta water quality, these results will directly aid water and resource managers regarding freshwater flow requirements for estuarine fishes.

## Objectives

The ultimate objective of the study is to define the environmental requirements and tolerances of Sacramento splittail for effective water/fish/activities management and restoration of this species. Specifically, the study will determine the: a) tolerance limits of Sacramento splittail to temperature, salinity and dissolved oxygen; b) effects of tolerable levels of these factors on the swimming performance of splittail; and c) physiological responses of splittail to varying levels of salinity, temperature, and dissolved oxygen content.

2

### **METHODS**

## Fish collection and maintenance

Juvenile and subadult/adult Sacramento splittail, are being collected from Suisun Marsh using 2.5 m otter trawl (mesh at cod and 6 mm bar) and/or 3 mm bar beach seine (Daniels and Moyle 1983) by Scott Matern of the UCD Fish Ecology Group. Fish had been collected from San Joaquin River by the Alosa and Longfin Groups headed by Kathy Heib and Jane Arnold of California Department of Fish and Game (CDFG) using mid-water trawls. Fish are also being collected by Lloyd Hess and Scott Siegfried at the Tracy Fish Collection Facility. Fish are transported in insulated coolers (with aeration) at ambient (collection) salinity and temperature to the Academic Surge Laboratory of the University of California, Davis. Sixteen subadult/adult splittail had been obtained through the kindness of Dr. Howard Bailey from his floating cages at the Goodyear Slough and were transported using insulated fiberglass tanks (with oxygenation) at ambient temperature at 10 ppt salinity and 10 ppm nitrofuracin (NFC). Fish are held in insulated fiberglass tanks with constant aeration at ambient temperature and salinity, and are gradually acclimated to test temperature and/or salinity 3-4 d before the experiment.

### **Tolerance tests**

Determination of temperature, dissolved oxygen and salinity tolerance limits of juvenile Sacramento splittail is being done following modifications of the Becker and Genoway (1979) method (4-6 replicates) defined by a loss of equilibrium. Loss of equilibrium in fish indicates the detrimental effects of the experimental variable so that the fish becomes physically disorganized and loses its ability to escape from conditions leading to its death (Becker and Genoway 1979). This kind of test criterion defines tolerance limits associated with increased disease susceptibility and decreased growth, reproduction, and survival, ultimately reducing rates of recruitment to succeeding life stages (Wedemeyer et al. 1990). Importantly, these tests can be conducted without sacrificing test fish.

Fish are held in individual plexiglass test vessels of a flow-through design (Cech et al. 1979).

1) Temperature: Fish are subjected to increasing or decreasing temperature starting from ambient (collection) temperature (1°C per 10 min) at 0 ppt salinity. The same procedure will be done at higher salinity based on salinity tolerance test results. Control fish are subjected to the same handling procedure and placed in identical plexiglass test vessels of the same salinity levels but without temperature changes. Inflow water is aerated to ensure high dissolved oxygen level.

2) Dissolved oxygen: Fish will be subjected to decreasing dissolved oxygen starting from 150 torr  $PO_2$  approximating air-saturation levels, (at 10 torr  $PO_2$  per 30 min) at ambient temperature and at higher temperature based on temperature tolerance test results at 0 salinity and at a higher salinity level based on salinity tolerance test results. Control fish will be subjected to the same handling procedure and placed in identical plexiglass test vessels of the

same temperature and salinity levels but without changing dissolved oxygen level.

3) Salinity: Fish will be subjected to increasing levels of salinity starting at 0 ppt (at 1 ppt per 20 min interval) at 12 and at a higher temperature based on temperature tolerance test results. Control fish will be subjected to the same handling procedure and placed in identical plexiglass test vessels of the same temperature and dissolved oxygen levels but in fresh water. Salinity tolerance tests will be conducted only if enough time and test fish are available.

