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**EXPERIMENTAL AND FIELD STUDIES  
TO ASSESS PULSED WATER FLOW  
IMPACTS ON THE BEHAVIOR AND  
DISTRIBUTION OF FISHES IN THE  
SOUTH FORK OF THE AMERICAN  
RIVER: SECOND YEAR**

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## Preface

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*Experimental and Field Studies to Assess Pulsed Water Flow Impacts on the Behavior and Distribution of Fishes in the South Fork of the American River: Second Year* is a final project report for the Ecological Impacts of Pulsed Flow Releases from Hydropower Facilities project (Contract Number 500-01-044) conducted by the Center for Aquatic Biology at the University of California, Davis. The information from this project contributes to PIER's Energy-Related Environmental Research Program.

For more information about the PIER Program, please visit the Energy Commission's website at [www.energy.ca.gov/pier](http://www.energy.ca.gov/pier) or contact the Energy Commission at 916-327-1551.



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## Abstract

This research evaluated the effects of pulsed flows from hydropower facilities on fish. Pulsed flows are large, rapidly increasing and decreasing discharges from hydropower facilities that may cause fish of all age classes to be stranded or washed away. Such discharges are used to meet peak energy requirements, recreational demands and sediment and vegetation management needs. To assess such effects, experimental and field investigations were conducted on two species: Rainbow trout (*Oncorhynchus mykiss*) and Hardhead (*Mylopharodon conocephalus*). This report presents the second year research results. First year results can be found in publication CEC-500-2005-172.

There were three phases to this year of study:

- Tracking the movement in a river of adult trout with radio transmitters to ascertain whether individuals are displaced longitudinally (downstream) in response to pulsed flows.
- Determining energy expenditures using radio telemetry and electromyogram (EMG) sensors (an electromyogram measures muscle activity) for an adult rainbow trout experiencing pulsed flow conditions.
- Ascertaining temperature preferences for adult rainbow trout and hardhead under laboratory conditions.

Tracking the movements of both small and large radio-tagged trout under frequent pulsed flow conditions in the South Fork American River, California, showed no significant relationships among fish movement and water flow variables, release site, location within river, fish size, or fish condition. Pulse stage was found to be statistically significant for energy expenditures; increasing pulse stage was correlated with increasing swimming speeds. The temperature analysis showed that the hardhead preferred a range of 19.6°–20.0°C, while the trout preferred a significantly cooler range: 16.0°–18.4°C.

The field and laboratory studies described in this report provide a description of the impacts of pulsed releases on hardhead and rainbow trout and recommended management measures to reduce these effects. This report also makes recommendations for further study.

**Keywords:** Pulsed flows, longitudinal displacement, American River, hardhead, rainbow trout, radio tracking, electromyogram telemetry (EMG)



# Executive Summary

## Introduction

Sharp increases and decreases in water flows are common in California's streams and rivers. Such flow fluctuations occur naturally in response to runoff patterns or, on the state's numerous regulated rivers, in response to flow releases from dams and reservoirs. These manufactured flows, also known as pulsed flows, are beneficial for generating electricity, providing irrigation water, ensuring flood control, flushing streambeds, and facilitating recreation such as river rafting. The effects of these pulsed flows on species present within these streams and rivers, however, are relatively unknown.

California native stream species have evolved with natural flow fluctuations that are characterized by infrequent but significant cool season pulse flows, especially those during the spring snowmelt. Manufactured pulsed flows, on the other hand, occur much more frequently and continue much later into warm seasons and thus deviate significantly from the natural flow patterns. Strong pulsed flows may have possible negative effects on fishes such as longitudinal displacement (forcing downstream of normal habitat) and lateral displacement (stranding along changing channel margins). For this reason, both laboratory and field studies were conducted to assess the impact of pulse flows on two fish species that inhabit California rivers.

## Project Objectives

This research identified the effects of pulsed flows on fishes. Laboratory and field investigations were conducted on two fish species: rainbow trout (*Oncorhynchus mykiss*) and hardhead (*Mylopharodon conocephalus*). Rainbow trout is a species native to California and consists of both anadromous (migrates from the ocean to spawn in freshwater streams and rivers) that is also known as steelhead trout and non-anadromous populations (year-round residents). Hardhead is also a native species that is resident year-round in lows to mid-elevation streams and rivers within the Sacramento and San Joaquin River drainages. Field work conducted for this project focused on rainbow trout while laboratory efforts focused both on rainbow trout and hardhead. This report presents the results of the second year of research.

The results of the first year of this study are reported in *Experimental and Field Studies to Assess Pulsed, Water-Flow Impacts on the Behavior and Distribution of Fishes in the South Fork of the American River*. This report can be found at [www.energy.ca.gov/2005publications/CEC-500-2005-172/CEC-500-2005-172.PDF](http://www.energy.ca.gov/2005publications/CEC-500-2005-172/CEC-500-2005-172.PDF).

The phases of the first year of this study were:

- Tracking the movement of juveniles and adults (greater than 15 centimeters total length), tagged with radio transmitters, in a river to ascertain whether individuals are displaced longitudinally in response to pulsed flows.
- Quantifying the distribution of juveniles (less than 15 cm) in a river from visual censuses of unmarked individuals and those marked with visible implant elastomer before, during, and after releases. Visible implant elastomer is a silicone based material

that is implanted beneath transparent or translucent tissue and remains externally visible.

- Determining the degree of longitudinal and latitudinal displacement, as well as substrate preference of juvenile fishes in varying flows in laboratory experiments.

The results of the first year of this study are:

- Radio-tagged rainbow and brown trout were tracked during a single pulsed flow. No significant differences were found between the distances moved before, during, and after the release. Fish numbers recorded in pools along this reach during snorkel surveys before and after this pulsed did not appear to differ before and after the pulse.
- Juvenile rainbow trout, hardhead, and Sacramento suckers were exposed to artificially pulsed flows within a longitudinal flume. Although fish moved either upstream or downstream, the most common (or mean) position of the individuals was close to the center of the flume during pulsed flows. The distribution of individuals was also determined in a lateral displacement flume, consisting of a rectangular tank separated into a main channel that never drained and a sloped bank that alternately flooded and became exposed. Only 3 (7.8 percent) of the 38 fish placed within the apparatus became stranded.

There were three components to the second year of this study:

1. Track the movement of larger adult rainbow trout (equal or greater than 25 centimeters total length), tagged with radio transmitters, to ascertain whether individuals are displaced longitudinally in response to pulsed flows in the river.
2. Assess and estimate the energetic costs of adult rainbow trout experiencing pulsed flows by implantation of an electromyogram (EMG) transmitter. Energetics in this case refers to a fish's energy (food) intake and expenditure (work). An electromyogram measures the electrical activity of muscles. When muscles are active, they produce an electrical current that is usually proportional to the level of the muscle activity. With this instrument, the fish's energetics can be calibrated in the laboratory and then used to track their movements and energetics in the river.
3. Assess the behavioral temperature preferences of adult rainbow trout and hardhead using a large laboratory annular apparatus.

## **Project Outcomes**

### **Radio Tracking**

The movements of 10 small (standard length 25.5–31.0 cm) and 10 large (standard length 32.0–38.5 centimeters) radio-tagged adult rainbow trout in response to frequent pulsed-flow releases in the South Fork of the American River (California) were tracked from July to October 2005. During this period the river had base flows of 5 cubic meters per second, with 4-hour midday releases of 40 cubic meters per second on most days for whitewater rafting, plus higher releases

on many days with peak flows up to 110 cubic meters per second. Fish were released into the river 12.9 and 16.1 kilometers upstream of Folsom Lake and tracked weekly. In the first week after being released, small trout moved only within 1 kilometer upstream or downstream of where they were released. Eight of the ten small trout moved little in the following eight weeks. Between weeks four and seven, one small trout moved 2.0 kilometers upstream, while between weeks five and seven, another small trout moved 2.0 kilometers downstream. In weeks 1 to 3, 8 of 10 large trout moved from 1.0 to 4.5 kilometers downstream. Between weeks 5 and 6, one large trout moved from a position 1 kilometers downstream to a position approximately 3.5 kilometers downstream, and then moved to a position 8.0 kilometers downstream of the release site between weeks 7 and 8.

Large trout spent most of their time in runs (41 percent), followed by pools (30 percent), and rapids (29 percent). Small trout were most often observed in runs (42 percent), followed by rapids (30 percent), and pools (28 percent). Repeated measures of analysis of variation (ANOVA) showed no significant relationships between fish movement and water flow variables, release site, location within river, fish size, or fish condition with a statistical level of significance greater than 0.05 ( $p > 0.05$ ) for all variables. ANOVA is a collection of statistical tests to differentiate variation. These results suggest that rainbow trout with standard lengths greater than 25 cm are not forced downstream by daily pulsed-flow increases from 5 to more than 40 cubic meters per second.

#### Electromyogram Telemetry

Radio telemetry with electromyogram sensors were used to study the energetic output of rainbow trout in response to pulsed flows. Investigations the previous year suggested that the fish within the watershed exhibit minimal directional movements. Nine rainbow trout (equal to or greater than 30 cm standard length) were implanted with these sensors to investigate movement patterns, swimming speed, and oxygen consumption of hatchery fish experiencing pulsed flows. Swimming activity was calibrated using a Brett-type respirometer, and fish were released into the river implanted with electromyogram sensors. A respirometer is a device used to measure the fish's rate of respiration by measuring the exchange of oxygen and carbon dioxide. Each individual's electromyogram outputs were recorded before the water pulse, as the water increased, stabilized, and decreased on three separate occasions. Electromyogram measurements were converted to swimming speeds by using laboratory calibrations. Factors potentially related to median swimming speeds, such as river discharge, time, sex, location, and pulse stage (no pulse, rising, peak, and decreasing pulses), were analyzed using a hierarchical mixed linear model. Pulse stage was found to be statistically significant; increasing pulse stage was correlated with increasing swimming speeds. In addition, above a river flow of 44 cubic meters per second, swimming activity decreased. These results indicate that the rainbow trout's ability to respond to pulsed flows without being displaced incurs other costs such as increased energy expenditure and decreased foraging opportunities at high flows.

## Temperature Preference

To effectively determine adult stream fishes' temperature preferences, a 3-meter-in-diameter, annular (ring-shaped) chamber of acrylic plastic was constructed. The annular chamber presents uniform light intensities, constant water depths and velocities, and stable vertical and horizontal temperature gradients to identify the temperature preferences of the experimental fish. Hardhead (mean total length: 36.2 centimeters) and rainbow trout (mean total length: 35.4 centimeters) were acclimated to 12°, 15°, and 18°C and tested, individually, in the 12° - 24°C annular gradient. The hardhead preferred temperatures in the range of 19.6 - 20.0°C, while the trout preferred a significantly cooler range: 16.0°–18.4°C. All of the hardhead avoided water less than 17°C in temperature, whereas the 12 and 15°C trout acclimation groups avoided water warmer than 19°C, and the 18°C trout avoided water less than 16°C and greater than 20°C. Presumably, stream fish temperature preferences can be used to optimize environmental characteristics in regulated systems for resident and migratory species.

## Conclusions

### Radio Tracking

The rainbow trout in this study did not appear to be displaced downstream by the pulsed flow regime of the South Fork American River in the summer of 2005, in spite of over 20-fold daily flow fluctuations. The fact that hatchery-reared rainbow trout were used for this study may have influenced the results. These fish would not have experienced natural pulsed flood flows during their development. Yet they did not permit themselves to be swept downstream during the increased flow velocities. It would be informative to repeat this study in a river reach that contained adequate numbers of wild trout of a size suitable for radio-tagging.

Over the time intervals that the fish were tracked, they did not seem to respond to increased flows by moving longitudinally upstream or downstream. However, the largest movements upstream were accomplished during weeks of lower pulsed flows (weeks 11 and 12). This suggests fish may move upstream more readily when pulsed flow peaks are lower, and that larger pulsed flows may limit the degree to which trout will move upstream. River regulators may wish to limit the magnitude of summer pulsed flow peaks at times when trout are expected to moving upstream in search of rearing habitat.

Initially after their release, the large trout in the study moved downstream, but in subsequent weeks, they tended to remain in approximately the same location. The smaller trout did not show this same initial downstream displacement. The larger fish would have had higher caloric requirements relative to the smaller trout. If the larger trout were unable to find habitat with an adequate food supply, they may have traveled downstream in search of areas with more food, thus spacing themselves out relative to the locally available food supply. It is possible that the pulsed flow regime of the river has indirect effects on trout feeding through impacts on the species composition and abundance of benthic macroinvertebrates. Benthic macroinvertebrates are small invertebrates occupying stream bottoms. It would be beneficial to study the impact of

pulsed flows on this community composition and biomass in conjunction with fish movement and fish diet in order to separate the direct (that is, velocity) and indirect (that is, food depletion) impacts of pulsed flows on fish in regulated systems. Also field tracking studies on the movements of juvenile rainbow trout (15–25 centimeters total length) would help the understanding of how pulse flows impact fish through various life stages.

### **Electromyogram Telemetry**

Electromyogram telemetry results suggest that rainbow trout are using more energy by increasing their swimming speed to maintain position when experiencing increasing flows. This increased energy output may alter a fish's metabolic balance and decrease resources available for growth and reproduction. The authors recommend that increases in flows be gradual or stepped to allow rainbow trout time to adapt to these changes in flow. In addition, based on their decreased activity at river flows above 44 meters per second, flows should not regularly exceed that level. More studies should be conducted with different fish species that are common in that stretch of river such as the Sacramento sucker (*Catostomus occidentalis*) and Sacramento pikeminnow (*Ptychocheilus grandis*). Efforts should also be made to investigate fish behavior at night, especially because late night pulses tend to be larger than pulses during the daylight hours. Temperature, turbidity, and increased macroinvertebrate drift associated with pulsed flows may also have relevance in determining fish behavior and energetics during pulsed flows.

### **Temperature Preference**

Consideration of the species' behavioral preferences is integral to explaining the distribution of native fishes in the South Fork American River. The temperature preferences of adult rainbow trout were elucidated in experiments conducted on three groups of fish, acclimated to different temperatures. The non-anadromous trout, that is those that do not return to the sea for breeding, preferred a cool or an intermediate water temperature throughout the seasons of the years. When ambient water temperatures are elevated, as is typical during California summers, the trout choose a narrower water temperature range of 15°–18°C. During this season trout avoid the coolest water temperatures in favor of intermediate temperatures. During the cooler parts of the year, the trout would presumably prefer cooler temperatures (less than 16°C). Because the 18°C-acclimated rainbow trout showed a bimodal locational preference in their seeking their 18.4°C preferred temperature, these results argue for temperature, rather than some other influence in the laboratory, to be the dominant behavioral cue in this apparatus.

Understanding the temperature preference of hardhead will require a greater understanding of their life history, swimming, metabolic, and growth performance throughout the South Fork. This ecological information will aid in determining why hardhead occurred in limited numbers at warmer temperatures in the South Fork, and occurred in large numbers in colder reaches of the river, while they preferred warmer temperatures in the laboratory experiments. Hardhead could seek refuge in colder river stretches and reservoirs due to: (1) resource competition from native or non-native fishes, (2) parasitic infections decreasing survival at warmer temperatures, or (3) lower quality forage in the warmer river. River water temperatures should be managed to

simulate the natural temperature range throughout the year, which best suits the native species present.

### **Benefits to California**

Human-manufactured water flow increases (pulses) are common within California's rivers. Although native stream species have evolved with seasonal fluctuations, the increased frequency (for example, for peaking hydroelectric operations) and late-summer timing (for recreational purposes) represents significant deviations from the natural flow patterns (also referred to as the natural hydrograph). The effects of flow pulses on the community of species present within the streams are relatively unknown. The field and laboratory studies described in this report provide a description of the impacts of pulsed releases of water for recreational and commercial purposes on hardhead and rainbow trout. The knowledge resulting from these studies may help agencies to manage their pulsed flows to minimize their effects on the resident fish.

**Note:** Unless otherwise indicated, all pictures and graphs in this report are the outcome of the research described herein.

## 1.0 Introduction

Human-controlled water flows (pulses) are common within rivers. There are many reasons for anthropogenic water discharges: (1) generating electricity, (2) flushing streambeds, (3) facilitating human recreation, (4) providing additional water for downstream irrigation diversions, and (5) flood control operations. A common rationale for controlling the flows of rivers is the production of electrical energy. Hydroelectricity makes up 25% of California's electrical capacity, and is thus a critical component of the state's electrical grid. Water from the Sierra snowmelt, which collects in reservoirs, is passed into large conduits that run laterally along ridges and then downward into secondary reservoirs within valleys, forcing the water to rotate turbines attached to generators to produce electrical power. These controlled flows provide additional electrical energy during hot summer days when electrical loads from air conditioners are highest (Hunter 1992). Flushing flows are brief and infrequent, but are sufficiently large that they move silt and sand downstream from the stream bed where they have settled due to slow water flow (Milhous 1994; Reiser et al. 1989). Recreational flows are water releases made to accommodate activities such as rafting and kayaking.

Although water releases provide obvious benefits to humans, the effects of these flow pulses on the aquatic community are relatively unknown. Native California fish species have evolved to cope with flow fluctuations, but their increased frequency (e.g., for electricity generation) and late-warm-season timing (for recreational purposes) represent significant deviations from a natural hydrograph. Thus, it is imperative that studies be conducted to determine the impact of pulse flows on the fish species that inhabit California's rivers.

### 1.1. Background

Strong pulsed flows may have possible negative effects on fishes such as longitudinal displacement (forcing downstream of normal habitat), lateral displacement (stranding along changing channel margins), and habitat temperature alterations. Downstream forcing could result in slower growth, decreased reproductive output, or mortality due to reductions in the availability of natural prey (e.g., macro-invertebrates) or habitat (e.g., cobble for juvenile protection, gravel for adult spawning). Lateral stranding could result in mortality due to high water temperatures and enhanced vulnerability to predation in shallow dewatering pools.

#### 1.1.1. *Longitudinal Displacement*

Strong natural and anthropogenic pulsed flows can force juvenile salmonids downstream (McCrimmon 1954; Erman and Leidy 1975; Ottaway and Clarke 1981; Ottaway and Forrest 1983; Heggenes and Traaen 1988; Crisp 1991; Crisp and Hurley 1991; Pearsons et al. 1992). Longitudinal displacement of juvenile coho salmon (*Oncorhynchus kisutch*) has been observed in streams during winter periods when floods are common (Bell et al. 2001; Shirvell 1994; Giannico and Healy 1998). Passive integrated transponder (PIT) tagged juvenile coho salmon moved mostly in the

downstream direction between 10 and 1,992 m (mean: 517 m) after a five-year recurrence flood in Prairie Creek, California (Bell et al. 2001). The higher recapture rates of the young coho salmon in more hydraulically protected habitat types (alcoves and backwaters), compared with those in main-channel pools, probably reflects the value of these off-channel habitats in minimizing the washout/dispersal of fishes downstream (Bell et al. 2001). Furthermore, there may be an interaction between the seasonal timing of pulsed flow releases, the life history stage of fish present, and the relative magnitude of pulses compared with normal flows for that time of year.

In contrast, the longitudinal displacement of larger fish seems less likely, due to their increased swimming performance compared with smaller fishes (Webb et al. 1999). Seventeen adult rainbow trout (TL 42.5 - 59.8 cm), equipped with radio transmitters were tracked in the San Juan River below Navajo Reservoir during an elevated, spring reservoir-discharge event (Gido et al. 2000). Twelve of the trout moved laterally into shoreline and side-channel habitats, and five were lost. Because many river species (e.g., members of the family Cyprinidae, the minnows and carps) may have less aerobic (red) muscle than salmonids (Bainbridge 1960; Bainbridge 1962), downstream displacement of larger minnows and suckers might be anticipated with a pulsed flow. Indeed, Sacramento suckers (*Catostomus occidentalis*) fitted with radio transmitters were displaced a mean of two kilometers (km) downstream after a flow pulse in the Mokelumne River during 2003 (Jeffres et al. 2006). However, in general, suckers swam upstream during pulses and downstream during flow reductions, perhaps in an attempt at upstream spawning migration behavior.

### **1.1.2. Lateral Displacement**

In rivers regulated for electrical power generation, sediment removal, or for recreational rafting, the flow of water may be increased for several hours. During this period, the water level rises and may form side channels. The cessation of water release can result in a rapid lowering of the water level as the river returns to its normal river channel (Cushman 1985; Hunter 1992). Stranding in shallow side channels has been observed in field studies (Maciolek and Needham 1952; Hamilton and Buell 1976; Bauersfeld 1977, 1978; Wooden 1984; Hvidsten 1985; Olson 1986; Olson and Mezgar 1987; Higgins and Bradford 1996) and laboratory investigations (Bradford 1997; Bradford et al. 1995; Monk 1989).

Laboratory investigations have provided insight to the impact of pulsed flows because potentially critical factors were varied individually to assess effects on fish behavior and identify problematic factors. For example, Bradford et al. (1995) identified stranding of juvenile coho salmon and rainbow trout on river bars caused by rapid decreases in river flow in an artificial stream channel under winter conditions. Many fish became stranded because they concealed themselves in the interstitial areas in the gravel substrate and were reluctant to leave when water levels receded. Coho salmon were more likely to be stranded than the rainbow trout. Also, juveniles of other species of salmonids responded

to manufactured flows in the Sultan River, Washington, with differing susceptibility to stranding at different times of day or night (Olson and Metzgar 1987).

### **1.1.3. Year 1 Laboratory and Field Experiments**

The research team conducted both experimental and field studies to assess the impact of these flows on four species of fishes that inhabit California Rivers. Radio-tagged rainbow trout and brown trout (*Salmo trutta*) were tracked during a single pulsed flow. No significant differences were found between the distances moved prior to, during, and after the release. Fish numbers were recorded in pools along this reach during snorkel surveys before and after the pulsed flow. The total fish density in each pool did not appear to differ before or after the pulse. The research team recorded the responses of juvenile rainbow trout, hardhead, and Sacramento suckers to artificially pulsed flows within a longitudinal flume. Although fish moved either upstream or downstream, the most common (or mean) position of the individuals was close to the center of the flume during pulsed flows. The distribution of individuals was also determined in a lateral displacement flume, consisting of a rectangular tank separated into a main channel that never drained and a sloped bank that alternately flooded and became exposed. Only three (7.8%) of the 38 fish placed within the apparatus became stranded. The field and laboratory studies described in the report provide an evaluation of the impacts of pulsed flows for recreational and commercial purposes on the behavior and movements of sub-adults and adults of these species of fishes.

More tracking of rainbow trout both in the presence and absence of pulsed flows within the Chili Bar Reach of the South Fork American River was needed. The research team angled for rainbow trout for a total of nine days during Year 1, totaling approximately 23 person-days. With this amount of effort, only three rainbow trout were caught of suitable size and condition for tagging. For this reason, the research team concluded that it was infeasible to track wild rainbow trout from the Chili Bar Reach of the main stem of the South Fork of American River. As an alternative, the team tagged and released rainbow trout raised at the local trout hatchery into the American River during Year 2.

### **1.1.4. Electromyogram Telemetry**

It was necessary to use both laboratory and field methods to determine the energetic costs of exposure to pulsed flow of various magnitudes elucidated via EMG sensors. After determining the relationships of swimming velocity, tail beat frequencies and oxygen consumption (metabolic) rate in a Brett-type swimming respirometer. It was then possible to estimate the costs (energy, food, or oxygen-based) associated with a flow pulse by measuring the tail beats, using special radio tags, over a flow pulse in the river. The field-based tail beat counts gave accurate estimates of stream-habitat energetic costs because the fish's behavior in a real stream (e.g., including the potential uses of hydraulic cover structures) is incorporated into the measurements.

Electromyograms (EMG) are the electric potentials, or voltage changes in musculature, which are roughly proportional to the extent and duration of muscular exertion (Sullivan et al. 1963). Aerobic metabolism within the muscles involved in muscular

tension govern the oxygen demand at any particular temperature. Thus, it is likely that EMG generated by each myomere will be closely correlated with oxygen consumption, and so will the activity of a whole segment of myomeres (Weatherley et al. 1982). The main swimming muscles in ectothermic species of fishes, which are fusiform in shape and do not actively elevate their body temperature, are in a band stretching along the length of the fish, which consist of bilaterally symmetrical series of upper and lower myomeres (Bone 1978). As a result, the EMGs recorded by electrodes embedded in the axial aerobic muscles of trout can be used to estimate swimming speed, and then correlated with tail-beat frequency, swimming speed, or oxygen consumption in laboratory tests.

A comprehensive review of this technique is given in Cooke et al. (2004). EMG telemetry has been used to determine the energetic cost of migration (Hinch 2003; Standen 2002), the activity exhibited during pulses of flow in a regulated river (Murchie and Smokorowski 2004; Geist et al. 2005), passage through potential barriers such as weirs, dams, and rapids (Hinch et al. 1996; Quintella et al. 2004), and to identify spawning activity (Brown et al. 2006). Hence, we chose this method to determine the energetic expenditure of trout in the presence of pulsed releases of water in the South Fork American River.

## **1.2. Temperature Preference**

Many factors influence the life history of stream fishes. In California's Sierra Nevada streams Moyle and Nichols (1973) and Moyle (2002) described elevation-related zones with different environmental properties that had characteristic assemblages of native fishes. Cech et al. (1990) examined temperature and dissolved oxygen, two properties that structure these elevation-related communities. These authors measured the oxygen consumption (energy turnover or metabolic) rate responses to several temperatures and dissolved oxygen levels in seven species, from the various zones described by Moyle and Nichols (1973). Together these data described optimal (fish showing metabolic homeostasis), sub-optimal (hypometabolic responses) or uninhabitable (mortality) stream conditions, based on these abiotic variables. While these "physiological limits" studies have value in describing boundaries on fishes' distributions, they do not consider biotic variables (e.g., predation, competition, parasitism) or the behavior of the fishes in question (Cech et al. 1990). Jobling's review (1981) showed that when given a choice, fishes select temperatures that are optimal for growth. Naturally, streams temperatures are influenced by flows (including those of reservoir releases in regulated systems), solar radiation (including influences of riparian cover), inputs from tributary streams and from springs, and air temperatures. Thus, in managing stream fish communities, the temperature preferences of fish should be considered (e.g., as the frequency distribution for temperatures occupied over time reviewed by (Coutant 1987), along with their physiological thermal limits.

Our model fishes, hardhead and rainbow trout, are both native species found in many California streams. Hardhead are omnivores capable of reaching 1 m TL. They are found

characteristically in deep clear pools and in runs with sand-gravel-boulder substrates in streams and rivers (Moyle 2002). Rainbow trout are sympatric with hardhead (e.g., in the American River, California, watershed) in 10-15° C water (Cooper 1983). Rainbow trout are also regularly planted by state-run hatcheries and are the most wide-spread fish in the state.

Traditional thermal preference apparatuses for fishes have significant drawbacks that limit their usefulness in studies of active or moderately active fishes. For example, the inherent presence of confounding variables such as chamber depth, fish-perceived cover, shifting thermoclines, or variations in light intensity can influence the results of behavioral preference experiments. Mobile fish also can move in and out of the modal preferred temperature in non-annular chambers. The annular chamber for aquatic animal preference studies conceived in Myrick et al. (2004) overcame these drawbacks. This design presented a non-stratified thermal gradient to the fish, via cross-channel flows of chilled, ambient, and heated water. Constantly moving water maintained the bimodal gradient and prevented vertical stratification. The annular design limited the possibility of the fish becoming distracted or lost from the influence of the thermal gradient, as in nature (Reynolds 1977).

### **1.2.1. Project Objectives**

The overall aim of this research was to identify the effects of pulsed flows on fishes. The research team conducted field and experimental investigations on rainbow trout and hardhead. There were three objectives to the study during 2005–2006:

1. Track the movements of radio-tagged adult trout (TL  $\geq$  25 cm) in the river during summer and early fall season pulsed releases to determine whether fish were displaced longitudinally.
2. Estimate the energetic costs of fish that experience pulsed flows by conducting laboratory calibrations and tracking adult trout (TL  $\geq$  25 cm) carrying EMG sensors during pulsed flows.
3. Investigate thermal preferences of adult rainbow trout and hardhead utilizing a large (3-m diameter) laboratory annular thermal preference apparatus.

### **1.3. Report Organization**

Section 2 describes the methods used in the study. Section 3 presents the results of Year 2 of the study. Finally, Section 4 discusses the results in the context of the results of prior studies, offering conclusions and recommendations. Throughout the report, the field studies will be described first and the laboratory studies second.



## 2.0 Project Approach

### 2.1. Study Site

Field studies of the effect of pulsed flows on trout were carried out in the South Fork American River (Figure 1). Of the 20 rainbow trout tracked; half were released at Henningsen-Lotus County Park (river mile: 8, river km: 12.9) and half at Mariah Wilderness Expeditions camp site (river mile: 10, river km: 16.1) on the South Fork of the American River. These fish were subsequently tracked from Marshall Gold Discovery State Historic Park to Gorilla Rock, upstream of Fowler's Rock Rapid. This 12.9 km reach of the river is characterized by strong pulsed flows and is a popular destination for whitewater rafting.

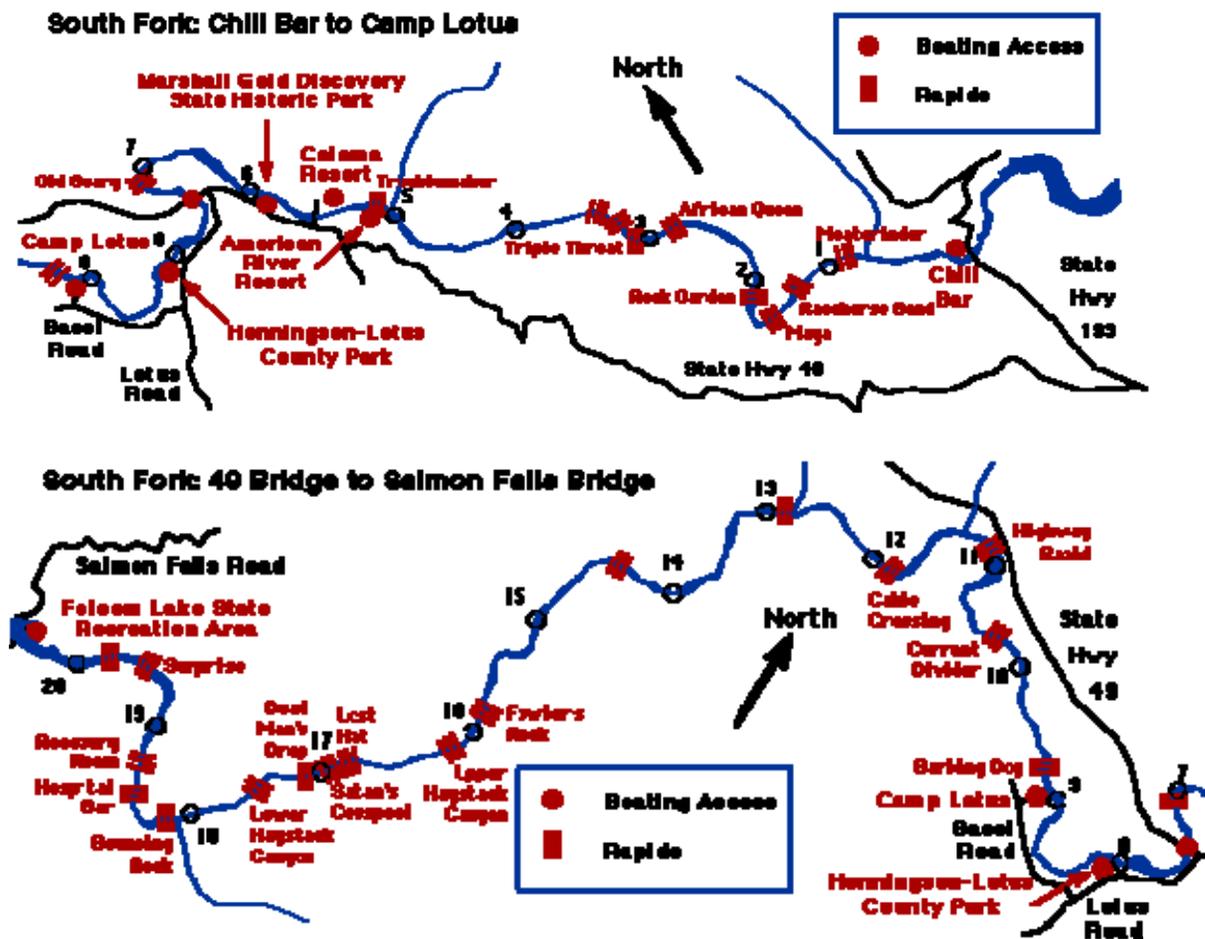
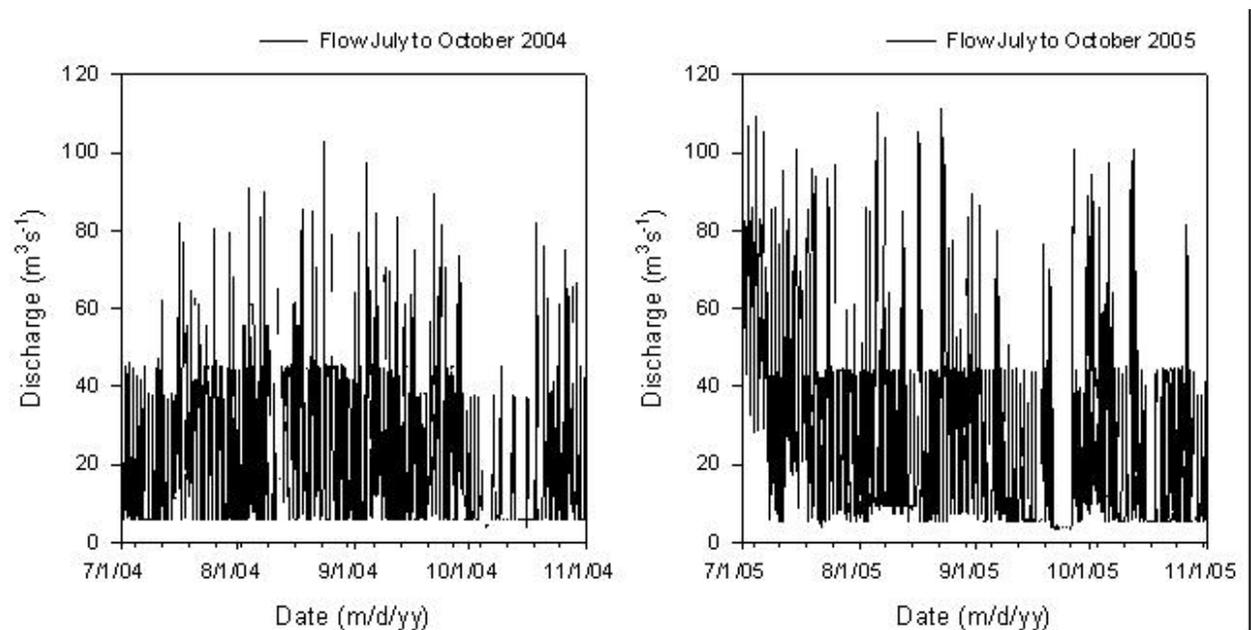


Figure 1. Shown are the upper (a) and lower (b) reaches of the South Fork of the American River.

Water is released at the Chili Bar Dam into the South Fork by Pacific Gas and Electric Company (PG&E). Based on an agreement with the whitewater rafting community, PG&E releases daily pulses of water into this reach of the river from May to September. The rate of water discharge from Chili Bar Dam is  $5.0 \text{ m}^3\text{s}^{-1}$  ( $176 \text{ ft}^3\text{s}^{-1}$ ). This rate is increased periodically during daytime to  $35.0 \text{ m}^3\text{s}^{-1}$  ( $1200 \text{ ft}^3\text{s}^{-1}$ ) on Tuesdays through Fridays; the rate of discharge is increased even further on Saturdays and Sundays, often exceeding  $40.0 \text{ m}^3\text{s}^{-1}$  ( $1400 \text{ ft}^3\text{s}^{-1}$ ). There is no agreement regarding the release of water on Mondays, and PG&E releases an amount of water into the river that meets the current hydropower demand.

Due to the large amount of rain during the winter of 2004 through 2005 the peak flows remained elevated ( $80 \text{ m}^3\text{s}^{-1}$  [ $2800 \text{ ft}^3\text{s}^{-1}$ ] and above) through the months of May, June and into July (Figure 2). The schedule of flows described above did not take effect until a sufficient amount of water is released from the upstream reservoirs. Photographic examples of the river at baseline and peak flow conditions are shown in Figure 3.



**Figure 2. The comparative rate of water discharge from Chili Bar Dam into the South Fork by PG&E during the 2004 and 2005 radio tracking surveys. Spring flows in 2005 remained elevated through June and into July due to the increased rainfall during the winter.**



**Figure 3. Photographs of the same reach of the South Fork (Camp Lotus): left taken in the absence of a pulse (baseline), right taken during the pulsed flow. Baseline flow was  $7.8 \text{ m}^3\text{s}^{-1}$  ( $275 \text{ ft}^3\text{s}^{-1}$ ), and peak flow was  $\sim 65.1 \text{ m}^3\text{s}^{-1}$  ( $2300 \text{ ft}^3\text{s}^{-1}$ ). Note how much of the riverbed is exposed with different flow magnitudes.**

## **2.2. Fish Collection and Maintenance**

Rainbow trout (mean TL: 25.4 cm) were obtained from the American River Trout Hatchery. The hatchery is run by the California Department of Fish and Game and located in Rancho Cordova. Fish were loaded into a large fiberglass transport tank filled with water from the hatchery (Figure 4). Oxygen was supplied continuously to the tanks via an oxygen cylinder connected to tubing and gas-diffusing stones within the transport tank. Fish were transported back to the Center for Aquatic Biology and Aquaculture (CABA) on the University of California, Davis campus, a ca. 40-min trip. The research team then transferred the trout to 550-l flow-through tanks at the appropriate temperature. The research team received two groups of rainbow trout, one group in mid-February 2005 and the other group in late June 2005. The first group was raised to a size capable of carrying larger radio and EMG tags.

Fish underwent a treatment regime of 200 ppm formalin static bath for 1 h followed with one to two days between treatments of 100 ppm oxytetracycline HCL (Terramycin 343 [Pfizer]) static bath for at least 2 h on three consecutive days. This treatment regime was outlined by William Cox (Fish Health Coordinator, Department of Fish and Game) for trout being released into the wild to minimize transfer of pathogens and comply with

U.S. Food and Drug Administration (FDA) regulations. One week after completing treatments the tank temperature was increased at a rate of 1°C per day until temperature reached ambient conditions (18°–19° C). Fish were fed formulated semi-moist pellets (Rangen, Inc., Buhl, Idaho) daily.



**Figure 4. American River Trout Hatchery hatchery manager and UC Davis researcher counting rainbow trout into the transport tank.**

The research team decided to use hatchery trout in our studies instead of wild rainbow trout based on the previous year's experience attempting to conduct studies on wild caught fish in the same stretch of river. We angled for rainbow trout for a total of nine days during Year 1, repeatedly fishing in the area between the Marshall Gold Discovery Site (River Mile 6) and Gorilla Rock (River Mile 15). Thus we fished for a total of about 23 person-days. With this amount of effort we caught ten rainbow trout. Of which only three rainbow trout were of suitable size and condition for tagging. For this reason, we decided to tag and release rainbow trout raised at the American River trout hatchery into the American River. It is of value to identify the effect of pulsed flows on hatchery-reared trout because they are released in the American River annually, and constitute the largest age class of trout within the river.

## **2.3. Radio Tracking**

### **2.3.1. Tag Description**

The radio tags utilized to track trout in the field were the smallest digitally encoded transmitters available for aquatic environments. A manual-tracking receiver (Lotek Wireless, Inc., SRX400) recognized beacons transmitting pulses of the same frequency by their unique digital codes. The use of a single frequency decreased the chance of observers moving out of range of a fish carrying a beacon of a particular frequency while scanning through other frequencies.

Two sizes of beacons were available for implantation in sub-adult and adult trout:

1. NTC-4-2L, 2.1 g in air, 8.3 mm diameter by 18.3 mm long, with a life span of 93 d and a pulse burst every 5 s.
2. NTC-6-2, 4.5 g in air, 9.1 mm diameter by 30.1 mm long, with a life span of 126 d and a pulse burst every 2 s.

For a given fish the research team chose one of the two tag sizes using the 2% rule of body mass as a guideline (Jepsen et al. 2002). The smaller tags were placed in the peritoneum of fish with a body mass between 105–500 g (25.5 – 31.0 cm SL) and larger tags placed in fish with a body mass of  $\geq 500$  g (32.0 – 38.5 cm SL).

### **2.3.2. Surgery**

The trout were transported from the outdoor fish housing tanks at CABA in 18.9-l buckets or a cooler depending on the size of fish. The research team implanted the tags using a surgery table placed on a small mobile cart. Individuals were transported a maximum distance of 50 m one way between the tanks and building.

The anesthetic solution was prepared in a 18.9-l, plastic bucket with 10 l of water, 27 g of sodium bicarbonate, and 10 ml of glacial acetic acid to buffer the pH as outlined in Prince et al. (1995), and Peake (1998). The pH of the anesthetic solution was neutral (7.0). Each fish was placed into the bucket and anesthetized to Stage 4 anesthesia, characterized by loss of orientation and slowing of gill movement. The fish was then removed from the solution of anesthetic, measured for weight and length, and its health assessed based on the fish's external body condition. The fish was then placed on the surgical table, ventral side up (Figure 5). The anesthetic solution was continuously passed over the gills with tubing connected to a recirculating, sump pump (Figure 6).



**Figure 5. Radio tag being inserted into the trout's body cavity through the incision site.**



**Figure 6. Fish awaiting sutures on the surgical table with an anesthetic solution circulating through the mouth and across the gills.**

An incision, 1–2 cm long, was made posterior to the rib cage and anterior to the pelvic girdle, off the fish's midline. To avoid damaging internal organs, a sheathed plastic 16 gauge (Ga.) catheter was used to puncture the body wall, creating an exit point for the whip antenna. The whip antenna was threaded through the plastic catheter sheath and fish's body wall until reaching the site where the antenna attaches to the cylindrical tag. The tag was then gently pushed through the incision into fish's body cavity. Three to four sutures were made along the incision site with synthetic, absorbable suture material (Vicryl™). Each suture was dotted with liquid topical tissue adhesive (Nexaband) to secure the knots. The fish was gently placed into the recovery tank after surgery and monitored for ventilation and tail beat frequencies, orientation, and swimming ability (Figure 7).



**Figure 7. Rainbow trout in the post-surgery recovery tank. Note the radio tag antenna wires trailing behind the fish.**

### **2.3.3. Releasing Fish**

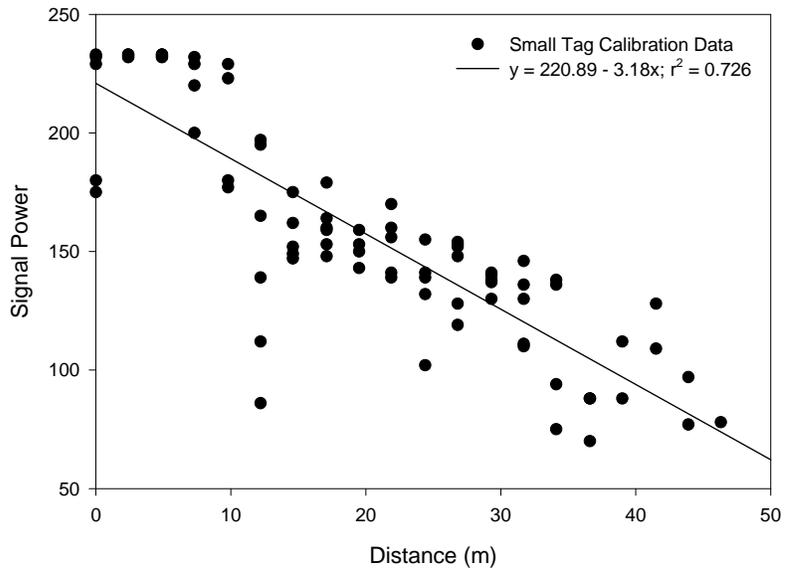
Fish were released into the South Fork American River approximately one-week post tag implant surgery. Radio-tagged fish were transported using the same methods as described in the Fish Collection section. Fish standard length, weight, and release date are given in Table 1. Transport and release of the radio tagged fish occurred during the late afternoon and evening for several reasons: (1) to avoid the peak summer temperatures during fish transport, (2) to avoid releasing fish during peak daytime use by recreational anglers, and (3) to allow fish to acclimate to the river as long as possible during non-pulsed or baseline flow levels.

**Table 1. Characteristics of fish implanted with radio tags and released into the South Fork American River. The table includes: length and weight (mean  $\pm$  SE), date tagged, date released, and tag type.**

<b>Release Group</b>	<b>Date Released</b>	<b>N</b>	<b>SL (cm)</b>	<b>Weight (g)</b>	<b>Tag Implanted</b>	<b>Dates Tagged</b>
1	Jul 7 05	10	35.7 $\pm$ 0.7	1088 $\pm$ 49	NTC-6-2	Jun 24 05
Large						Jun 30 05
2	Aug 12 05	10	28.8 $\pm$ 0.6	409 $\pm$ 29	NTC-4-2L	Aug 4 05
Small						

Each set of radio-tagged fish was split into two groups of five and released at two different sites (Henningesen-Lotus County Park or Mariah Wilderness Expeditions) to (1) avoid introducing fish in high densities, thereby minimizing displacement of individuals already present, and (2) separate tags with the same frequencies in order to minimize tag frequency collisions while tracking.