#### Swimming performance

Critical swimming velocities will be determined at different salinity and temperature combinations depending on tolerance test results using a modified Brett-type swimming apparatus (Brett 1964) with a recirculating water flume incorporating a variable-speed motor. Juvenile fish will be placed in the swimming chamber and, after 10-min acclimation period. critical swimming velocity will be measured by step increases of 10 cms<sup>-1</sup> in water velocity at 10 min intervals starting at 10 cms<sup>-1</sup> until the fish is fatigued (Beamish 1978). Fish will be considered fatigued when it is "pinned" 3 times at the rear of the chamber. Critical swimming velocity, defined as the maximum velocity fish could maintain for a precise time period, will be calculated using the formula (Brett 1964):

$$U_{crit} = U_i + (U_{ii} \cdot T_i \cdot T_{ii})$$

where:

= critical swimming velocity

U <sub>crit</sub>	= critical swimming velocity
U <sub>i</sub>	= highest velocity maintained for the prescribed
	time period
U <sub>ii</sub>	= velocity increment

- Ti = time elapsed at fatigue velocity (in min)
- T. = prescribed swimming period

Swimming tests have been used to define limits of acclimation to stresses associated with salinity (Wakeman and Wohlschlag 1979), temperature (Berry and Pimentel 1985), and dissolved oxygen (Kutty and Saunders 1973) and have been used as tests of fish condition (Smith 1982; Flagg et al. 1983).

#### Physiological responses

Adult/subadult splittail are arterially cannulated to determine physiological responses (changes in blood PO<sub>2</sub>, PCO<sub>2</sub>, pH, hematocrit, plasma cortisol, lactate, and osmolality levels) to varying levels of salinity, temperature and dissolved oxygen content. Fish are mildly anesthetized with MS-222 and the dorsal aorta cannulated through the posterio-lateral section of the fish (Cech and Rowell 1976). Fish are allowed to recover from surgery in a plexiglass test vessel for a period of 3-5 days before experimentation.

Fish are subjected to varying temperature, dissolved oxygen or salinity as in the

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juvenile fish studies. Control fish are cannulated as in the test fish and placed in identical plexiglass test vessels but not subjected to changes in temperature, dissolved oxygen or salinity. Blood samples are collected via the cannula protruding from the controls and test fish just before the start of the experiments and will be referred to as "resting" samples, and subsequent samples will be collected every 2-4 hr. Final samples are collected at the end of the tolerance tests. Changes in the blood PO<sub>2</sub> (partial pressure of oxygen), PCO<sub>2</sub> (partial pressure of carbon dioxide), and pH are determined using a Radiometer or Instrumentation Laboratories blood gas analyzer. Capillary tubes containing blood samples are separated by centrifuged at 11,500 rpm for 3 min and hematocrit read. Plasma samples are separated by centrifugation in microcentrifuge tubes, transferred to plastic freezer vials, and frozen for later analyses of osmolality, lactate and cortisol. Packed red blood cells are resuspended and reinjected into the fish to prevent anemia. Osmolality will be measured using a calibrated Wescor (Logan, UT) 5100B Vapor Pressure Osmometer. Lactate levels will be determined using a calibrated YSI (Yellow Springs, OH) 27 Lactate Analyzer. Cortisol levels will be measured using a modified enzyme immunoassay (ELISA, Munro and Stabenfeldt 1985).

Changes in physiological responses will identify mechanisms defining tolerance thresholds in adult/subadult Sacramento splittail. The blood gas values and blood pH will show how fish cope with oxygen depletion (hypoxia). Specifically, we will examine arterial PO<sub>2</sub> against environmental PO<sub>2</sub> slope changes and patterns of pH and PCO<sub>2</sub> associated with hypothesized hyperventilation, to identify critical PO<sub>2</sub>s (Cech et al. 1979). Changes in plasma cortisol will indicate primary stress responses, while changes in lactate, osmolality and hematocrit will indicate secondary stress responses (Wedemeyer et al. 1990). Prolonged cortisol elevation is a major factor contributing to the damaging effects of stress on survival, growth and reproduction (Pickering 1992) and indicates reduced fitness resulting in higher susceptibility to diseases (Robertson et al. 1963; Kent and Hedrick 1987; Woo et al. 1987). Blood lactate will be used with pH and PCO<sub>2</sub> to determine degree and type (metabolic or respiratory) of acid-base imbalances. Hematocrit and osmolality measurements will define within fish or fish-environment fluid shifts, leading to osmoregulatory dysfunctions (Wedemeyer et al. 1990).