Fish were transferred from the transport tank into coolers or buckets and carried to the river. The cooler was then filled with river water and fish were allowed to swim away out of their own volition. River temperature and GPS locations were recorded. While fish acclimated to the river the calibrated distance from the tagged fish and signal power with the receiver and antenna was determined. Calibrations were used to determine the fishes' position while tracking in the river and also to estimate telemetry error. A regression based on the calibrations was calculated for the smaller radio tags (NTC-4-2L) (Figure 8). Unfortunately, due to time constraints the large radio tags (NTC-6-2) were not calibrated. However, based on their experience with the tags, especially when fish were visually observed while tracking, the authors estimated their calibrations by averaging the small radio and EMG tag calibrations. The maximum signal power for the tags is 250. The highest signal power we recorded while calibrating and tracking fish was 233.



**Figure 8. Calibration data and linear regression for fish carrying small radio tags. Data are from two fish, one at each release location on the South Fork of the American River.**

When releasing the larger group of radio-tagged trout there were some difficulties with the receiver settings, so the research team was able to identify the tag frequency of each fish that was released, and its body morphology, but not the individual identification code. Fish release site was extrapolated based on the tag frequencies and morphology of fish released at each site, location and frequency of the fish when tracked the following day (July 8, 2005) and the location, frequency, identification code and behavior of tagged fish tracked on July 14, 2005, and July 21, 2005. The research team was unable to determine the release location of RT5 and RT6 because their locations were between the two release sites, which means that they could have moved upstream from Mariah or downstream of Henningsen-Lotus. Accordingly, RT5 and RT6 movement data start at July 14, 2005, instead of July 7, 2005.

### 2.3.4. Positioning Fish

A Pelican™ case with foam fitting snugly around the equipment was secured into the back of the raft with cam straps. After losing the receiver on July 28, 2005 when the raft

high-sided, the case was retrofitted with a plexiglass window bolted to the case and sealed with silicon caulk to ensure that the equipment remained secure in the watertight case, while the receiver's screen could still be viewed by researchers (Figure 9).

The research team programmed the receiver to continuously scan between several frequencies at 5.5-s intervals (tag pulse interval + 0.5 s) and display the identification code and signal power of the tag if within range. The team floated downstream in the river, searching for the tagged fish by moving the antenna (3-element Yagi) back and forth between banks of the river (Figure 10).

Once a fish was detected by the receiver, the raft was rowed into an eddy or beached in order to determine the upstream location bearing of the tagged fish. It was difficult to accurately distinguish the direction where the strength of the signal was greatest. For that reason, the tracker stood in the same spot and rotated the vertically oriented antenna back and forth, recording each direction where the signal strength began to attenuate, and also recorded the best estimate of the maximum signal strength. The research team recorded at least two sets of bearings for each fish, one upstream and the other downstream. An attempt was made to locate the fish from both sides of the river when possible. Bearings were taken to the fish from points on the riverbank. Fish location was estimated by triangulation of lines of position based on the bearings recorded. If the upstream and downstream bearings seemed disparate or an exact fish position was not obtained, another fish location bearing was taken. A GPS location was recorded at each site for which bearings were determined.



**Figure 9. Telemetry equipment: receiver, gel cell battery, GPS, headphones, antenna, and datasheets. The receiver fit snugly into the foam lining of a Pelican™ case which was strapped to the oar frame of the raft.**



**Figure 10. (a) Research team floating the river and listening as the receiver scans continuously for radio-tagged fish. (b) Researchers taking and recording bearings with a compass to triangulate a fish's location.**



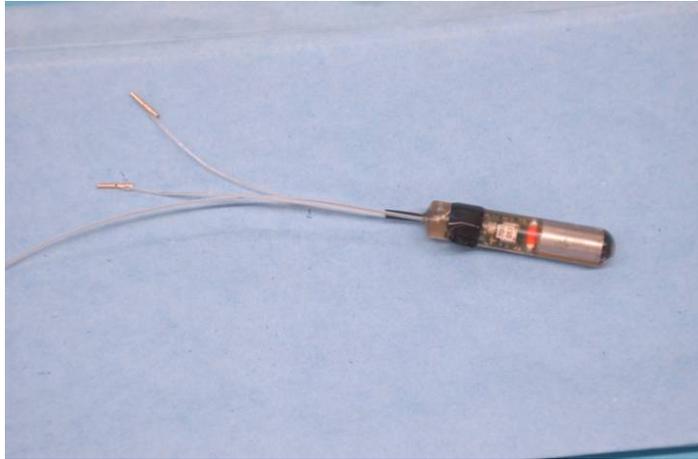
**Figure 11. Determining radio-tagged fish's river location and recording its location and habitat characteristics.**

In addition to the upstream and downstream bearings, fish location was also determined based on the tag's transmitted signal power as the research team was floating down the River. When the signal power for the tag was  $> 160$ , a GPS location was recorded, as well as signal power, gain, habitat (rapid, run, pool), and location (river right, left, or middle). Tag calibration regressions (see Figure 8) were combined with the GPS accuracy estimate to calculate the amount of error associated with each fish's exact GPS location point. Fish location was also marked on a map of the river, and a more specific map of the area where each fish was found was drawn (Figure 11).

## **2.4. Electromyogram Telemetry**

### **2.4.1. Tag Description**

The EMG radio tags by Lotek Wireless, Inc., are designed to detect the electrical voltage changes associated with muscle contraction and in that way researchers are able to measure changes in the muscle activity of free swimming fish. These transmitters have two Teflon-coated stainless steel electrodes attached to one end. Attached to each electrode is a gold plated sensor, used to hold the electrodes in the musculature permitting the detection of EMG signals (Hinch et al. 1996; Thorstadt et al. 2000) (Figure 12). The transmitters detect signals  $> 1-2$   $\mu\text{V}$  in amplitude, at a  $150$   $\mu\text{V}$  factory-ascertained threshold (Kaseloo et al. 1992). These signals are then processed based on a 1-s interval, and the tag transmits a muscle activity output based on a scale of 0–50 (50 being the maximum activity).



**Figure 12. Electromyogram (EMG) radio tag (CEMG-R11-18). Note the two gold plated electrodes and coated wire antenna protruding from the base of the tag.**

Two types and sizes, which were ordered at separate, of Coded EMG radio tags were implanted in the trout:

1. CEMG-R11-18, 10 g in air, 11 mm diameter by 54 mm long, with a life span of 56 d at 5 s continuous burst rate.
2. CEMG-R11-25, 12 g in air, 11 mm diameter by 62 mm long, with a life span of 40 d at 2 s continuous burst rate.

Between ordering each set of tags, Lotek Wireless, Inc. changed the design of their EMG tags due to a backorder in one of the key components and based on feedback given by previous researchers who had used the tags. Larger 12 g tags were ordered due to the delay as rainbow trout would be larger and capable of carrying heavier tags.

Additionally, after discussions with researchers (R. Brown and S. Cooke) with EMG experience, and based on their recommendations, we also adjusted the pulse burst rate to a shorter interval (2-s continuous burst rate). Using a shorter pulse rate interval increased our ability to capture the quick changes in movement and activity of tagged fish while tracking, better than the longer 5 s burst rate.

#### **2.4.2. Surgery**

One to two weeks before EMG-tag-implant surgeries, small groups of five to ten fish were transported in the CABA transport tank to the Academic Surge Building's room 1381 (Fish Physiology Laboratory) on the UC Davis campus. A sub-set of large adult rainbow trout were kept at this location to remain in close proximity to the Fish Physiology Laboratory's Brett-type respirometers for calibrations. Fish were maintained in the same manner as when held at CABA.

The EMG-tag-implant surgeries were conducted with a similar approach as the radio-tag-implant surgeries previously described. Trout were netted from their holding tanks and placed in 18.9-l buckets with water from their holding tanks and 27 g of sodium bicarbonate, and 10 ml of glacial acetic acid to buffer the pH as outlined in Prince et al.

(1995) and Peake (1998). Fish remained in the anesthetic solution for at least five minutes and removed when anesthetized to Stage 4 anesthesia; characterized by loss of orientation and slowing of gill movement. Length and weight were measured and the fish placed on the surgical table, ventral side up. The anesthetic solution was continuously passed over the gills with tubing connected to a recirculating sump pump.

Electrode placement affects the signals obtained from the transmitters; therefore, their location was standardized according to the suggestions of Beddow & McKinley (1999) in order to facilitate consistent comparisons of activity between individuals. An incision, 2-3 cm long, was made posterior to the rib cage and anterior the pelvic girdle, off the fish's midline; more specifically half way between the tip of the pectoral fins and origin of the pelvic fins. An effort was made to standardize the relative position of the incision. To avoid damaging internal organs, a sheathed plastic 16 Ga. catheter was used to puncture the body wall, creating an exit point for the whip antenna. The whip antenna was threaded through the plastic catheter sheath and fish's body wall until it reached the site where the antenna attaches to the cylindrical tag. The tag was then gently pushed through the incision into fish's body cavity and into the most anterior portion of the body cavity.

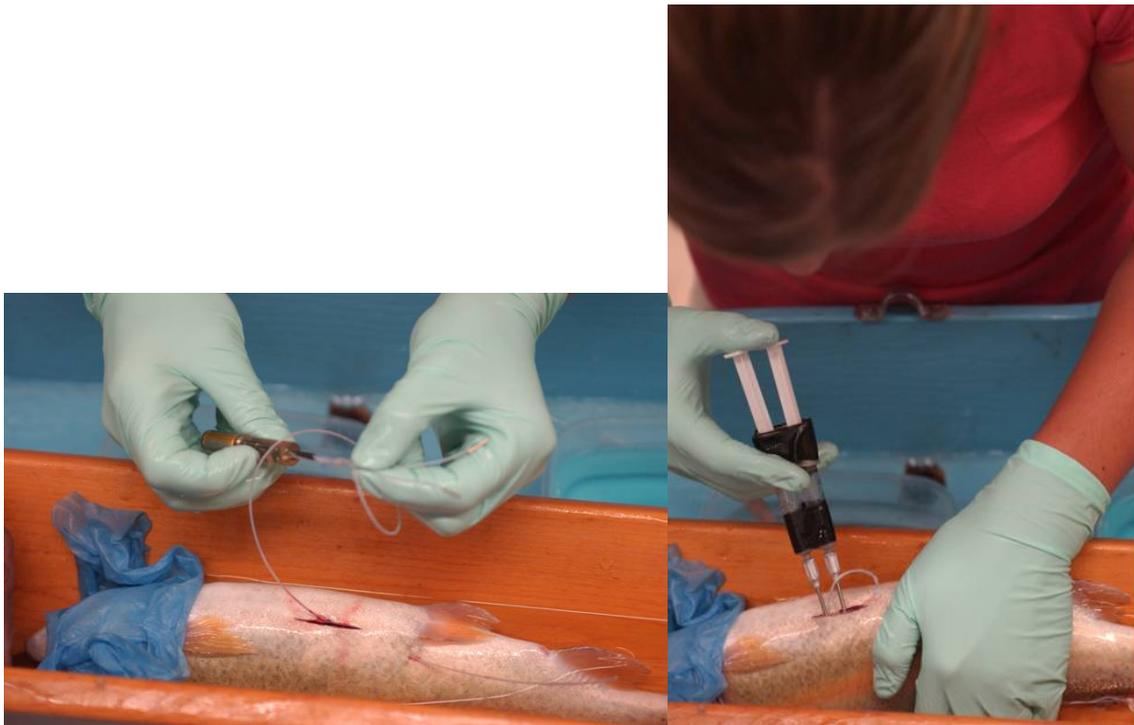
The gold-plated electrodes of the EMG tag were fitted into the grooves of a tool described in Bunt (1999). The EMG electrode wires were twisted to prevent wire movement and irritation to the fish. The implant tool was constructed of two, 3-ml syringes fixed together with surgical steel rods (20-Ga, 7.5 cm long) attached to the rubber plunger (Figure 13). The rods were designed to slide through 14-Ga hypodermic needles attached to the tips of the syringes with grooves routed into their tips large enough to hold the gold-tipped electrodes. The rods acted as a plunger to force the electrodes tips into the muscle.

Once the electrodes were placed into the tool, the tool was inserted into the incision site perpendicular to the fish's body (Figure 14). The surgeon guided the tool with the electrodes with one hand and felt the location of the tool and electrodes in the tissue with the other. When the electrodes were in the correct place (just ventral to the lateral line) the plunger was pressed to insert the gold plated electrodes. The electrodes tips were implanted into the red (aerobic) axial muscle. Electrodes were placed in the same relative location, halfway between the tips of the pectoral fins and origin of the pelvic fins.

Four sutures were made along the incision site with synthetic, absorbable suture material (Vicryl™). Each suture was dotted with liquid topical tissue adhesive (Nexaband) to secure the knots. The surgery took 8 to 15 min. The fish was gently placed into the recovery tank after surgery and monitored for gill ventilation rate, tail beat, orientation, and swimming ability. Fish were allowed 2 to 5 d to recover from surgery before starting laboratory calibrations.



**Figure 13. Electromyogram implant tool: gold-plated tag electrodes are placed into the grooves of two hypodermic needles and then forced out with 21-Ga steel rods attached to the syringe's rubber plunger.**



**Figure 14. (a) Twisting the probe wires before inserting the electromyogram tag into the body cavity; (b) Placing the electrode tips using the electrode implanting tool.**

### **2.4.3. Laboratory Calibrations**

To test whether carrying EMG radio tags affected the swimming abilities of the rainbow trout, the research team conducted incremental velocity tests on 10 fish of the same size without tags and 10 with tags. Critical swimming velocity ( $U_{crit}$ ) and the maximum sustained (aerobic) swimming speed (Hammer 1995) were compared between the two groups of rainbow trout (t-test, evaluated at  $\alpha < 0.05$ ). In addition, to verify that repeated

exposure to the swim tunnel would not alter swimming performance, we ran a subset (N = 6) of the untagged fish twice. We compared the two trials for each of the 6 fish's  $U_{crit}$  and tail beat frequencies (t-test, at  $\alpha < 0.05$ ).

Geist et. al (2002) found that the same EMG radio tag did not produce the same results, based on a calibration curve of the EMG electrical output at varying speeds for different fish. This suggests that results are more accurate when the same fish is calibrated and released, rather than applying calibrations from a different group to the fish that are released. However, the concern in using the same fish for calibrations and release is that it increases the amount of handling, thus increasing the chances that the electrodes will dislodge from the correct position in the muscle (Steven Cooke and Rich Brown, pers. comm.). Since 2002, however, Lotek Wireless has redesigned their EMG radio tags to output a number based on an algorithm of the tags' laboratory measurements and calculations, instead of transmitting a pulse every time a voltage threshold is reached.

The research team conducted a series of preliminary tests with the EMG radio tags. Depending on tag placement and fish health, groups of two to three fish were used. The research team investigated whether the EMG outputs varied between fish. EMGs were compared at each velocity for two different fish implanted with the same tag. Fish that were tested more than once in the swim tunnel were compared, using critical swimming velocity calibration and active metabolism tests, to investigate whether the EMGs varied over time. The team also attempted to test the effects of handling by calibrating fish both before and after a mock handling. Fish were loaded into the transport tank and driven for one hour, then returned to their tanks and swum again. However, in between the mock handling and second swim in the swim tunnel, the receiver was lost during a river survey, and the team was unable to test these fish immediately after handling.

#### **2.4.4. Critical Swimming Velocity and Active Metabolism Tests**

Critical swimming velocity ( $U_{crit}$ ) and active metabolic rates were examined using a 655-l, Brett-type recirculating swim tunnel located in the Department of Wildlife, Fish, and Conservation Biology's Fish Physiology Laboratory at the University of California, Davis (Figure 15). Experimental fish were carefully removed from their holding tanks using a bucket and net so that the fish remained in water during transport. Fish were anesthetized using carbon dioxide (0.55 ml glacial acetic acid/l  $H_2O$  and 1.35 g sodium bicarbonate/l  $H_2O$ ) to minimize the stress of handling and decrease the chance of dislodging the EMG electrode tips. After 5 min in the anesthetic bath, when stage 4 anesthesia was attained, the fish's weight and length were measured before it was placed within the chamber of the swim tunnel. Fish were manually revived until gill ventilatory movements became steady and the fish could maintain equilibrium.

Fish were allowed to acclimate in the chamber for 1 h (15 min at 5 cm/s, followed by an additional 45 min at 10 cm/s). The  $U_{crit}$  experiments began when flow was increased to 26.0 cm/s (approx. 0.75 body length/s, bl/s) and consisted of 30 min intervals with stepwise increases of 13.3 cm/s per interval, where 13.3 cm/s corresponds to 0.25 bl/s (Hammer 1995; Beamish 1978). During each interval, tail-beat frequency was recorded at

15, 20, and 25 min into each period. When EMG-tagged fish were used, the EMG signal was also manually logged for the duration of the experiment (Figure 16). An EMG reading was recorded every minute, and 30 readings were recorded while the fish was swimming routinely, maintaining position in the chamber with continuous, regular body undulations and tail beats. EMGs recorded during swim tests were used to calculate calibration curves, relating EMG and swimming velocity.

Beginning with the 4<sup>th</sup> time period, corresponding to velocity 65.9 cm/s, the chamber was sealed off by closing all the valves and turning off 4 submersible pumps, which supplied fresh water, and an water sample was taken at the beginning and end of each time period in order to determine the pO<sub>2</sub>. Since it is generally accepted that metabolic performance of fish decreases below approximately 70% air saturation, if the oxygen saturation in the chamber fell below 70%, the chamber was flushed with air-saturated water (Hammer 1995).

Fish underwent four to six velocity steps over two to three hours. The experiment ended when fish fatigued. Fatigue was defined as when the fish impinged against the back screen three times in one time period or if the fish continuously contacted the back screen for three minutes and would refuse to swim after the flow was stopped and started three times.  $U_{crit}$  was calculated according to the equation  $U_{crit} = V_p + ((t_f / t_i) * V_i)$ , where  $V_p$  is the penultimate velocity at which the fish swam before fatigue (cm/s),  $V_i$  is the velocity increment,  $t_f$  is the elapsed time from velocity increase to fatigue, and  $t_i$  is the total time of each velocity interval (Brett 1964). Active metabolic rate was calculated according to the equation  $MO_2 = [(CO_2(A) - CO_2(B)) * V] / T$ , where  $MO_2$  is O<sub>2</sub> consumption rate (mg O<sub>2</sub>/h),  $CO_2(A)$  is O<sub>2</sub> concentration in water (mg O<sub>2</sub>/h) at the start of the measurement period,  $CO_2(B)$  is O<sub>2</sub> concentration in water (mg O<sub>2</sub>/h) at the end of the measurement period,  $V$  is the volume of the respirometer (L), and  $T$  is the time elapsed during measurement period (Cech 1990).

In addition to  $U_{crit}$  tests, separate active metabolism tests were conducted for a subset of three fish per group of fish released. Active metabolisms tests were conducted similarly to the  $U_{crit}$  tests with a few differences. Time intervals were extended to 60 min to ensure a measurable drop in the pO<sub>2</sub>. In addition, pO<sub>2</sub> was measured at three velocities, 26.0, 39.3, and 52.6 cm/s, rather than ending with fatigue. The pO<sub>2</sub> was measured with an electrode and/or a radiometer. Thirty EMG readings were logged (one minute interval and during routine swimming) during the first 30 min of each velocity tested when possible. Active metabolic rate was calculated (Cech 1990). Active metabolism estimates during swim tests were used to calculate calibration curves relating EMG and swimming velocity as well as active metabolism and swimming velocity.

Temperature was decreased from 19°C to 16°C during the second set of EMG calibrations, using the CEMG-R11-25, 2-s, pulsing tags, because the temperature of the river had decreased as well. Temperature was decreased 1 °C per day, and fish were allowed to acclimate to the new temperature for two to five days before  $U_{crit}$  trials and at least one week before metabolism trials.  $U_{crit}$  tests were conducted at 19°C for two of the

fish before temperatures were decreased to compare  $U_{crit}$  and EMGs at the different temperatures.



**Figure 15. Rainbow trout swimming during a critical swimming velocity ( $U_{crit}$ ) test in Brett-type respirometer.**



**Figure 16. Researchers observing fish swimming during critical swimming velocity ( $U_{crit}$ ) tests. The swim chamber was covered by curtains and the fish was observed on a live feed from a video camera. The receiver was placed on top of the video monitor so that the fish and EMG output could be viewed simultaneously.**

### 2.4.5. Electromyogram Telemetry

The protocol for releasing was similar to the description above for releasing radio-tagged fish into the river. EMG-tagged fish were released on three different dates: August 26, September 27, and October 18, 2005 (Table 2). Fish were released at Henningsen-Lotus County Park or Camp Lotus, in the morning (0730 – 0800) before the pulsed flow began for the day. The research team conducted range tests for both types of tags (Figure 17), and tracking commenced as soon as the fish were released into the river.

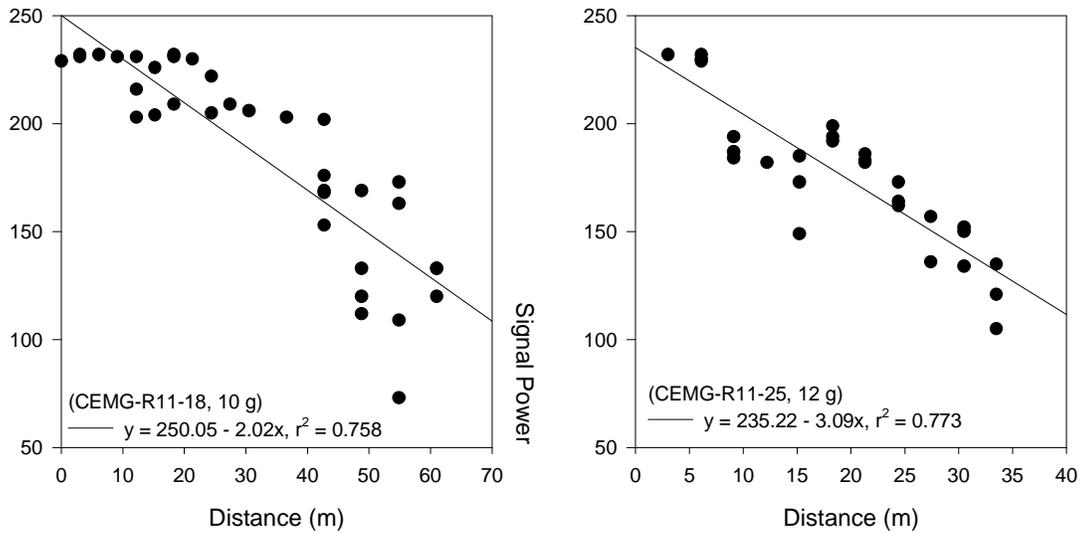
**Table 2. Characteristics of fish implanted with electromyogram radio tags and released into the South Fork American River. The table includes: length and weight (mean  $\pm$  SE), date tagged, date released, and tag type.**

Release Group	Date Released	N	SL (cm)	Weight (g)	Tag Implanted	Rate (sec)	Dates Tagged
1	Aug 26 05	4	33.9 $\pm$ 1.3	889 $\pm$ 127	CEMG-R11 18	5	Aug 15 05 Aug 16 05 Aug 19 05
2	Sep 27 05	1	39.4	1279	CEMG-R11-18	5	Sep 20 05
3	Oct 18 05	4	44.4 $\pm$ 0.9	1932 $\pm$ 44	CEMG-R11-25	2	Sep 28 05 Oct 3 05

A manual tracking receiver (SRX 400A or SRX 32, Lotek Wireless) was utilized to transmit the EMG output from the EMG radio tags. Sixty EMG outputs were recorded (approx. 10 min in length) every half hour throughout the day for each fish detected by receiver. In October, the research team was able to record EMGs using a continuous data logging receiver (SRX 600, Lotek Wireless) in addition to using the manual tracking receivers.

Fish location was determined once per hour based on the tag's transmitted signal power. When the signal power for the tag was  $> 180$  (based on tag calibration regressions, 180 was chosen to best determine position), a GPS location was recorded as well as signal power, gain, habitat (rapid, run, pool), and location (river right, left or middle). The research team remained in the same location as the fish they were tracking for that day's pulsed flow. Tag calibration regressions (Figure 17) were combined with the GPS accuracy estimate to calculate the amount of error associated with each fish's exact GPS location point.

EMGs and fish location were collected before the pulse (baseline), as the water rose, stabilized, and decreased (if possible) for a minimum of five hours, but usually for at least seven hours. The research team would follow one fish throughout an entire pulse. If more than one fish with an EMG transmitter was detected in the same location, those fish would be tracked as well. Fish were tracked during three separate pulsed flows, unless the research team was unable to locate the fish.



**Figure 17. Electromyogram tag calibration curves. The smaller 10 g, 5 s pulsing CEMG tag range tests (left), the larger 12 g, 2 s pulsing CEMG tags range test (right). Both range tests were conducted at the same location: Henningsen-Lotus County Park.**

## 2.5. Temperature Preference

### 2.5.1. Fish Collection

Beginning in January 2005, site surveys were conducted to identify a feasible collection site for hardhead (Figure 18). Survey locations were chosen based on those previously cited in the literature and by communications with other researchers. Due to the heavy rainfall during the end of 2004 and beginning of 2005, most rivers and creeks were excessively swollen or flooded when surveyed and, for that reason, infeasible to consider as collection sites until flows diminished. Sites surveyed were the Lower and Upper American River, South Fork American River above and below Chili Bar Dam, Cache Creek, Upper San Joaquin River, and the Kern River.



**Figure 18. Hardhead (*Mylopharodon conocephalus*) caught by rod and reel at Slab Creek Reservoir.**

At Slab Creek Reservoir, South Fork American River, the extra precipitation created a deep pool (approximately 10 m deep) at the head of the reservoir. During most of the year the area is a run or rapid at the head of the reservoir. The deep pool created conditions allowing adult hardhead to gather in significant groups, possibly readying for their spring upstream migration to spawn. This behavior made capture by rod and reel feasible. Hardhead (N=22) were captured between April 4, 2006, and June 6, 2006, using rod and reel techniques. Fish were transported in large, aerated coolers of river water at up to two fish per cooler, with up to two coolers per fishing trip. The coolers' temperatures were monitored and maintained throughout the fishing day by adding fresh river water, including just prior to the ca. 2-h vehicle transport to CABA. At CABA fish were transferred to an aerated 555-l tank held at 12°C, with continuous flows of well water, until experimental acclimations. Salmon roe (N=13 caught) and halved earth worms (N=10 caught) were found to be the most productive baits compared to using spinning tackle or fly fishing.

By-catch, at Slab Creek Reservoir consisted of 8 rainbow trout, 7 Sacramento pikeminnow and 24 Sacramento suckers. Interestingly all the pikeminnow were caught on salmon roe, the trout on worms and spinning tackle, and suckers were caught on all bait types. Together, along with hardhead, these large growing fish represent the native fish assemblage of the South Fork.

The experimental rainbow trout (N=41, ca. 15-mo-old "Coleman" strain) were obtained in April 2006 from the American River Hatchery (California Department of Fish and Game), where they were held in 11°C lower American River water. The trout were transported to CABA in a large, air-equilibrated aluminum transport tank and divided into two, aerated 555-l tanks (12°C) with continuous flows of well water, until experimental acclimations.

### **2.5.2. Fish Care and Feeding**

Hardhead were noted to eat crayfish at the reservoir, when fed crustaceans (local trapped crayfish, store-bought shrimp, and commercial krill) they showed little interest, except for live brine shrimp (*Artemia*). Live brine shrimp was supplemented for the first month at CABA, when several variations of commercial feed, *Daphnia*, freeze-dried worms, feeder fish, and earthworms, were fed to the hardheads. They responded very favorably to diced earth worms. Subsequently, due to the sporadic summer availability of brine shrimp, they were weaned to earthworms and to SilverCup™ 5 mm commercial pellets. At the time of the experiments, all the hardhead accepted earthworms, and several readily accepted the commercial pellets. Hardhead tanks were always covered with heavy landscape fabric that was firmly clamped down, as they are an exceptional jumping species. Rainbow trout were fed, ad libitum, SilverCup™ 3 and 5 mm commercial feed, daily. Temperatures of all the tanks were checked daily, and tanks were cleaned as necessary.

Upon arrival at CABA both hardhead and trout were treated with nitrofurazone (NFZ) (10 g/l) for 45 min, daily, over 10 d. This treatment was to prevent bacterial infection

from handling and transport, especially in hardhead, which developed hook-site infections. After the treatment period most fish showed no signs of external bacterial infection. Six hardhead died before the experiments, and one died after the experiments started. Of the seven hardhead that died three had external microscopic gyrodactylid flukes and three had severe internal tapeworm infestations. Although earthworms were not fed to the fish prior to the tapeworms being noted, they were not believed to be the source of the infection. Due to the short time in captivity before the observance of the flukes and tapeworms in the hardhead, the likely source was their collection site. Hardhead were treated for the ectoparasites with Chloramine-T (200 ppm) in a 4-h static bath three times from May 4 to July 10, 2006. To treat for tapeworms, the hardhead tanks were treated with 111 ml/555 l of 37% formaldehyde solution for 1 h. The rainbow trout required no NFZ treatments, and no trout died during the holding or acclimation periods.

### **2.5.3. Annular Experimental Chamber**

The temperature preference chamber was constructed of clear acrylic plastic and featured three concentric and perforated circular walls separating receiving/mixing chambers, swimming chamber, and effluents chamber within the 3-m diameter, solid outer first wall (Figure 19).

The chamber's base was acrylic, blacked out with window tinting, on an acrylic sub frame (3.05 m x 3.05 m by 2 cm thick), mounted on a painted steel frame with large, leveling feet to prevent any chamber movements or vibration during experiments (Figure 20). The area between the outermost two walls was divided into 8 adjoining chambers for receiving and mixing the incoming water. Water was distributed to the receiving/mixing chambers via PVC manifolds and ball valves from 16, constant-head 17-l reservoirs containing either chilled (11.5°C), ambient (18°C), or heated (24°C) water. Heated well water was directed to two gas equilibration columns, which prevented gas supersaturation, prior to its distribution into the receiving/mixing chambers. The 30-cm-wide swimming channel, between the second and third outermost walls, was kept at a constant, 15.2-cm water depth. The swimming channel presented an unobstructed, large-radius (2.4 m) path for the fish. To measure temperature, 64, YSI 400 series thermistors coupled to several model 46 TUC YSI Tele-thermometers or Fisher Traceable digital thermometers were positioned symmetrically around the swimming channel. As the water moved across the swimming channel, it passed into eight effluent chambers, directing the water to the chamber's center area and drain.

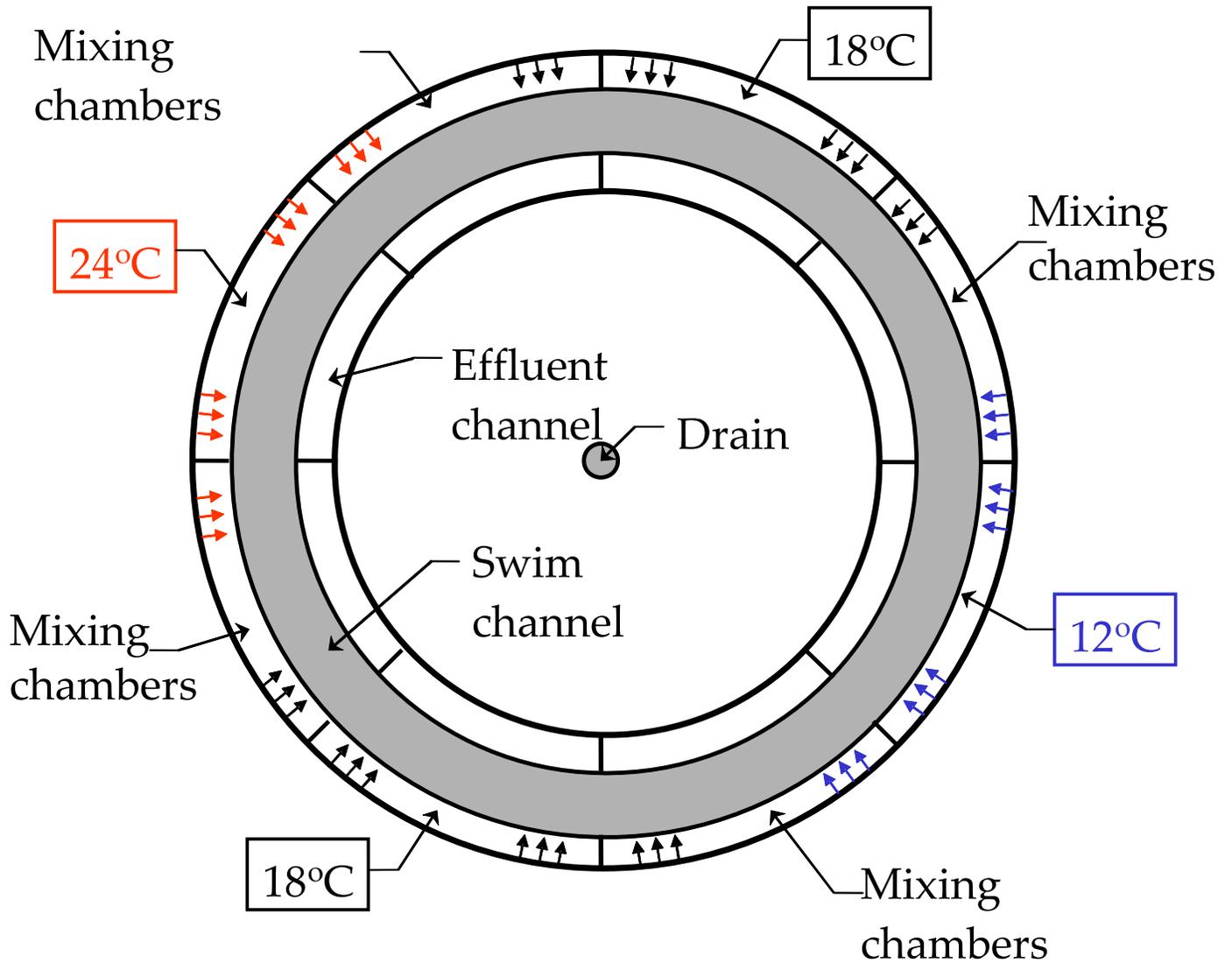


Figure 19. Top, diagrammatic view showing the temperature-preference apparatus' mixing chambers, swimming channel, effluents channel, and centered drain. The direction of incoming water temperatures is color-coded with each arrow representing a valve-manifold with 3 valves per manifold.

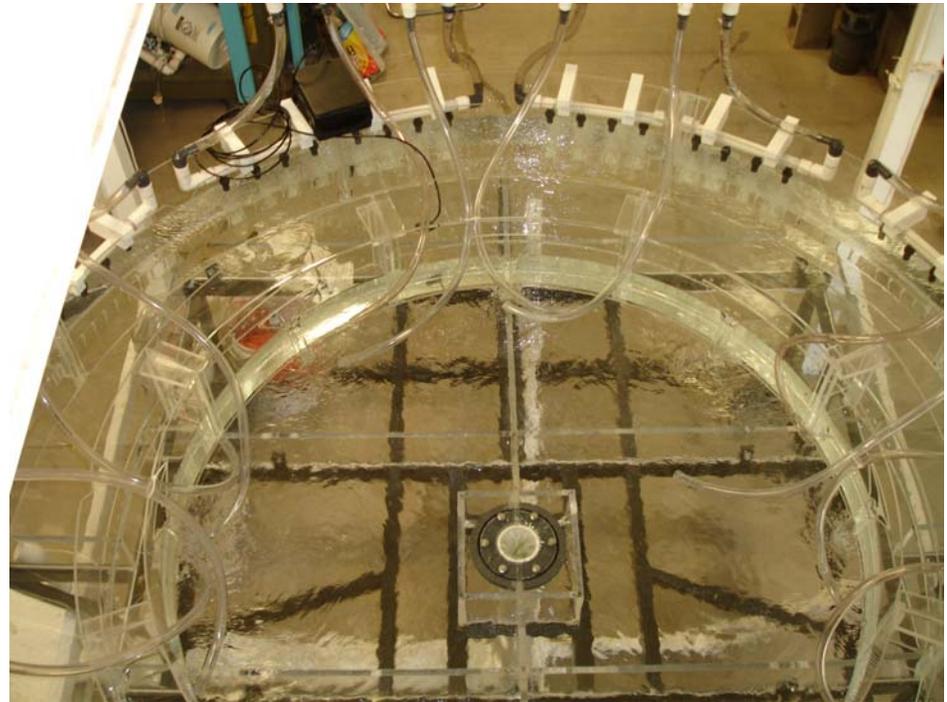


Figure 20. (a) Side view of temperature-preference apparatus and steel frame); (b) top view of same apparatus.

The chilling system consisted of two Heat Controllers, Inc. 15-horsepower heat exchangers. A 3800-l reservoir maintained a constant supply of cold water which was delivered to the mixing valves via a 1.75-horsepower pump. This system supplied a stable supply of 11.5°C water to the apparatus. Two Mobius (model: T-M1 Takagi) on-demand, tankless gas boilers plumbed in series heated the ambient well water. Heated water was directed to two gas equilibration columns. Two lift pumps (model: March Manufacturing Inc. 5C-MD), in a 50-gal reservoir, then supplied gas-equilibrated, heated water to the apparatus.

The heated and chilled supply lines, directly before the apparatus, were controlled by Belimo (model: LRB24-SR) and Honeywell (model: ML7984) mixing valves, respectively. The mixing valves were both controlled by separate Omron (model: E5AK) digital controllers that dampened water temperature oscillations. Mixing valves were fitted with an 18°C (ambient) line and either heated or chilled line, depending on the system. This allowed us to supply water of many temperatures within the 11.5°C to 24.0°C range, using the mixing valves. The system then had a range of authority for maintaining target temperatures. These systems proved very reliable and consistent in supplying water at the desired temperatures.

The distribution system consisted of several sub-systems to allow very fine water control to the apparatus (Figure 21). At the apparatus, the heated, chilled, and ambient lines were run to a valve manifold and a 760-l reservoir. The reservoir here, powered by a 0.5 horsepower water pump, allowed for mixing of any combination of the 3 source waters to get temperatures between 11.5° to 24°C for acclimations. The valve manifold allowed for selective water distribution control into the sixteen, 19-l reservoirs located above the apparatus.



**Figure 21. Water preparation and distribution systems.**

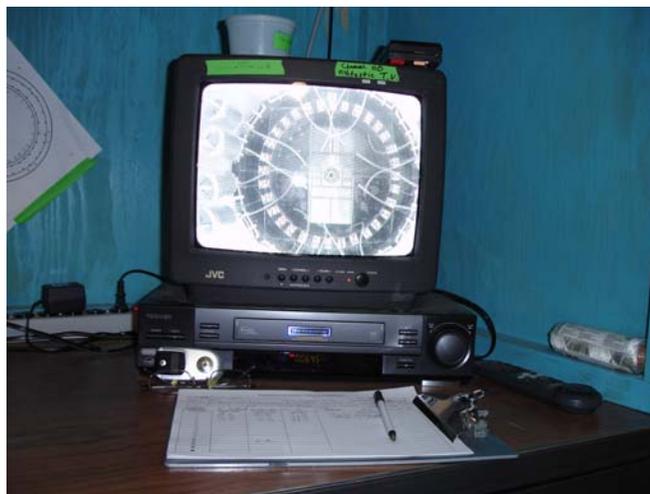
Overhead reservoirs were split into three groups by makeup water lines: ambient (8), heated (4), and chilled (4) reservoirs (see Figure 20 for position of reservoirs around apparatus). The

overhead reservoirs were equipped with an overflow standpipe to maintain constant head pressures and flows, and a submerged bulkhead fitting connected to valve manifolds in the apparatus' mixing chambers. Valve manifolds were placed symmetrically, with two in each of the apparatus' eight mixing chambers. Each reservoir was individually mounted to a pulley system to set the hydraulic head. During a 1-h experiment, approximately 17,000 l of water from the chilled, heated, and ambient systems was used.

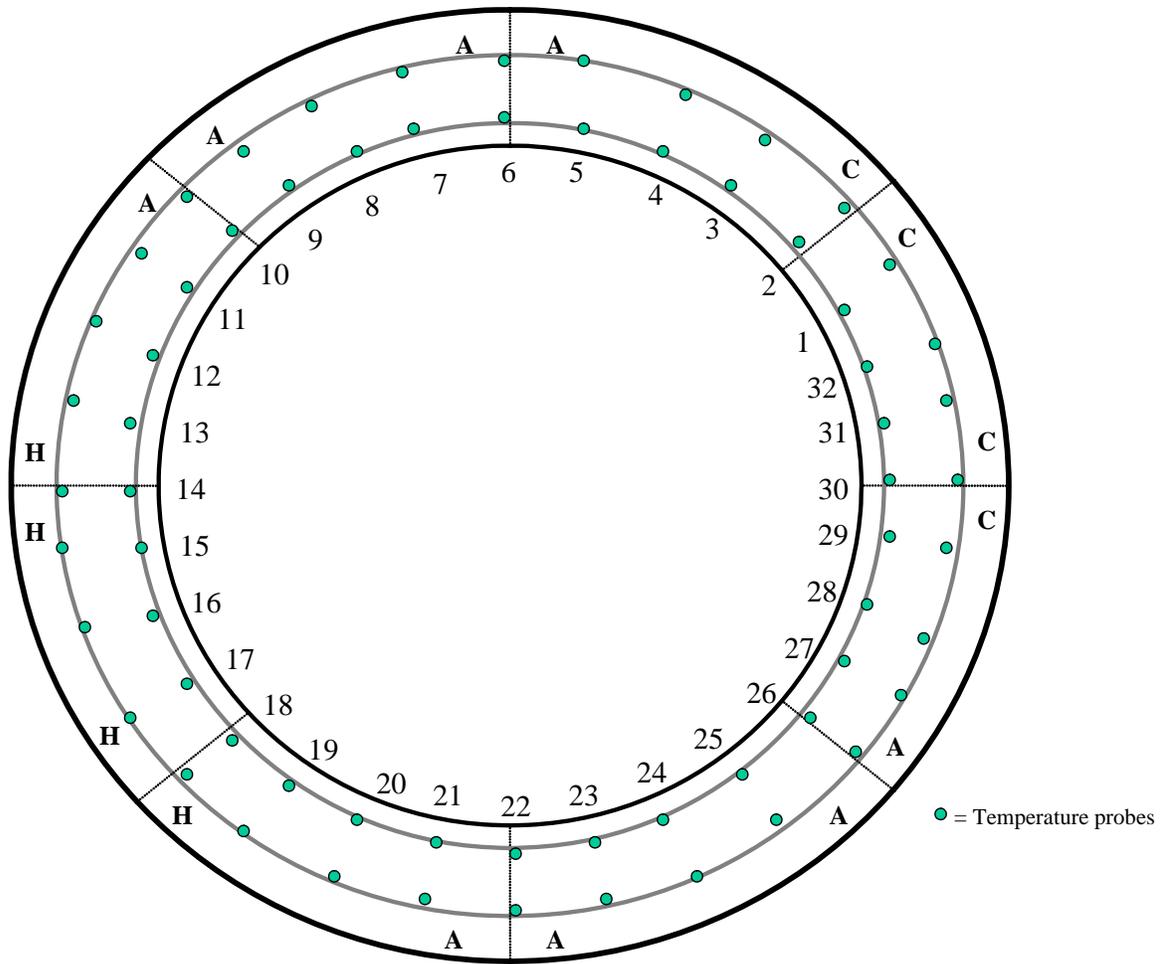
#### **2.5.4. Experimental Design and Statistical Analysis**

Non-organism tests were performed in the annular preference chamber, along with the fish tests. Three repeated non-organism tests confirmed the apparatus' ability to maintain a repeatable temperature gradient for the hour-long experiments. Due to limited availability, the same hardhead were used for the 12°, 15°, and 18°C acclimation groups. The hardhead were kept at 12°C for 5 months, 15°C for 22 d, and 18°C for 17 d prior to the start of the respective experiments. Trout were separated into three acclimation groups: 12°, 15°, and 18°C. The 12°C trout were held for 4 months at acclimation temperature prior to starting the group's experiments. The 15°C fish were held for 15 d, and the 18°C fish were held for 17 d at the acclimation temperature before starting the groups' experiments.

A remote camera coupled to a video monitor was used to observe the fish without disturbing their behavior, and the perimeter of the apparatus was covered in shade cloth (Figure 22). The apparatus was virtually segmented into 32 areas, each encompassing 11.25° arc of the swimming channel (Figure 23). Each fish was released into the apparatus at a randomly selected location after the total apparatus' temperature was stabilized at the fish's acclimation temperature. Fish were given 20 min at the acclimation temperature with 4 mixing chambers supplying water to the swimming channel. After acclimation, all three source waters (chilled, ambient, and heated) were supplied to the relevant mixing chambers, producing the temperature gradient for the 1-h experiment. Typically the temperature gradient was stabilized in < 5 min.