#### Statistical analyses:

Analyses of variance (ANOVA) and Tukey's test (using SYSTAT software) will allow quantitative comparisons of the variables between sample times within a test (e.g. temperature test at 0 ppt salinity) and between tests (e.g. temperature tests at 0 and 18 ppt salinities). Cortisol values will be transformed to  $\log_{10}$  before conducting ANOVA to increase homogeneity of variance and the value of 1 added to each cortisol datum before log transformation to avoid negative values (Young and Cech 1993).

5

#### **PROGRESS TO DATE**

Fish collection and maintenance

In September 1993, we were notified by Dr. Perry Herrgesell (Study Manager of the Interagency Ecological Study Program) regarding the availability of funds for the project. Immediately after, materials needed for fish collection such as 4 80-liter capacity coolers, 4 medical-type oxygen tanks and regulators, 11 battery-operated air pumps, rechargeable batteries and battery recharger were purchased. At the same time, arrangements were made with the Alosa and Longfin Groups headed by Kathy Heib and Jane Arnold of the CDFG, with Geir Aasen (CDFG), Mark Pierce (USFWS), Lloyd Hess of the Tracy Fish Collection Facility, and Scott Matern of the Fish Ecology Group at UC Davis. Four coolers with one oxygen tank and regulator each were brought to the CDFG and USFWS Stockton office for use in the splittail transport.

Table 1 shows the date of collection, number of fish, place of collection, and person/group responsible for fish collection, and survival to date of Sacramento splittail. The fish were arbitrarily grouped into juvenile (10-100 g), subadult (100-400 g), and adult (>400 g body weight).

The fish collected by the Alosa Group of the CDFG by mid-water trawl on October 5, 1993 had sores and bruises on the dorsal and lateral parts of the body. The fish were transported to the UCD laboratory and were treated with 10 ppm NFC and 10 ppt salinity. The smaller fish had a parasitic copepod on its lateral side near the base of the anal fin and the fish died the next day. The other fish was fed with live adult brine shrimp (*Artemia salina*). A week later the fish showed signs of infection on the sores at the lateral and posterior parts of the body. It was treated with 10 ppm NFC and 10 ppt salinity for 3 days but did not recover.

The two juvenile fish collected by the Longfin Group by of the CDFG by mid-water trawl on October 6, 1993 appeared to be in good condition. They were also treated with 10 ppm NFC and 10 ppt salinity, maintained at  $\sim 17^{\circ}$ C and were fed with live adult brine shrimp. Nine days later, these two fish were used for upper thermal tolerance test. After the test, both fish appeared to be in good condition. However, after removal of an algal-like growth near the mouth of one fish caused its death.

The two juvenile and one subadult fish collected by Scott Matern of UCD by otter trawl from Suisun Marsh on October 21, 1993 had sores on the body and had algal-like growth near the base of the dorsal fin. All were treated with 10 ppm NFC and 10 ppt salinity. However, the two juvenile fish died 2 days later. The subadult fish was maintained at  $\sim 17^{\circ}$ C and were fed with live adult brine shrimp. Eight days later, it was used in upper thermal tolerance test and recovered well. A month later, the fish was found dead outside the rearing tank.

On October 25, 1993, 16 subadult/adult fish were kindly donated by Dr. Howard

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Bailey from his floating cages in Goodyear Slough. These fish were transported in an insulated fiberglass tanks with 10 ppt salinity and 5 ppm NFC. All fish appeared to be in good condition and trained to feed on Silver Cup trout pellets. All fish were maintained at  $\sim 17^{\circ}$ C and were fed with live adult brine shrimp. Two weeks later, one adult and one subadult fish were cannulated. The adult fish died the next day, while the subadult one recovered and was used for upper thermal tolerance test. However, during the test, the fish appeared to be stressed and did not recover from the test. Two more adult fish were cannulated. One fish did not recover from cannulation, while the other recovered within minutes after cannulation. This fish was used for upper thermal tolerance test. However, during the test, no blood could be drawn from the cannula. Repeat tugging on the cannula to loosen it a little for blood collection appeared to have stressed the fish, so that it did not recover. It seemed that the remaining fish have to be reared to an increased size before any cannulation is attempted. A larger fish would recover from cannulation better and would be less stressed during the test.

The fish collected from Suisun Marsh in December, 1993 and January 1994 appeared to be in good condition. However, they appeared to have difficulty switching diet from live adult brine shrimp to trout pellets. They panicked easily and would jump through a small gap between the tank and its cover.

In February 1994, a total of 5 subadult/adult fish were collected by Lloyd Hess and Scott Siegfried from the Tracy Fish Collection Facility. These were transported to UCD in 10 ppt salinity and 5 ppm NFC. Some fish showed bruises and sores. One adult fish died the next day and the rest were treated with 10 ppt salinity and 5 ppm NFC for three days. Two of the fish appeared to have fungus-like infection.

The main problem for fish collection is the availability of fish. We expect to be able to collect young-of-the-year splittail during the spring and summer seasons. We are also rearing the subadult/adult fish to a larger size so that testing on physiological responses can be conducted.

## Thermal tolerance test

Table 2 shows the preliminary data on body weight, origin, acclimation temperature, upper tolerance limit and 24-h survival after thermal tolerance tests of Sacramento splittail. Results show that the test in itself is not lethal to the fish and good results can be obtained by the Becker and Genoway (1979) method. The limited results show that the upper thermal tolerance limit of splittail increases as the acclimation temperature increased. For juvenile fish, those acclimated at 12°C had a mean upper thermal tolerance of 20.25°C, a difference of 8.25°C; and one acclimated at 17°C had an upper thermal tolerance limit of 25.5°C, a difference of 8.5°C. However, more fish are needed to be tested before any further statistical analyses can be done. Installation of high-capacity chiller units and modifications of the thermal tolerance set-up are ongoing for testing subadult fish at lower temperature.

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Date of collection	Number of fish	Place of collection	Collected by	Survival to date
10-05-93	2 subadult/adult	San Joaquin River	Alosa Group (CDFG)	0
10-06-93	2 juvenile	San Joaquin River	Longfin Group(CDFG)	0
10-21-93	1 subadult, 2 juvenile	Suisun Marsh	Scott Matern (UCD)	0
10-25-93	16 subadult/adult	Goodyear Slough	Howard Bailey (UCD)	8 subadult, 4 adult
12-09-93	4 subadult	Suisun Marsh	Scott Matern (UCD)	2 subadult
12-10-93	3 subadult	Suisun Marsh	Scott Matern (UCD)	2 subadult
01-06-94	2 subadult, 1 juvenile	Suisun Marsh	Scott Matern (UCD)	1 subadult
01-07-94	1 subadult, 4 juvenile	Suisun Marsh	Scott Matern (UCD)	1 subadult
02-14-94	2 subadult/adult	Tracy Fish Facility	Scott Siegfried (Tracy)	2 subadult/adult
02-16-94	3 subadult, 1 adult	Tracy Fish Facility	Llyod Hess (Tracy)	2 subadult

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Table 1. Date of collection, number of splittail, place of collection and person/group responsible for collection.

Fish Weig no. (g)	Weight	Origin	Temperature °C		24-h	Notes
	(g)	Ũ	Acclimation	Upper limit	survival	
J1	15.1	San Joaquin River	- 17	25.5		died after removal of algal-like growth
J2	10.6	San Joaquin River	17	control .	+	
<b>S</b> 1	220.3	Suisun Marsh	.17	25.3	+	had algal-like growth near dorsal fin
S2	304.8	Goodyear Slough	17	control	+	
<b>S</b> 3	384.6	Goodyear Slough	17	22.2	-	cannulated; did not recover from test
J3	16.9	Suisun Marsh	12	control	+	
J4	23.4	Suisun Marsh	12	20.1	+	
J5	15.4	Suisun Marsh	12	19.4	+	· .
J6	18.2	Suisun Marsh	12	21.3	+	
J7	18.0	Suisun Marsh	12	20.2	· • • +	

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Table 2. Body weight (g), origin, acclimation temperature (°C), upper thermal tolerance limit (°C) and 24-h survival after test of Sacramento splittail.

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