**Figure 22. Video monitor displaying the experimental subject within the 32 segmented areas of the temperature-preference apparatus.**



**Figure 23. Overhead view of the apparatus' temperature probe locations, also showing the virtual positions. The letters represent the type of water in each of the eight mixing receiving/chambers (A=ambient, C=cool, and H=heated).**

During the acclimation and experimental periods, fish snout locations and corresponding temperatures were recorded every 2 min. Fish could easily swim around the entire annular ring in < 15 s, minimizing possible space and time autocorrelations (Figure 24).

Descriptive statistics and a one-way ANOVA on preferred temperatures were performed using Sigmapstat 3.0™. Circular statistics and histograms of fish locations were completed using Oriana 2™ including mean vector ( $\mu$ ), length of mean vector ( $r$ ), concentration, Rayleigh Test ( $Z$ ), Rao's Spacing Test ( $U$ ), Watson's  $U^2$  Test (Uniform,  $U^2$ ), and the Kuiper's Test (Uniform,  $V$ ). Statistical significance was considered at  $\alpha < 0.05$ .



**Figure 24. Rainbow trout swimming around the annular ring during temperature-preference experiment.**



## 3.0 Project Outcomes

### 3.1. Field Studies

#### 3.1.1. Radio Tracking

The research team tracked fish with radio tags once a week on Thursdays from July 7, 2005, to October 13, 2005. River locations for Large and Small size groups during the tracking study are shown in Figures 25 and 26 (see also Tables 3 and 4). However, not all twenty fish were detected each tracking day. RT6, RT11, and RT14 were caught by anglers who sent the radio tags to the local Department of Fish and Game branch. It is probable that some of the other missing fish, which were not caught by anglers, moved upstream or downstream of the study reach, or lost due to predation (e.g. river otters). The missing fish may have also moved upstream of the upper end of the study reach or downstream of the lower end of the study reach. The day the receiver was lost (July 28) the only fish detected were those upstream of the location where we lost the receiver. On September 15, the river was not pulsed according to the regular schedule. Fish locations were determined based on where we could access the river on foot. If a fish was detected in the same approximate location as the previous week, that location was used. The research team was not able to locate RT12 and RT16 on September 15. The last three large tags were no longer detected after September 29. At this date the fish had been in the river 84 d, and the tags had been active 92 to 98 d. While 92 to 98 d is shorter than the tags estimated lifespan of 126 d, it is close enough to assume that the tags' batteries expired during the week of September 29 to October 3. This assumption is also supported by the fact that both tags became undetectable on the same date and they were in different locations within the river, far from either the upstream or downstream endpoints. Telemetry error varied for each individual depending on river location, how many fish were in the same area, and how close to the fish the research team was able to get (see Tables 3 and 4). Telemetry error was higher for large tags (range: 4 to 49 m) than small tags (range: 4 to 36 m).

The Large group was released into the river with a higher health assessment index value (mean  $\pm$  SE:  $14.1 \pm 0.6$ , range: 11 to 17) than the Small group (mean  $\pm$  SE:  $11.8 \pm 0.3$ , range: 11 to 13) ( $p = 0.003$ , t-test). Fish health was assessed using a cumulative index based on each fish's external characters, such as fins, scales, abrasions. The higher the health assessment index number the more damage was found to the fish. Whereas a health assessment value of 11 indicates that the fish was in good health with no damage, 58 is the maximum value indicating severe body damage. The majority of the damage recorded for the "Large" group was abrasions, or splitting or wearing of their caudal fin. This increased damage to the caudal fin is probably due to the lengthened time the "Large" group was held in captivity.

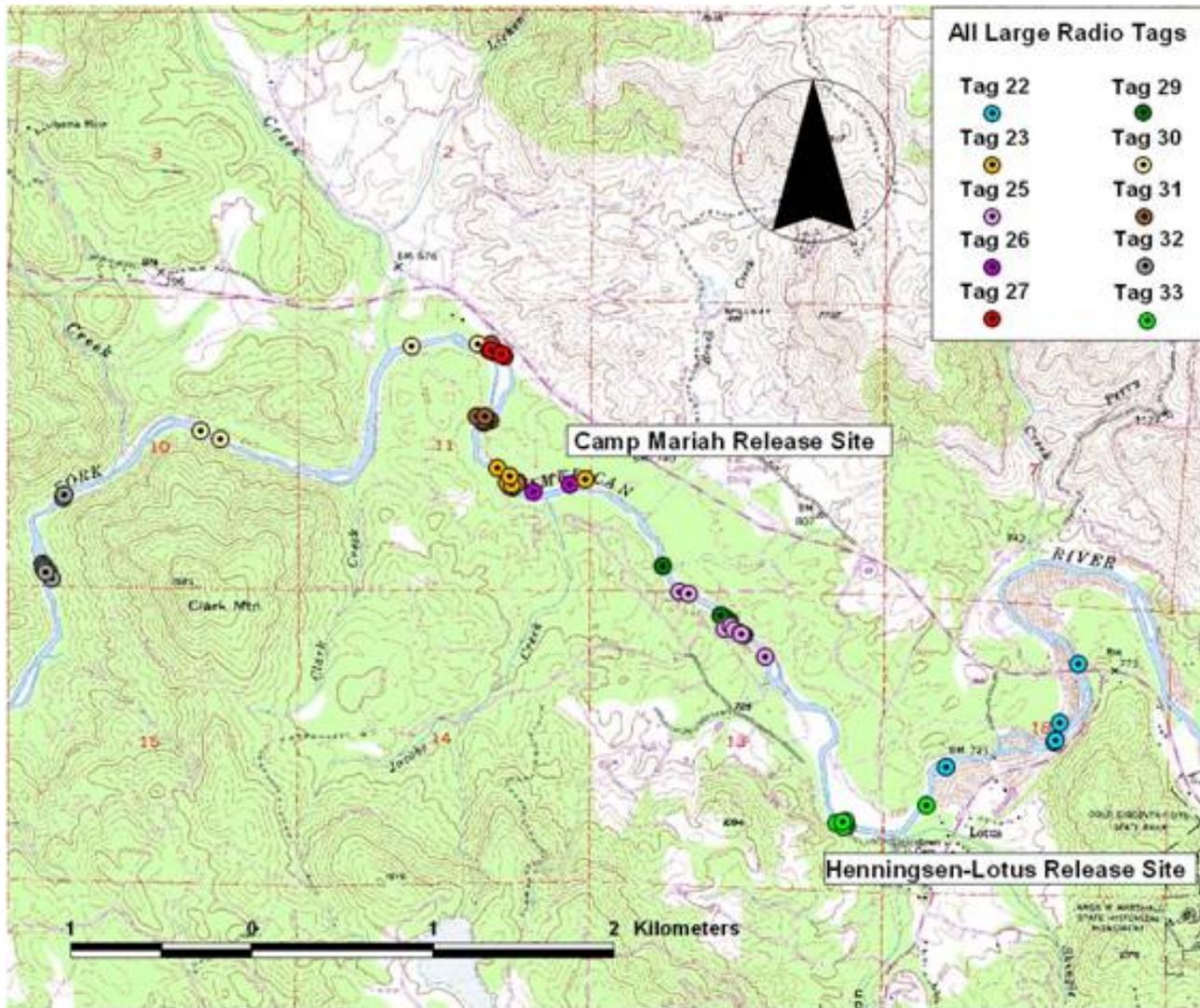


Figure 25. Arcview map of the locations of radio-tagged trout in the large-size group during tracking study. Trout are identified by tag code (e.g., Tag 22 is RT1). Flow moves downstream from right to left.

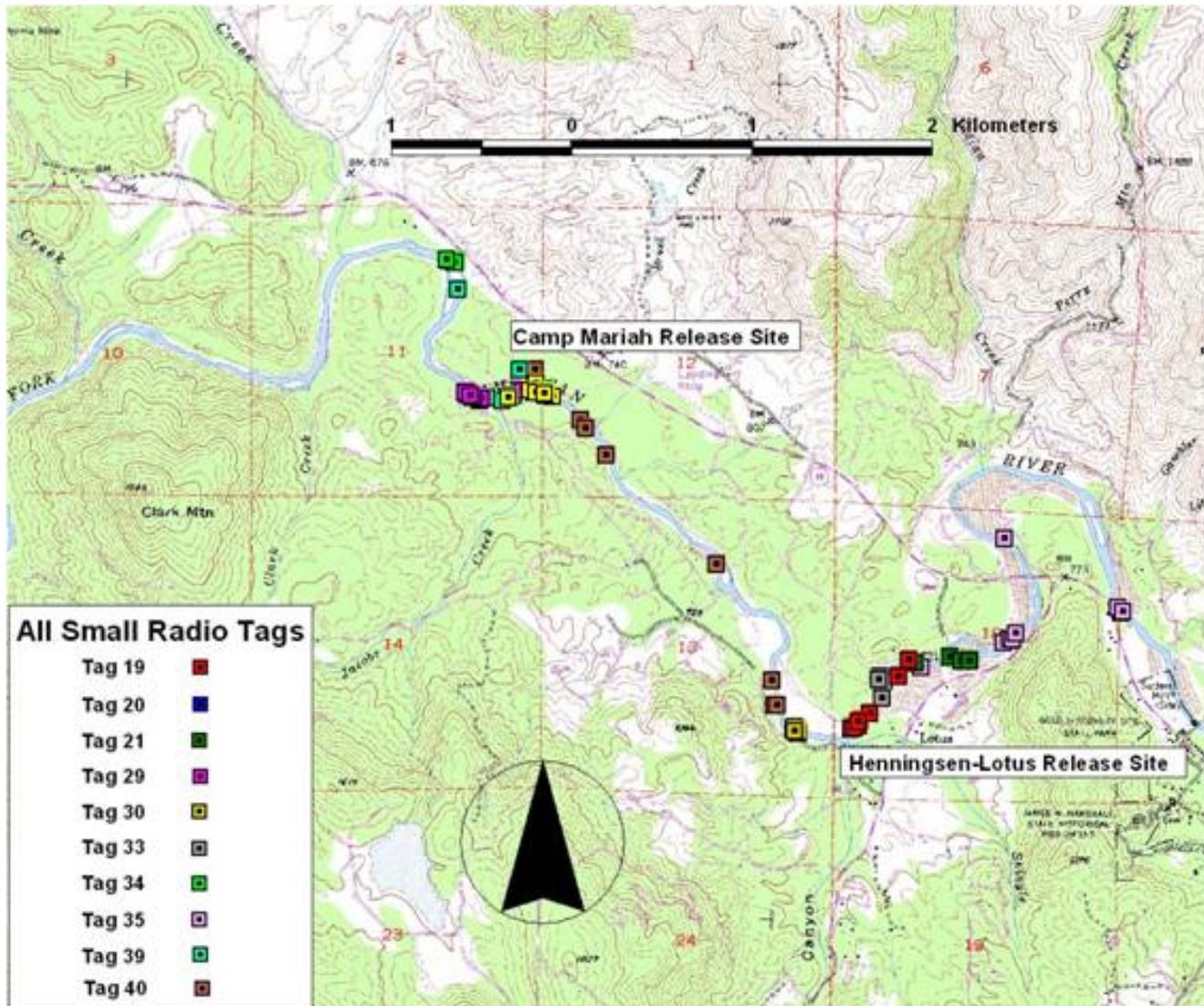


Figure 26. Arcview map of the locations of radio-tagged trout in the small-size group during tracking study. Trout are identified by tag code (e.g., Tag 19 is RT13). Flow moves downstream from right to left.

**Table 3. Identification numbers, tag codes, frequencies, telemetry errors, length, weight, and dates of detection for large rainbow trout.**

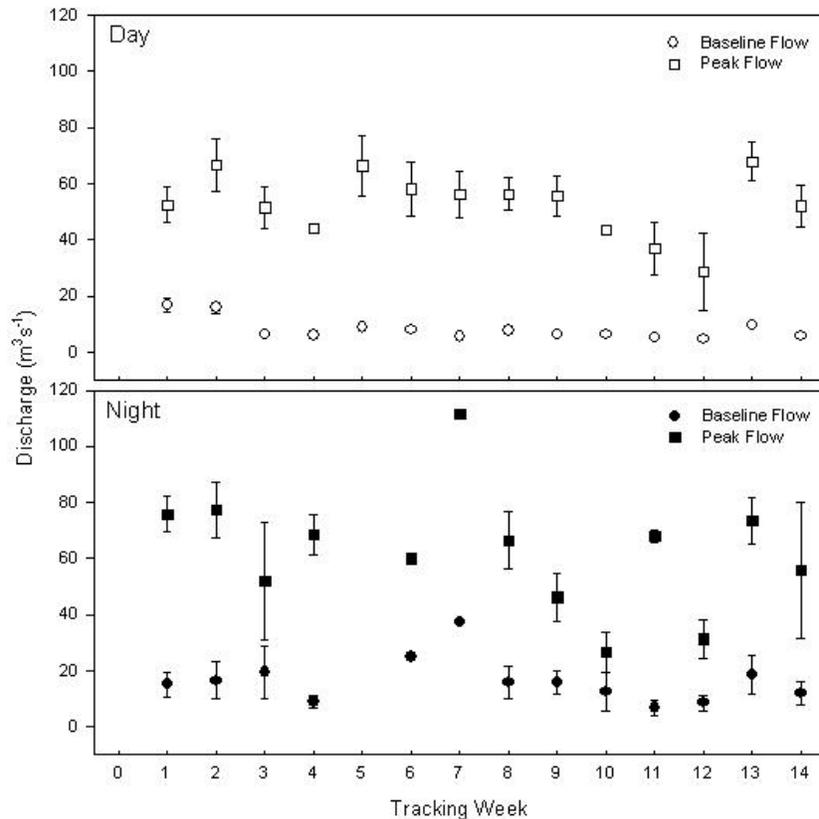
Fish ID	Tag Code	Frequency (MHz)	Telemetry Error (m) Mean ± SE	SL (cm)	Weight (g)	Dates Radio Tags Detected												
						July				August			September					
						7	14	21	28	8	11	18	25	1	8	15	22	29
RT1	22	151.400	15.0 ± 3.1	38.5	1242	X	X	X	X	X								
RT2	33	151.420	14.5 ± 2.9	35	970	X	X	X	X	X	X	X	X					
RT3	25	151.400	15.1 ± 2.8	34	909	X	X	X	X	X	X	X	X	X	X	X	X	X
RT4	29	151.420	15.4 ± 3.3	36	1020	X	X	X	X	X	X	X	X					
RT5	26	151.400	15.9 ± 2.8	32	864	X	X	X		X	X	X	X	X	X	X	X	X
RT6*	23	151.400	20.3 ± 3.7	36	1050	X	X	X		X	X	X						
RT7	31	151.420	12.6 ± 1.9	38	1205	X	X	X		X	X	X	X	X	X	X	X	X
RT8	30	151.420	18.3 ± 4.7	34	1115	X	X	X		X	X							
RT9	27	151.400	20.8 ± 2.7	36	1235	X	X	X		X	X	X	X	X	X	X	X	X
RT10	32	151.420	20.9 ± 5.0	34	980	X	X	X		X	X	X	X	X	X	X		

(referred to in text as “Large” group). \* Denotes fish recovered by fishermen.

**Table 4. Identification numbers, tag codes, frequencies, telemetry errors, length, weight, and dates of detection for small rainbow trout (referred in to in text as “Small” group). \* Denotes fish recovered by fishermen.**

Fish ID	Tag Code	Frequency (MHz)	Telemetry Error (m) Mean ± SE	SL (cm)	Weight (g)	Dates Radio Tags Detected									
						August			September				October		
						12	18	25	1	8	15	22	29	6	13
RT11*	21	151.360	11.9 ± 2.9	28	365	X	X	X	X	X					
RT12	35	150.640	10.2 ± 1.6	27	341	X	X	X	X	X		X	X	X	X
RT13	19	151.360	10.5 ± 1.9	31	500	X	X	X	X	X	X	X	X	X	X
RT14*	33	150.640	11.4 ± 1.7	29	459	X	X	X	X	X	X	X	X		
RT15	30	150.600	10.0 ± 1.7	28	374	X	X	X	X	X	X	X	X	X	X
RT16	40	150.640	12.8 ± 2.3	27.5	335	X	X	X	X	X		X	X	X	X
RT17	29	150.600	15.0 ± 1.8	30.5	520	X	X	X	X	X	X	X	X	X	X
RT18	34	150.640	12.5 ± 1.3	30	474	X	X	X	X	X	X	X	X	X	X
RT19	20	151.360	10.5 ± 1.9	25.5	242	X	X	X	X	X	X	X	X	X	X
RT20	39	150.640	16.0 ± 3.8	31	479	X	X	X	X	X	X	X	X	X	X

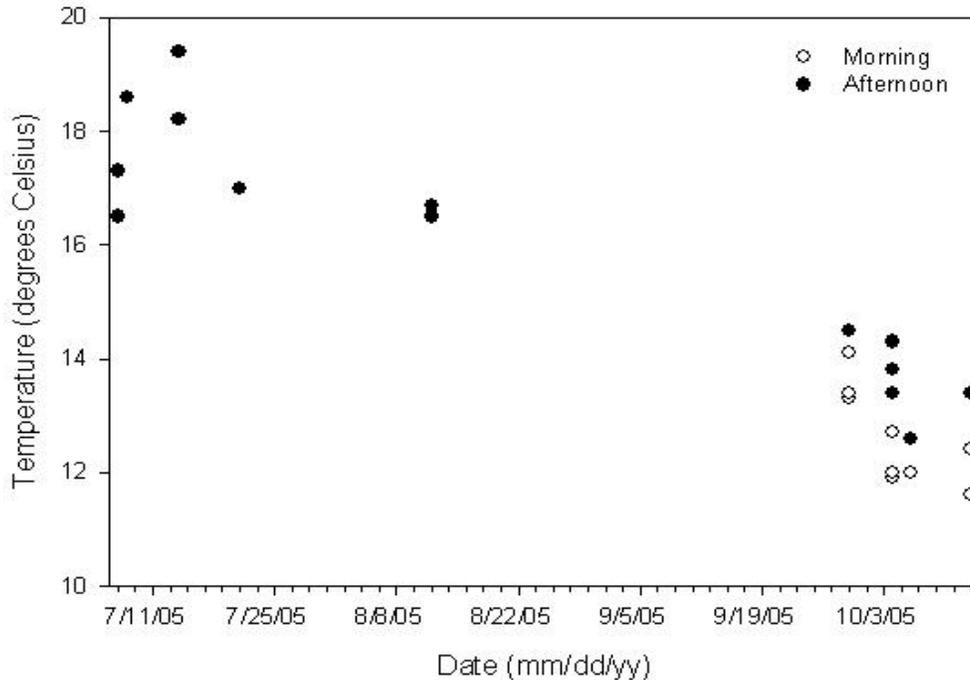
Discharge ( $\text{m}^3\text{s}^{-1}$ , river flow) during the tracking period (Figure 27) oscillated between the non-pulsed (i.e., baseline) flow in the morning before the day's pulsed flow began and the pulsed flow's peak. The pulsed flow began between 9:00 and 11:00 am depending on the research team's location on the river. The pulse took about 4 to 4.5 h to reach Camp Lotus, and 5 h to get to Mariah Wilderness Expeditions, 1 mile downstream. Throughout most of the summer the river was pulsed regularly during the day (Table 5). During the weeks of September 15 and 22 (tracking weeks 11 and 12) daytime pulsed flows were more irregular and less frequent. Around August 5 (tracking week 5) the Sacramento Municipal Utility District (SMUD) finished releasing spring runoff flows (Bill Center, pers. Commun.; see American River website postings). This timing corresponds to the week without any night pulsed flows. Night pulsed flows were generally larger in magnitude (see Figure 27) than pulsed flows during the day and the frequency varied greatly from week to week (see Table 5). River temperature ranged during radio telemetry studies from  $19.4^\circ\text{C}$  to  $11.6^\circ\text{C}$  (Figure 28). However, daily variation in temperature while the research team moved downstream was approximately 1 to  $2.5^\circ\text{C}$  from upstream sites in the morning to downstream sites in the afternoon. Overall, river temperature decreased from the beginning of the summer tracking to the end of the tracking period in October.



**Figure 27. Discharge or river flows that coincided with the days radio-tagged fish were tracked in the river.**

**Table 5. The number of pulsed flows (peaks) each week during the electromyogram telemetry study during the day and the night.**

Tracking Week	Dates	Pulsed Flows (#/week)	
		Day	Night
1	July 7 - 13	7	7
2	July 14 - 20	7	4
3	July 21 - 27	7	3
4	July 28 - Aug 3	7	5
5	Aug 4 - 10	7	0
6	Aug 11 - 17	7	2
7	Aug 18 - 24	7	1
8	Aug 25 - 31	7	4
9	Sept 1 - 7	7	4
10	Sept 8 - 14	7	2
11	Sept 15 - 21	5	2
12	Sept 22 - 28	3	2
13	Sept 29 - Oct 6	8	4
14	Oct 7 - 13	7	3



**Figure 28. River temperatures (°C) measured during radio tracking study from July 7 to October 13, 2005.**

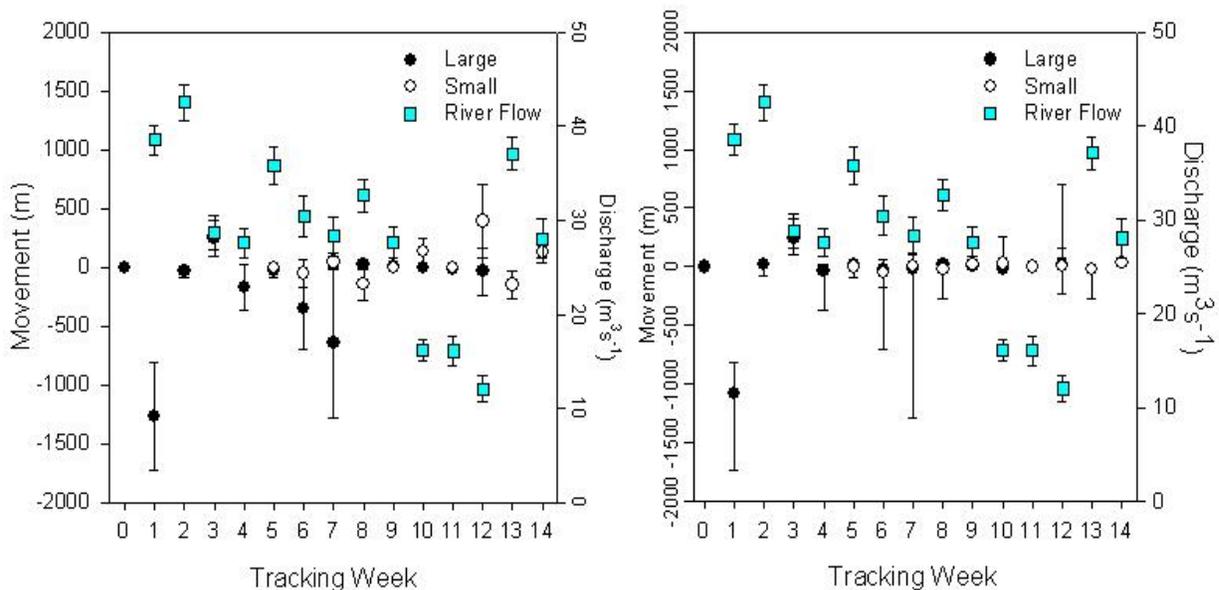
The data were analyzed using a repeated-measures analysis of variance, with fish as subjects, movement per week (m) as the dependent variable, repeated measures over time, and the following fixed main effects: size (group: large, small), habitat (rapid, run, pool), weight (g), health assessment index, location within the river (middle or margin), release location (Henningesen-Lotus County Park, Mariah Wilderness Expeditions), mean daytime peak flow, mean nighttime peak flow, mean weekly flow, and mean weekly flow range. The

distribution of the data precluded analyzing the data using linear model theory. None of the terms investigated were statistically significant ( $\alpha \leq 0.05$ ). An analysis was performed on the ranked data using the same structure. The model fit was adequate; however, no terms were statistically significant (Table 6). An analysis was also performed restricting movement to less than 500 m, which produced the same basic results. Although model fit was better in this analysis, it was still not wholly acceptable. Further analysis was performed using the log (absolute distance moved), which also produced the same basic results. The additional analyses performed on ranks described above were applied in an attempt to minimize the influence of outliers. This model did not include RT5 or RT6 because the release location was unknown, and their locations could not be extrapolated as described previously.

**Table 6. Summary of results from the mixed model applied to the radio telemetry data. The linear model applied to the data evaluated whether the rate of movement was affected by the factors in column one. Categories for each factor are shown in the lower right hand corner of cells in the first column.**

Effectors	DF	F	P	Significant $\alpha \leq 0.05$
Size Large, Small	1	0.01	0.9289	No
Weight g	1	0.02	0.8944	No
Health Index 11 to 58	1	0.00	0.9907	No
Release Location Henningsen-Lotus, Mariah	1	0.22	0.6535	No
Week 1 to 12	11	0.99	0.4581	No
Habitat Rapid, Run, Pool	2	1.57	0.2166	No
River Location Main Channel, Margin	1	1.85	0.1774	No
Mean Weekly Day Peak Flow $m^3 s^{-1}$	1	1.74	0.1903	No
Mean Weekly Night Peak Flow $m^3 s^{-1}$	1	1.82	0.1807	No

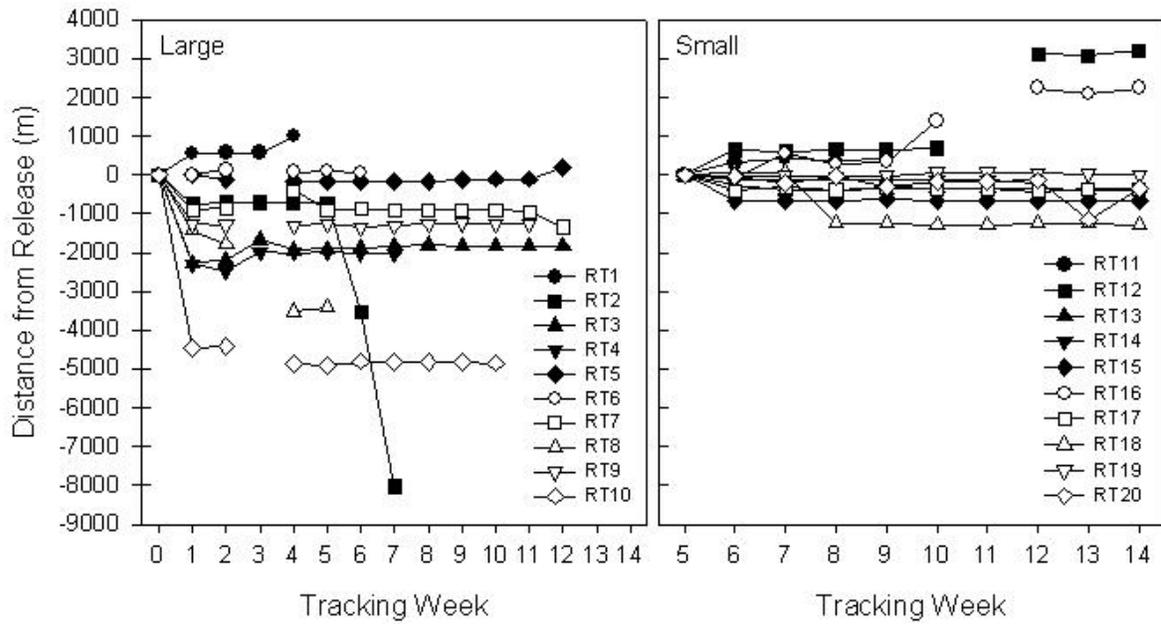
Based on results of the statistical models, the weekly movements of the radio-tagged rainbow trout were not affected by any of the variables the research team measured. In context of the research team's study objectives, the magnitude of pulsed flows ( $P = 0.1903$  day;  $P = 0.1807$  night) and the weekly mean flow ( $P = 0.4581$ ) did not affect weekly fish movement (Figure 29). The large-size group of radio tagged trout (RT1-10) was displaced during the first week after being released in the river; however, once their positions were established they only moved small distances in the following weeks, with a few individual exceptions. Mean movement was influenced by a few longer individual movements and median movements are shown as well to diminish the effects of those few outlying values.



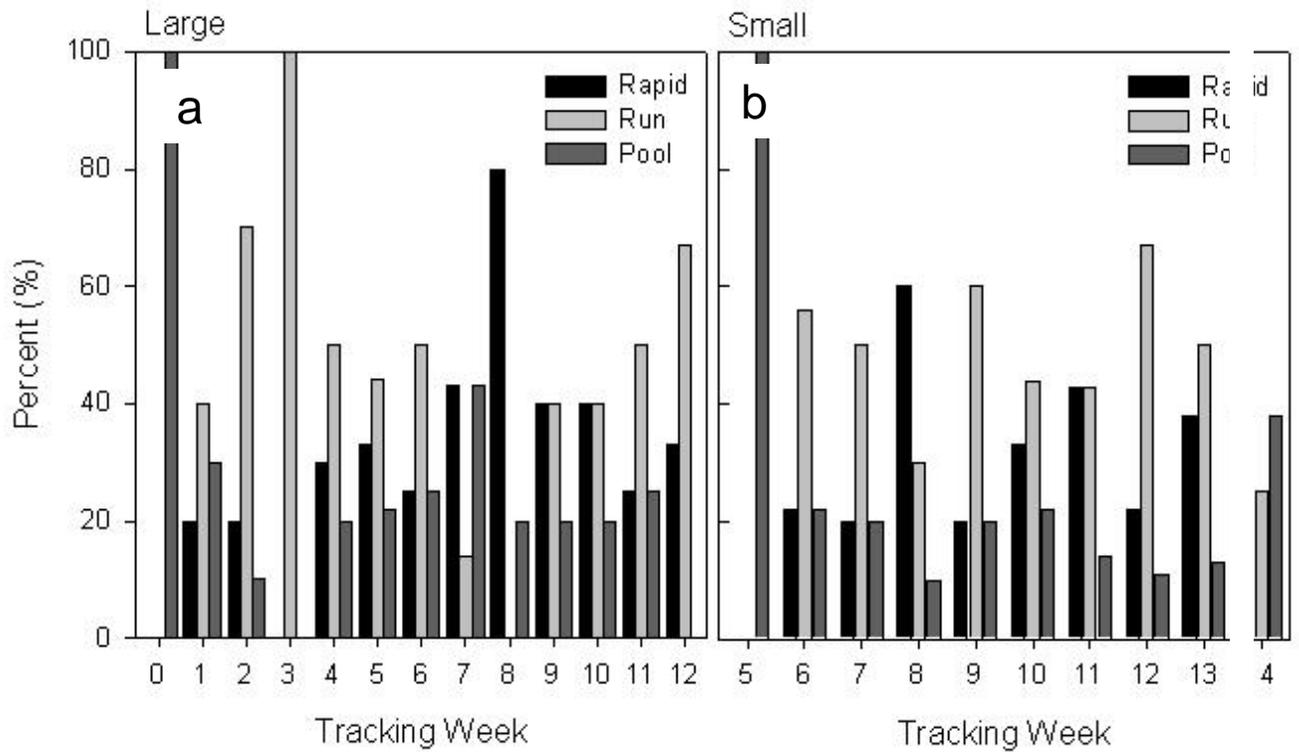
**Figure 29. Fish movement (left: mean  $\pm$  SE; right: median  $\pm$  SE) and discharge (mean  $\pm$  SE) during radio-tracking studies in the South Fork American River. The “large” group of radio-tagged trout (black circles) was released five weeks before the “small” radio-tagged trout (white circles). During weeks 13 and 14 no fish in the large-size group were detected.**

Each fishes’ location in relation to its release location is depicted in Figure 30. In this figure, individual large movements are easily identified. RT2’s downstream movement coincides with the release of the small group. RT15 was detected in RT2’s previous location on the date that RT2 was detected further downstream. RT2 was probably displaced by RT15. During the September 15, 2005 tracking day (week 11), RT12 and RT16 were not detected in the same area as the previous week’s location (September 8, 2005). The following week (September 22, 2005), both fish were detected upstream of their locations (Figure 30). Weeks 11 and 12, when these fish were moving upstream, correspond to lower flows (see Figures 27 and 29), which may have facilitated these upstream movements.

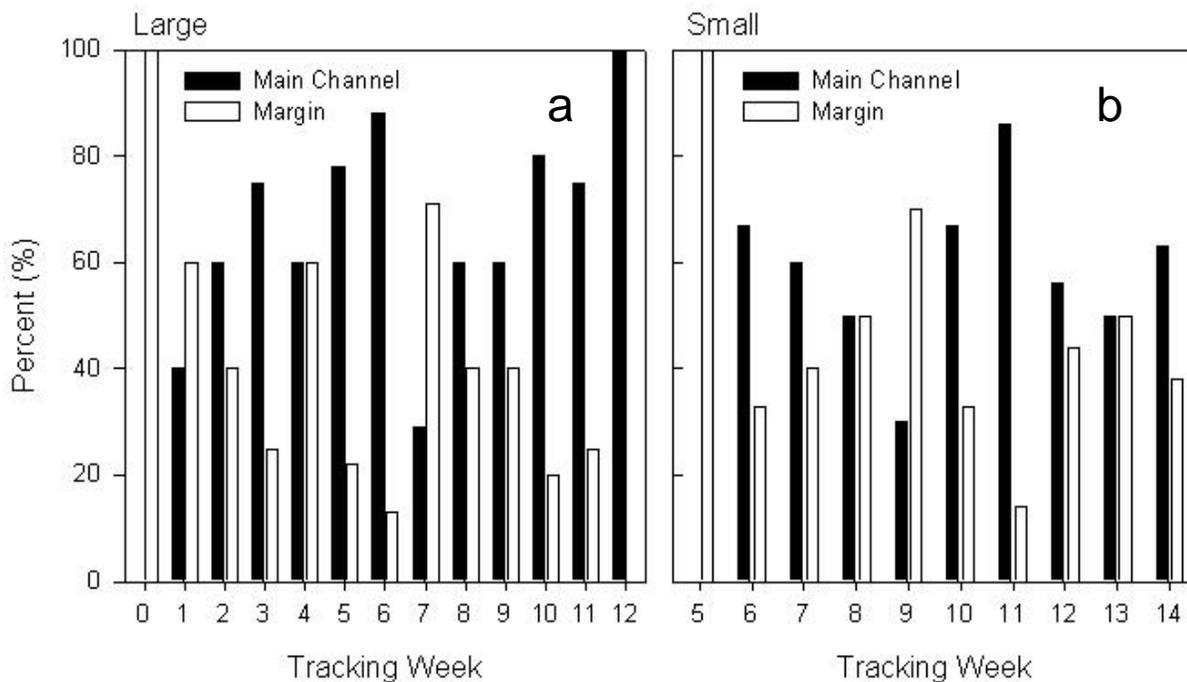
Movement was not influenced by whether radio tagged fish were in a rapid, run, or pool habitat ( $P = 0.2166$ ) (Figure 31) or were in the main channel (main flow) or margins of the river ( $P = 0.1774$ ) (Figure 32). The “large” group was located in runs the most (41%), followed by pools (30%), and rapids (29%). The “small” group was located in runs most often (42%), then rapids (30%), and pools (28%). Both Large (56%) and Small (51%) groups were located in the main channel slightly more often than the river margins, although as noted above these differences were not statistically significant.



**Figure 30. Fish locations shown as distances upstream (+) or downstream (-) from release location for individuals in the large-size (left) and small-size (right) groups of radio-tagged rainbow trout released in the South Fork American River.**



**Figure 31. Weekly percentages of time when the large-size group (a) and small-size group (b) of radio-tagged trout were located in each habitat type (rapid, run, pool).**



**Figure 32. Weekly percentages of time when the large-size group (a) and small-size group (b) of radio tagged trout were located either in the main river channel or river margins.**

## ***Electromyogram Telemetry***

### ***Preliminary Tests***

There was no significant difference between the critical swimming velocity ( $U_{crit}$ ) of EMG-tagged rainbow trout versus the  $U_{crit}$  of untagged trout during preliminary tag studies (Table 7). Tail beat frequency also did not differ significantly when comparing tagged to untagged trout, except at a velocity of 52.6 cm/s (t-test:  $P = 0.036$ ). Tagged trout swimming at 52.6 cm/s had a higher tail beat frequency than untagged trout. Critical swimming velocity of the first and second swim tests of untagged rainbow trout were not significantly different (t-test:  $P = 0.127$ ). Tail beat frequency also did not differ significantly, except at the 65.9 cm/s velocity interval. During the second swim, fish tail beat frequency was faster than the tail beat during the first swim (t-test:  $P = 0.034$ ). The morphology (SL, weight, and health assessment index) of the three groups of trout tested were not significantly different (ANOVA:  $P = 0.380, 0.801, \text{ and } 0.348$ ).

The authors did preliminary tests of the EMG radio tags to determine whether the research team's handling and transport approach affected EMG values and placement. Three fish were initially tested in the flume following the methods of  $U_{crit}$  tests between July 20 and 26, 2005. After the first  $U_{crit}$  tests, fish were loaded into the transport tank, driven around for an hour, and then unloaded back into their original tank to simulate transport to the study site. On the July 28, 2005, the receiver with the capability to detect EMG radio tags was lost in the river during radio telemetry surveys. A replacement receiver capable of reading these tags was not received until August 15, 2005. Post-transport fish were tested August 15 and 16,

2005. The condition of the electrode implant site in the muscle for the three fish tested invalidated the post-transport tests. Two fish had fluid and tissue encasing the electrodes, and the third fish's electrode was starting to wear through the skin.

The research team also investigated the validity of applying EMG-velocity calibrations of one fish to other individuals with the same tag. Being able to apply one set of calibrations to all subsequent individuals with that tag would allow the research team to minimize the handling of fish released for field studies, thus decreasing the chance of dislodging implanted electrodes. EMG radio tags were tested in four pairs of fish. Standard length and weight of the first group (mean  $\pm$  SD: SL = 36.4  $\pm$  4.5 cm, weight = 979  $\pm$  371 g) and second group (mean  $\pm$  SD: SL = 35.0  $\pm$  3.9 g, weight = 957  $\pm$  323 cm) implanted with the tags were not significantly different (t-test: P = 0.659, 0.934). However, the size difference between pairs of fish with the same tag ranged from 3.6 cm to 11.9 cm for SL and 46 g to 927 g for weight as fish were chosen at random from holding tanks. EMGs were significantly different (Tables 8 to 11) between fish with the same tag swimming at the same velocity (t-test or non-parametric equivalent on ranks). Calibration regressions from  $U_{crit}$  swim tests for each pair with the same tag are shown in Figure 33. Due to the significant differences between EMGs from the same tag implanted into different fish, the research team decided to calibrate each fish it released into the river.

**Table 7. Summary of the swimming performance ( $U_{crit}$  and Tail beat frequency) and morphology (standard length, weight, and health assessment index) of preliminary swim tests investigating the effects of tags and multiple exposures to a swim tunnel. Mean ( $\pm$  SE) is shown for parameters in the table.**

Group	N	SL (cm)	Weight (g)	Health Index	$U_{crit}$ (cm/s)	Tail beat Frequency (beat/min)				
						26 cm/s	39.3 cm/s	52.6 cm/s	65.9 cm/s	79.2 cm/s
Untagged 1 <sup>st</sup> Swim	10	34.6 $\pm$ 0.9	874 $\pm$ 88	15.2 $\pm$ 0.4	79.7 $\pm$ 2.3	85.7 $\pm$ 2.9	121.1 $\pm$ 3.1	152.7 $\pm$ 2.6*	184.6 $\pm$ 3.6	203.0 $\pm$ 6.4**
Untagged 2 <sup>nd</sup> Swim	6	35.8 $\pm$ 1.2	887 $\pm$ 122	14.8 $\pm$ 0.8	85.3 $\pm$ 2.3	89.9 $\pm$ 2.5	117.7 $\pm$ 2.5	153.2 $\pm$ 1.9	189.6 $\pm$ 2.9	218.4 $\pm$ 2.4**
EMG Tagged	5	34.0 $\pm$ 1.0	777 $\pm$ 91	13.5 $\pm$ 1.0	76.1 $\pm$ 5.2	94.4 $\pm$ 2.9	129.3 $\pm$ 4.0	162.8 $\pm$ 4.1*	196.0 $\pm$ 3.4	205.3 $\pm$ 1.6

\* P = 0.036, \*\* P = 0.034

**Table 8. Summary of the electromyograms recorded at the same velocities for two fish tagged with the same EMG radio tag, code 11.**

Code	EMG Radio Tag		Velocity (cm/s)	EMG mean $\pm$ SE		P
	Frequency (MHz)	Type		Trout A	Trout B	
11	150.400	CEMG-R11-18	26.0	5.5 $\pm$ 0.1	10.5 $\pm$ 0.1	< 0.001
			39.3	6.2 $\pm$ 0.1	10.9 $\pm$ 0.2	< 0.001
			52.6	8.0 $\pm$ 0.1	14.7 $\pm$ 0.4	< 0.001
			65.9	8.4 $\pm$ 0.2	16.7 $\pm$ 0.3	< 0.001
			79.2	9.6 $\pm$ 0.4	17.8 $\pm$ 0.2	< 0.001

**Table 9. Summary of the electromyograms recorded at the same velocities for two fish tagged with the same EMG radio tag, code 13.**

Code	EMG Radio Tag		Velocity (cm/s)	EMG mean $\pm$ se		P
	Frequency (MHz)	Type		Trout A	Trout B	
13	150.700	CEMG-R11-18	26.0	3.3 $\pm$ 0.1	9.9 $\pm$ 0.1	< 0.001
			39.3	4.5 $\pm$ 0.1	11.0 $\pm$ 0.2	< 0.001
			52.6	6.7 $\pm$ 0.1	12.0 $\pm$ 0.3	< 0.001
			65.9	8.9 $\pm$ 0.2	15.5 $\pm$ 0.6	< 0.001
			79.2	12.5 $\pm$ 0.3	19.7 $\pm$ 0.3	< 0.001

**Table 10. Summary of the electromyograms recorded during calibrations tests for two fish tagged with the same EMG radio tag, code 15.**

Code	EMG Radio Tag		Velocity (cm/s)	EMG mean $\pm$ se		P
	Frequency (MHz)	Type		Trout A	Trout B	
15	151.000	CEMG-R11-18	26.0	5.7 $\pm$ 0.1	10.1 $\pm$ 0.1	< 0.001
			39.3	7.2 $\pm$ 0.2	10.7 $\pm$ 0.1	< 0.001
			52.6	8.6 $\pm$ 0.1	11.4 $\pm$ 0.1	< 0.001
			65.9	16.3 $\pm$ 0.6	12.6 $\pm$ 0.4	< 0.001

**Table 11. Summary of the electromyograms recorded during calibration tests for two fish tagged with the same EMG radio tag, code 16.**

Code	EMG Radio Tag		Velocity (cm/s)	EMG mean $\pm$ se		P
	Frequency (MHz)	Type		Trout A	Trout B	
16	151.000	CEMG-R11-18	26.0	3.7 $\pm$ 0.5	5.9 $\pm$ 0.1	< 0.001
			39.3	4.7 $\pm$ 0.3	6.4 $\pm$ 0.1	< 0.001
			52.6	6.2 $\pm$ 0.5	7.5 $\pm$ 0.1	< 0.001
			65.9	6.7 $\pm$ 0.1	8.7 $\pm$ 0.2	< 0.001
			79.2	8.7 $\pm$ 0.2	13.3 $\pm$ 0.2	< 0.001

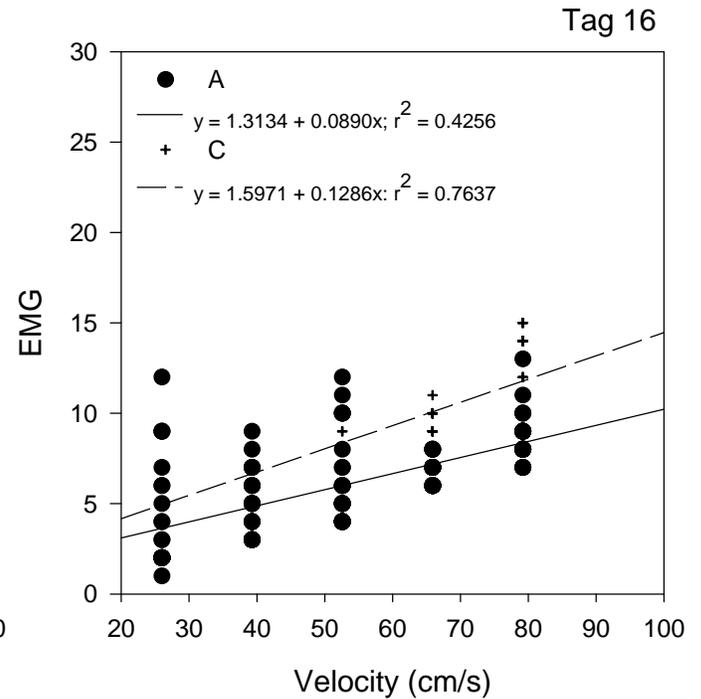
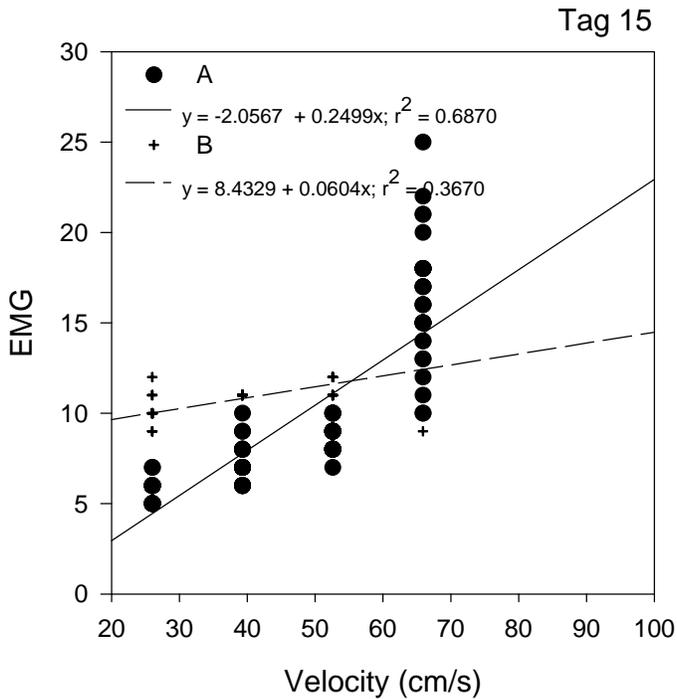
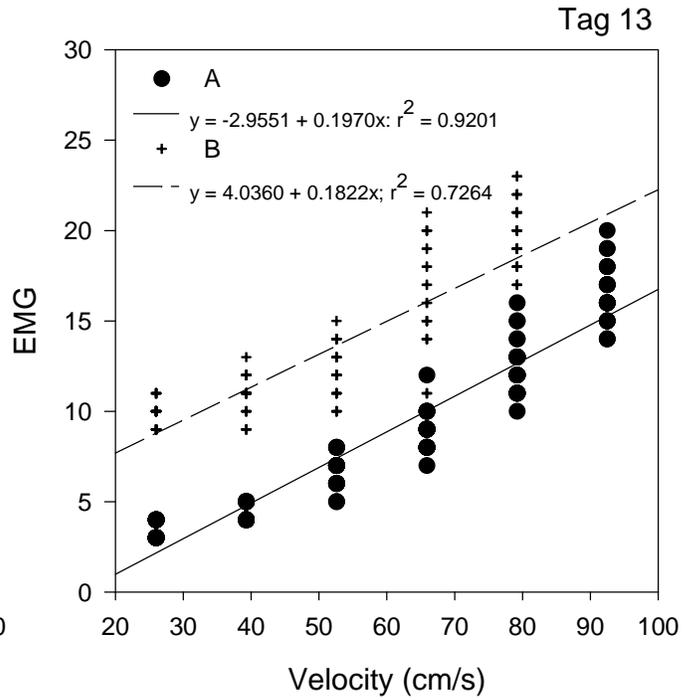
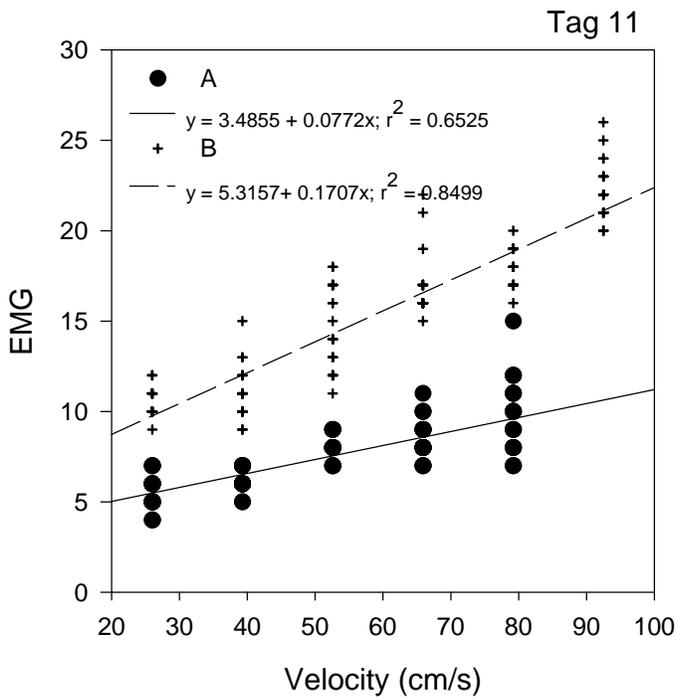
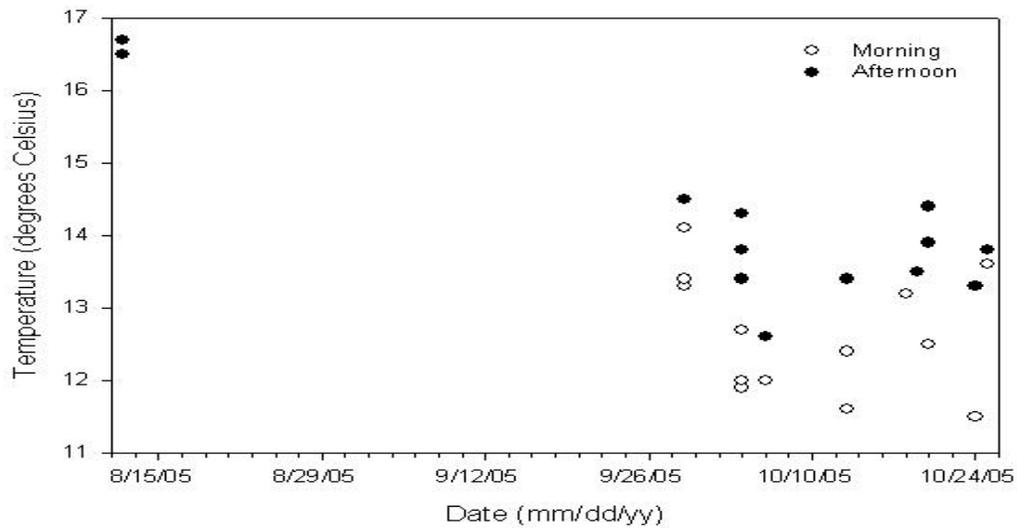
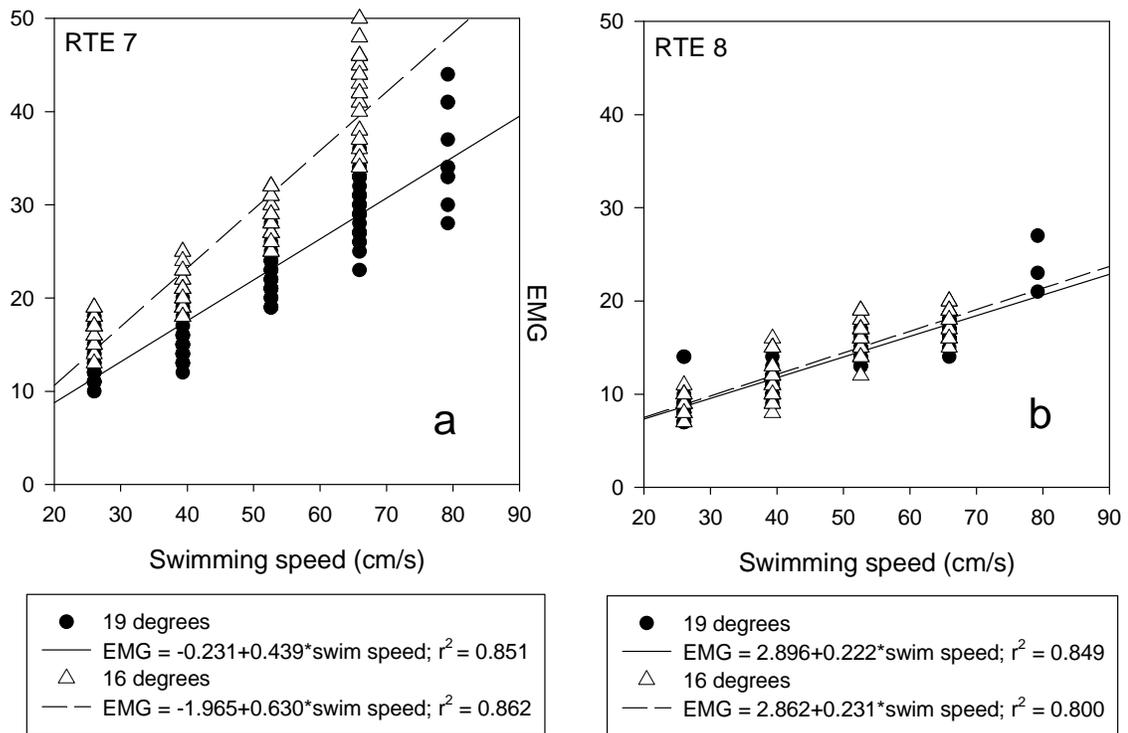


Figure 33. Calibration electromyograms and regressions of different fish implanted with the same tag.



**Figure 34. River temperature (°C) during electromyogram telemetry field studies. Temperatures recorded in the morning and afternoon are denoted with white and black circles, respectively.**



**Figure 35. Swimming test linear regressions for RTE 7 (a) and RTE 8 (b) at two different temperatures, 19°C (black circles) and 16°C (open triangles).**

While the river's temperature decreased throughout the summer (Figures 28 and 34). Efforts were made to keep the holding temperatures in the laboratory tanks as close as possible to the temperatures measured in the field. Swimming and metabolism trials for the second group of EMG-tagged rainbow trout (fish that were released October 18, 2005, with the CEMG-R11-25 tags) were conducted at a lower temperature than the first group, 16°C versus 19°C. Before decreasing tank temperatures to 16°C swimming tests were at 19°C for two EMG-tagged rainbow trout (RTE 7 and 8). Calibration regression intercepts and slopes were not significantly different for RTE 8 (Table 12, Figure 35), however, the critical swimming velocity was lower at the lower temperature. RTE 7 calibration regression slopes were significantly different ( $P < 0.001$ ); EMG increased more with increasing velocity when temperature was lower. Critical swimming velocity for RTE 7 was also 8.8 cm/s less at 16°C than at 19°C.

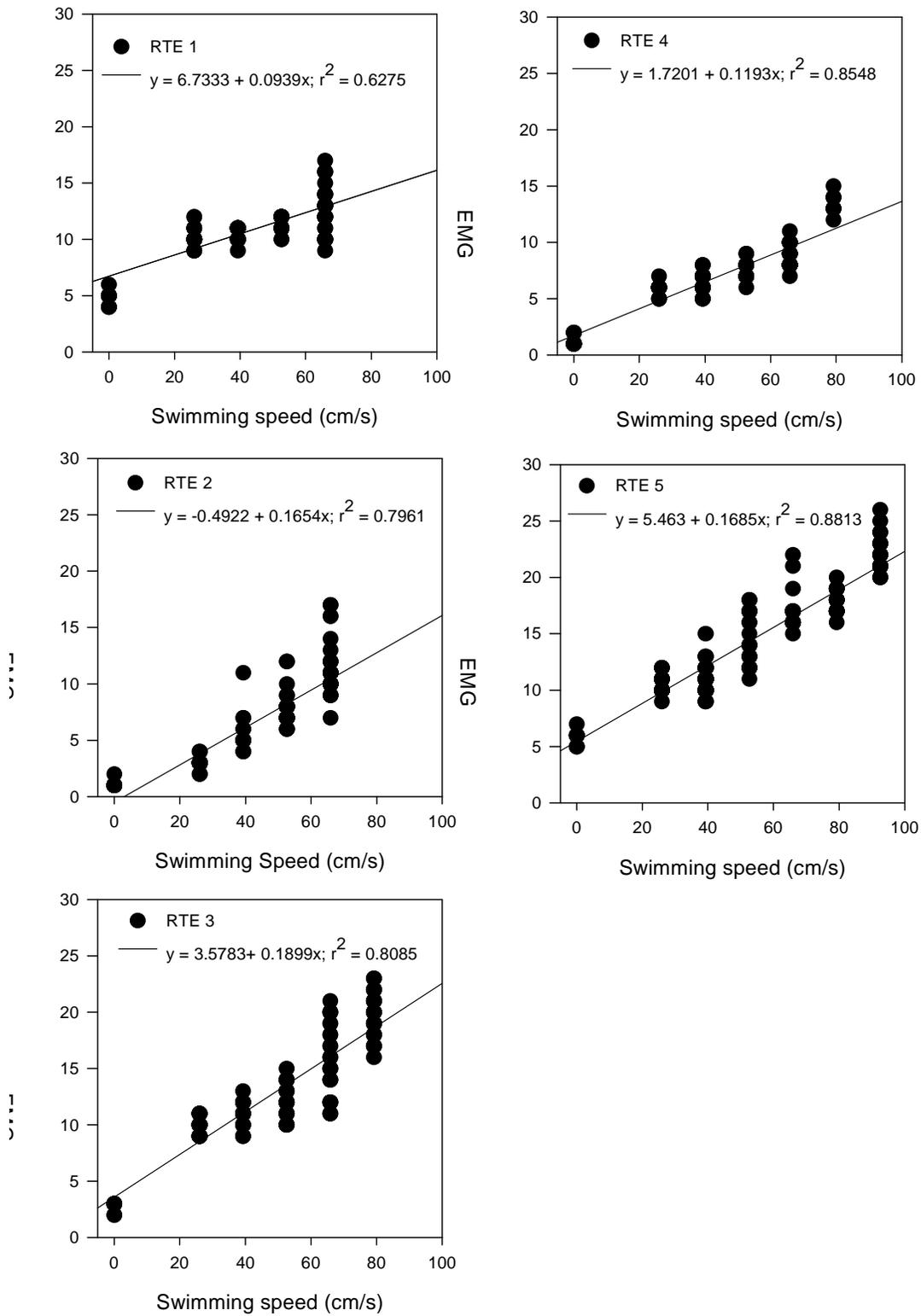
**Table 12. Comparison of  $U_{crit}$  swim trial calibration regressions for RTE 7 and 8 at two temperatures, 19°C and 16°C.**

Fish ID	Temperature (°C)	$U_{crit}$ (cm/s)	Intercept ( $\pm$ SE)	Slope ( $\pm$ SE)	$r^2$	$P_{intercept}$	$P_{slope}$
RTE 7	19	71.6	-0.023 $\pm$ 0.833	0.439 $\pm$ 0.016	0.851	0.161	<0.001
RTE 7	16	62.8	-1.965 $\pm$ 1.121	0.630 $\pm$ 0.023	0.862		
RTE 8	19	73.2	2.896 $\pm$ 0.420	0.222 $\pm$ 0.009	0.849	0.959	0.508
RTE 8	16	69.9	2.862 $\pm$ 0.514	0.231 $\pm$ 0.011	0.800		

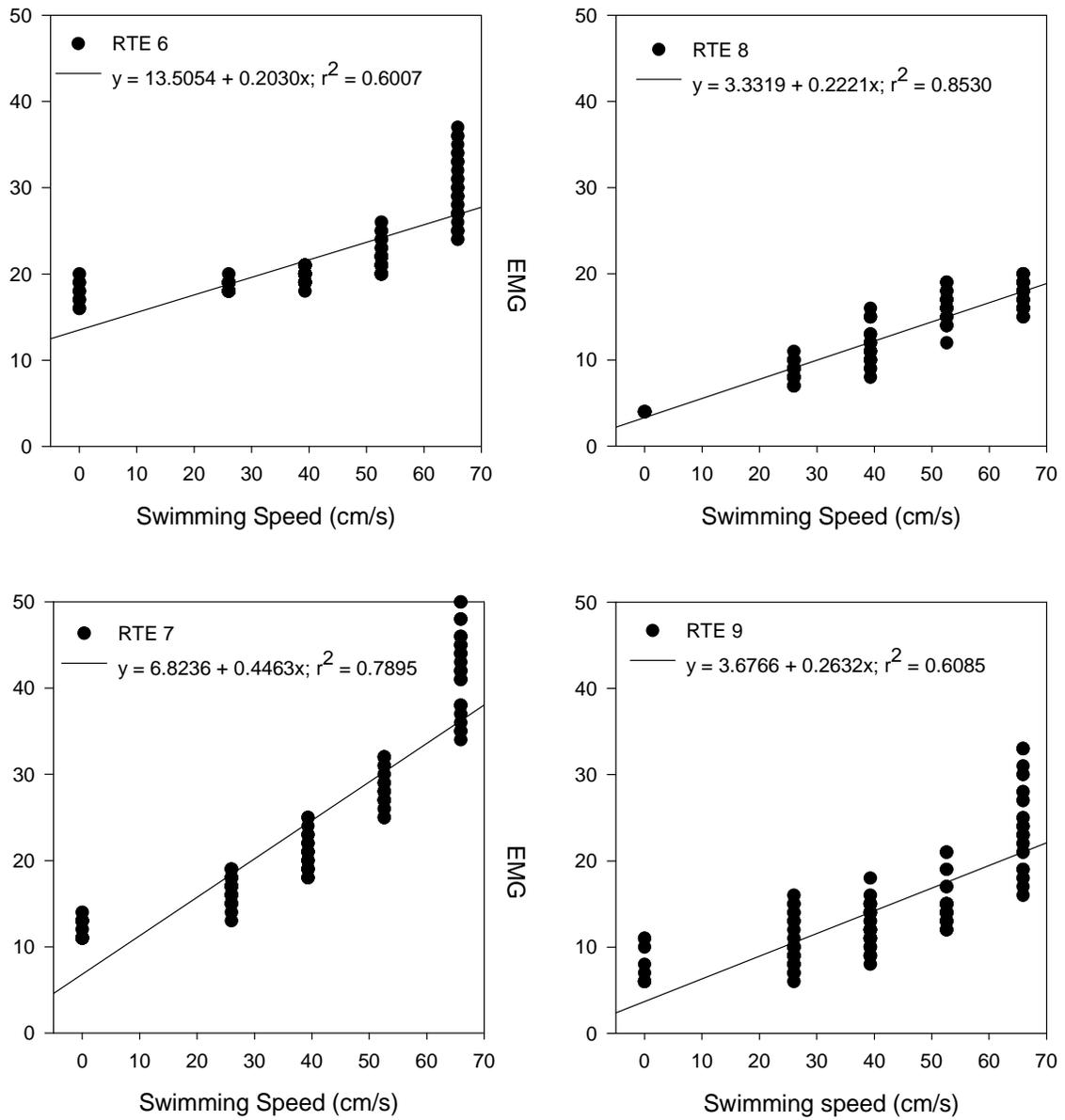
### Calibrations

Each of the nine fish released with EMG tags underwent swimming trials, testing critical swimming performance and relating EMG and swimming speed. Calibrations for fish are shown in Figures 36 and 37. Regression equation slopes, intercepts, and  $r^2$ , as well as  $U_{crit}$ , are shown for all EMG-tagged fish in Table 13. Swim tests and metabolism trials were conducted at 19°C for RTE 1 through 5 and at 16°C for RTE 6 to 9. EMG activity increased with increasing swimming velocity; however, EMG ranges overlapped between the tested velocity steps.

Mean estimated oxygen consumption also increased with increasing swimming speed (Figures 38 and 39). First group mean values are based on  $n = 12$ . However, not all fish that swam in the flume completed all velocity steps, five fish completed at least three velocity steps, while the remaining fish tested completed fewer steps. Regressions for groups 1 and 3 were based on the mean oxygen consumption measured at each velocity. Oxygen consumption was only measured for one fish in group 2. Linear regressions for each EMG group of fish released are shown in Table 14 and Figure 38.



**Figure 36. Calibrations of EMG versus swimming speed for fish (RTE 1 to 5) with CEMG-R11-18 tags, which burst every 5 s.**



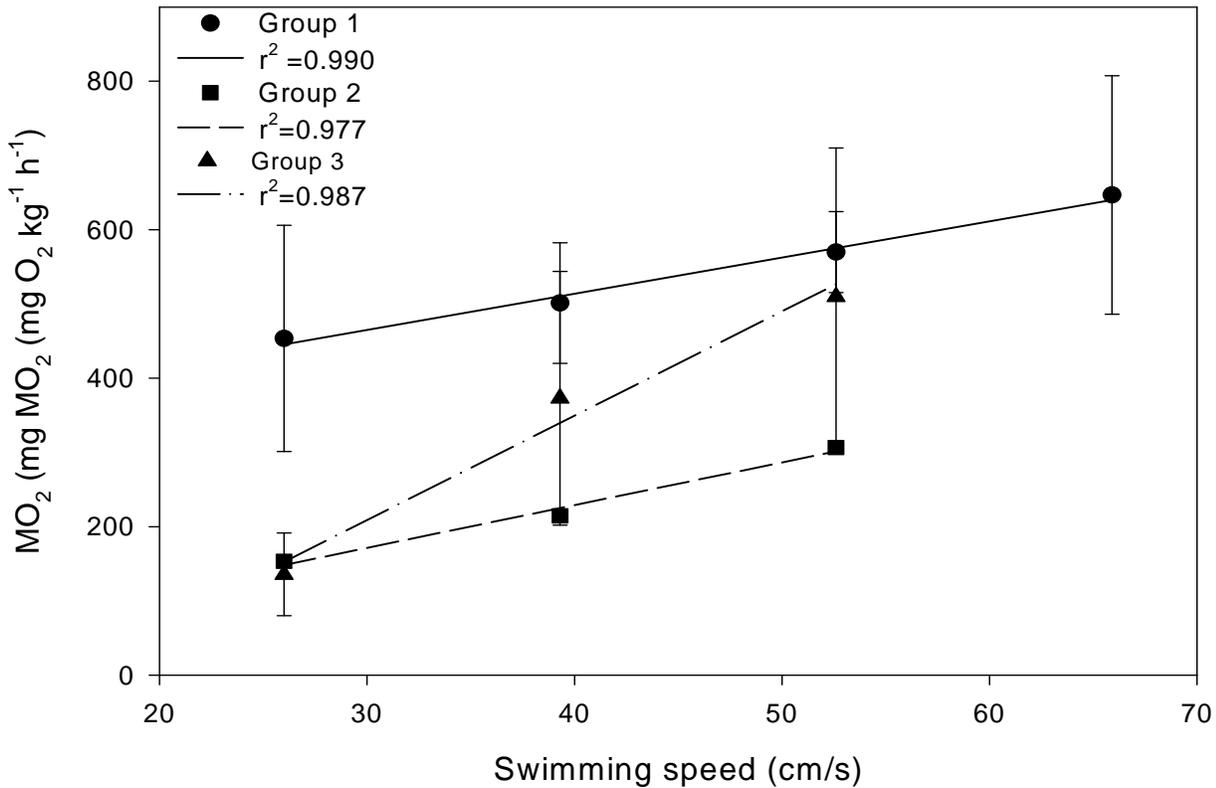
**Figure 37. Calibrations of EMG versus swimming speed for fish (RTE 6 to 9) with CEMG-R11-25 tags, which burst every 2 s.**

**Table 13. Laboratory calibration regressions for EMG and swimming speed (cm/s) before fish were released into the South Fork of the American River. Morphology (standard length, weight, sex, and health assessment index) and critical swimming velocity are also shown with individual tag characteristics.**

Fish ID	SL cm	Weight g	Sex	Health Index	Code	EMG Tag		Ucrit cm/s	Linear Regressions		
						Frequency	Type		Intercept	Slope	r <sup>2</sup>
RTE 1	30	510	-	11	15	151.000	CEMG-R11-18	67.4	6.7333	0.09388	0.6275
RTE 2	35	1007	-	14.5	14	150.700	CEMG-R11-18	61.9	-0.4922	0.1655	0.7961
RTE 3	36	996.7	-	12.25	13	150.700	CEMG-R11-18	79	3.5783	0.1899	0.8086
RTE 4	34.5	1044	-	16	16	151.000	CEMG-R11-18	78.3	1.7200	0.1193	0.8548
RTE 5	39.4	1279	M	15	11	150.400	CEMG-R11-18	94.9	5.4628	0.1685	0.8813
RTE 6	46	2007	M	15.5	19	150.400	CEMG-R11-25	60.0	13.5054	0.2031	0.6007
RTE 7	42.5	2083	M	17.5	22	150.700	CEMG-R11-25	62.8	6.8237	0.4463	0.7895
RTE 8	45.7	2039	F	14	20	150.400	CEMG-R11-25	69.6	3.3319	0.2221	0.8530
RTE 9	43.5	1600	F	15	21	150.700	CEMG-R11-25	61.5	3.6766	0.2632	0.6085

**Table 14. The intercept, slope, and r-square for linear regression of oxygen consumption at various swimming speeds calculated for electromyogram-tagged rainbow trout.**

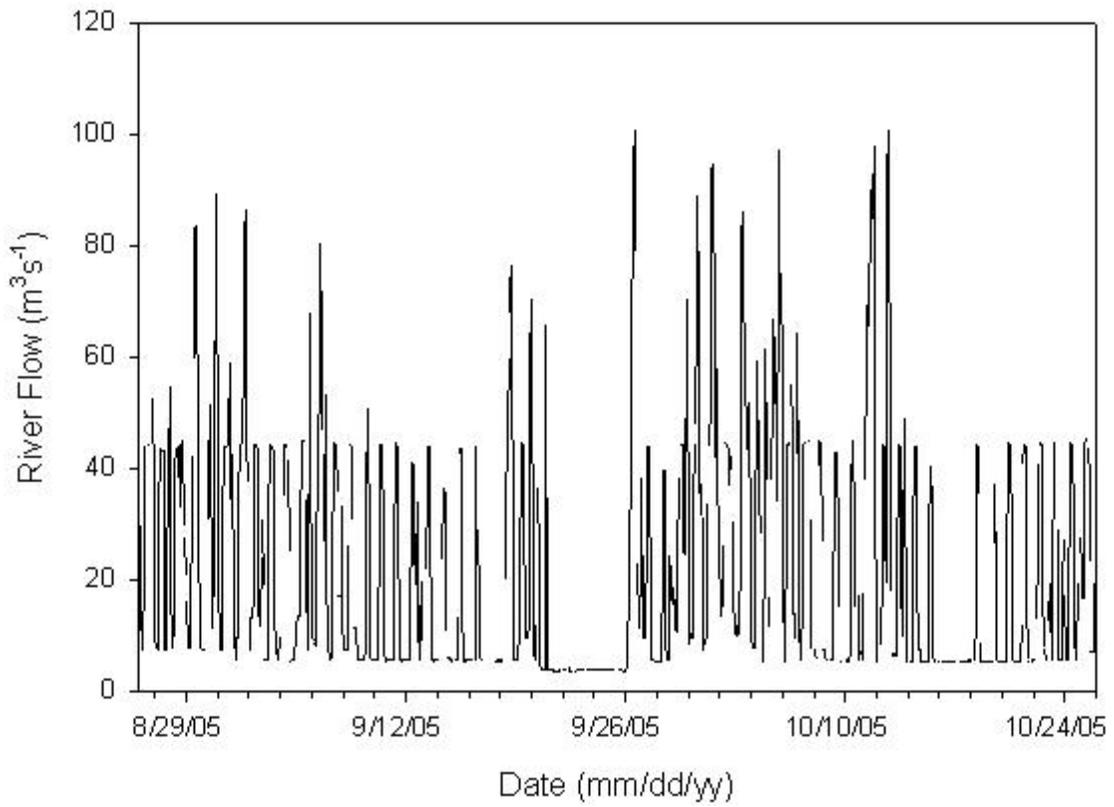
Group	N	Intercept	Slope	r <sup>2</sup>
1	12	318.750	4.878	0.990
2	1	-1.559	5.763	0.987
3	3	-213.128	14.063	0.977



**Figure 38. Estimation of oxygen consumed by trout when swimming at different speeds in the laboratory. The regression line for group 1 was calculated from the speeds of multiple trout (mean + SE); the regression line for group 2 is based on an individual in 19°, that was tested in the laboratory; the regression line for group 3 is based on RTE 6, 7, and 8 (mean ± SE) when swimming at three different swimming speeds.**

### **Telemetry**

Three groups of fish were released for the EMG telemetry study (N = 4, 1, 5). As long as the research team was able to locate the EMG-tagged fish, fish were tracked (EMGs and location recorded) for a minimum of three days (Table 15). On days when the pulsed flow was released later in the day or not at all, fish were tracked an additional day. After the Labor Day holiday, in mid-September, river flows were more variable and unpredictable (Figure 39). There were several weeks with less than one pulsed flow peak per day. On October 19, 2005, the research team was unable to locate RTE 19, one day after its release into the river. The team searched for the missing fish over the entire study site, but the fish was still not detected. The fish was last detected in a deep pool immediately above a rapid on October 18, 2005. Figure 40 shows the locations fish were detected during the EMG telemetry studies.



**Figure 39. River flow (discharge) from Chili Bar Dam during the electromyogram telemetry studies in the South Fork American River.**

**Table 15. Days each electromyogram-tagged rainbow was tracked during pulsed flows in the South Fork American River. Dates in bold denote days on which fish were released.**

Fish ID	Tag Code	Release Location	Telemetry Error (m) (mean±se)	Tracking Dates for Electromyogram Tagged Fish																		
				August			September						October									
				26	29	30	31	7	9	12	13	14	19	27	3	4	18	19	20	24	25	
RTE 1	15	Henningsen-Lotus	14.7 ± 1.4	X			X	X														
RTE 2	14	Henningsen-Lotus	20.7 ± 2.8	X					X	X				X								
RTE 3	13	Camp Lotus	15.8 ± 1.8		X				X	X			X									
RTE 4	16	Camp Lotus	15.0 ± 1.2			X					X	X										
RTE 5	11	Henningsen-Lotus	9.1 ± 0.1											X	X	X						
RTE 6	19	Henningsen-Lotus	8.3 ± 0.3														X	X	X			X
RTE 7	22	Henningsen-Lotus	10.5 ± 0.3														X	X	X	X		
RTE 8	20	Henningsen-Lotus	10.4 ± 0.9														X	X	X			
RTE 9	21	Henningsen-Lotus	10.4 ± 1.6														X					

\* RTE 8 moved out of detection range on October 20, 2005, as the water level increased.

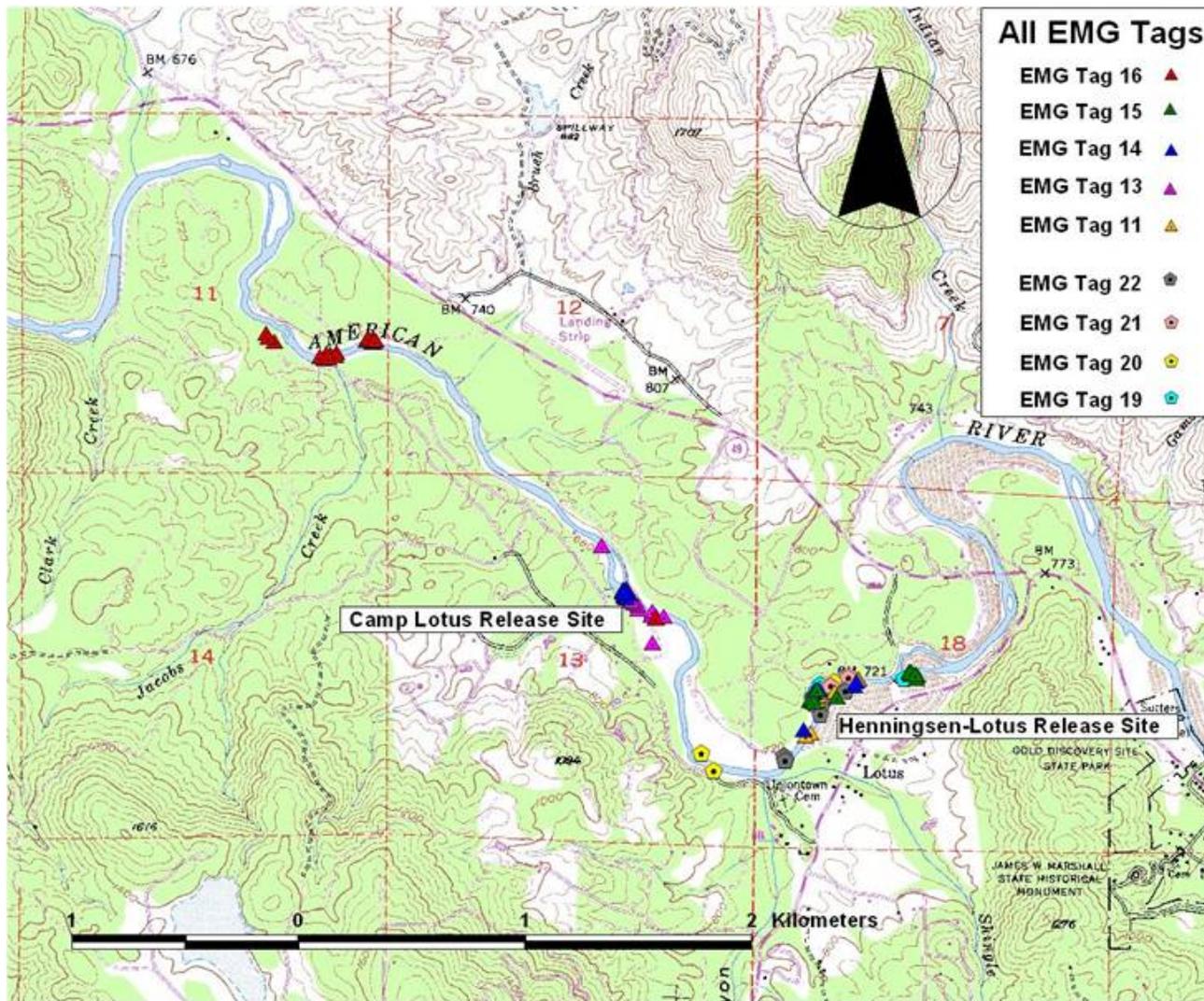


Figure 40. Locations where electromyogram-tagged fish were detected. Fish tagged with code 13 and 16 were released at Camp Lotus, the rest of the fish were released at Henningsen-Lotus. River flow moves from upstream on the right to downstream on the left.

Linear regressions from laboratory calibrations (see Figures 36 and 37) were used to convert recorded field EMGs to swimming speed estimates in the field. The swimming speeds for each tracking interval (60 EMG data points recorded) were summarized by the median value (median EMG velocity). These data were analyzed using a mixed linear model with main effects on median swimming speed only. Fixed effects included: sex (male, female, unknown),  $U_{crit}$  (cm/s), standard length (cm), weight (g), river mile, discharge (cfs), pulse stage (no pulse, rising, stable/peak, decreasing) at time EMG measured, days in the river, and rate of movement (m/h). A random effect was modeled for each fish to capture individual differences in activity. Time dependence was modeled using a repeated-measures analysis with compound symmetry structure for each fish by date combination. Model fit was assessed using residual analysis.

Pulse stage was statistically significant in this analysis ( $P = 0.0002$ ; Table 16). Once significant factors were identified, non-significant terms were eliminated, and the model was reduced to include median swimming speed and pulse stage. Second-order interactions were studied during the final stages of this process with no significant interactions noted. Very little explanatory power was lost in the reduced model compared to the full model. Pulse stage was still statistically significant in the reduced model ( $P = 0.0001$ ). Rising pulse stage was associated with the largest increase in median swimming velocity (+ 4.7390). There was also a slight increasing trend with no pulse or baseline flow conditions (+ 0.7893). Stable or peak pulse stage was negatively associated with median swimming velocity (-1.1536). Model fit was assessed using residual analysis. Statistical models were designed and run in SAS by Jerome Braun at UC Davis' Statistical Laboratory.

**Table 16. Mixed model analysis results evaluating the factors potentially related to swimming speed during the electromyogram field studies. The table includes degrees of freedom (DF), F statistic (F), and P value (P).**

Factors	DF	F	P	Significant $\alpha \leq 0.05$
Sex male, female, unknown	2	0.03	0.9700	No
$U_{crit}$ cm/s	1	1.17	0.2796	No
Standard Length cm	1	0.75	0.3887	No
Weight g	1	0.54	0.4650	No
River Mile mi	2	2.44	0.0890	No
Discharge $f^3s^{-1}$	1	0.67	0.4135	No
Pulse Stage No Pulse, Rising, Peak/Stable, Decrease	3	6.95	0.0002	Yes
Days in the River day	1	0.34	0.5628	No
Rate of movement $mhr^{-1}$	1	0.06	0.8031	No

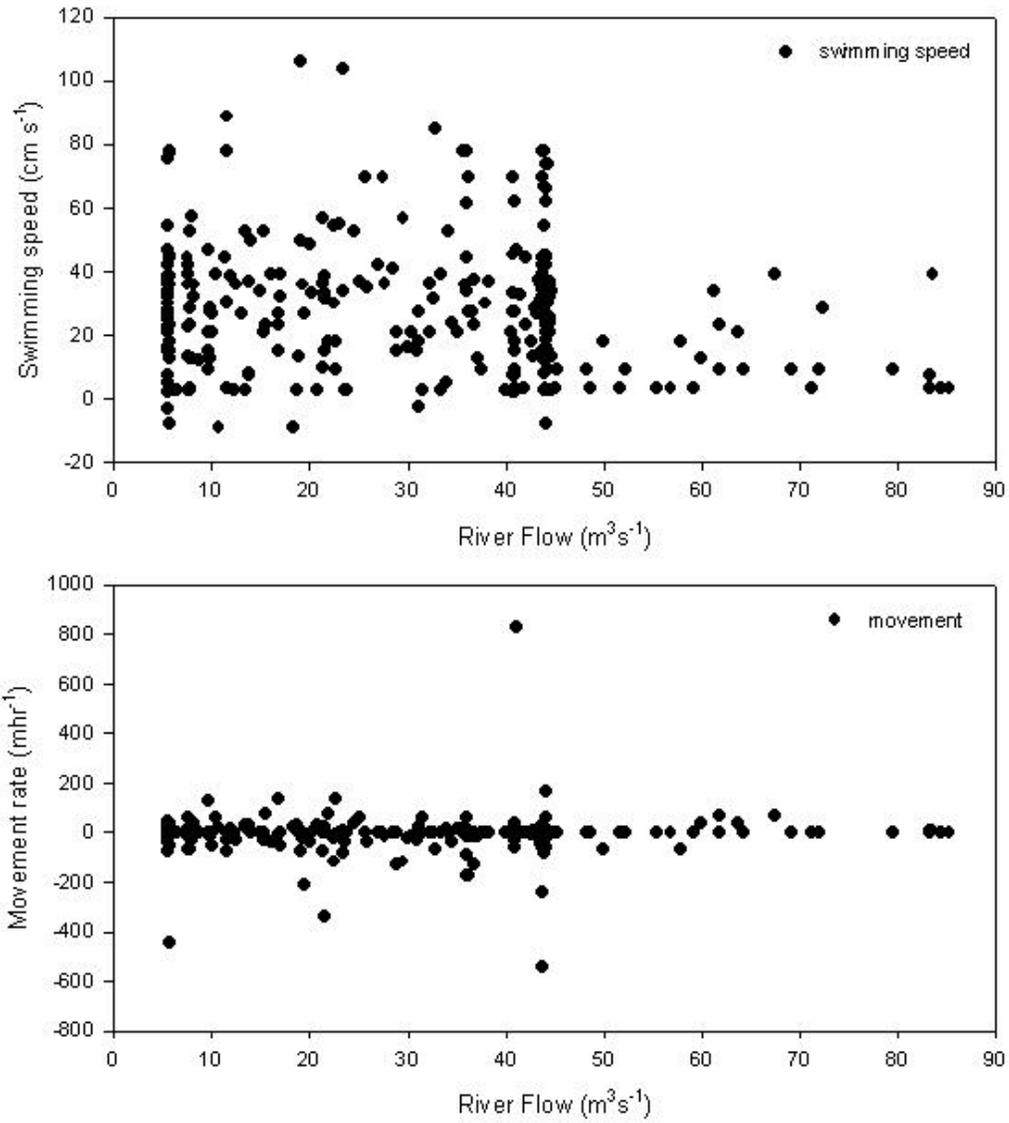
The proportions of EMG-tagged trout that were swimming at less than 50% and less than 100% of their  $U_{crit}$ , as well as those exceeding their highest calibration speed, were calculated (Table 17). The percentage of trout swimming at less than half their  $U_{crit}$  was lowest during the rising stage of the pulse, 54.2%, consistent with the trout exerting more effort during the rising phase of the pulsed release of water. Furthermore, the percentage of trout exceeding the highest calibration speed was highest at this stage, also consistent with trout expending greater effort as the water flow increases and the water rises. Aside from this there was little difference between the percentages of trout swimming at speeds between 50-100% of their  $U_{crit}$ .

**Table 17. Mean ( $\pm$  SE) percentages of electromyogram-tagged trout that were swimming at velocities below 50% and below 100% of their critical swimming velocity during pulsed flows in the field, and the percentage of trout that were swimming faster than their highest laboratory calibration speed.**

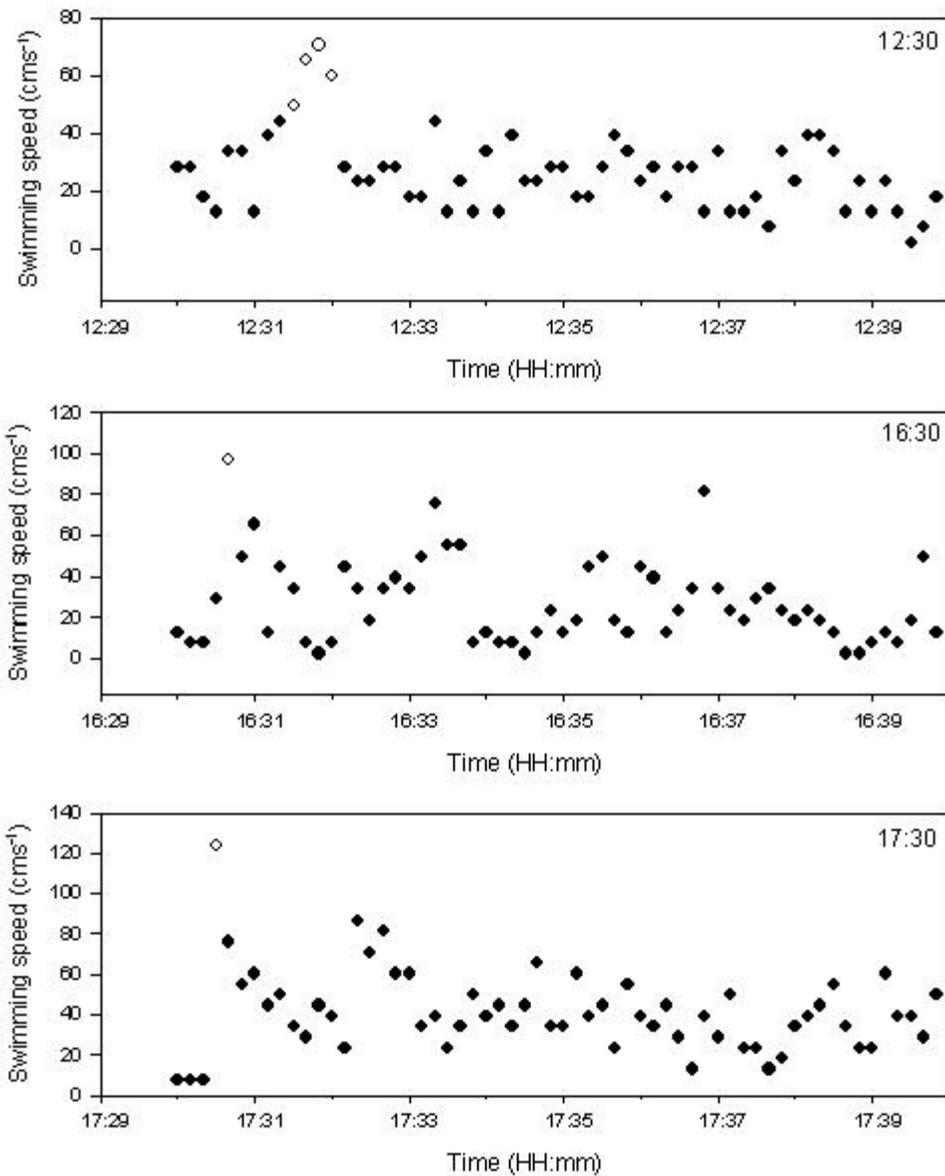
<b>Pulse Stage</b>	<b>&lt; 50 % <math>U_{crit}</math> Mean <math>\pm</math> SE</b>	<b>&lt; 100 % <math>U_{crit}</math> Mean <math>\pm</math> SE</b>	<b>&gt; Highest Calibration Speed Mean <math>\pm</math> SE</b>
No Pulse	62.5 $\pm$ 11.4	90.4 $\pm$ 5.9	7.5 $\pm$ 4.4
Rising	54.2 $\pm$ 11.7	86.0 $\pm$ 8.5	12.3 $\pm$ 7.6
Stable Peak	62.7 $\pm$ 10.8	87.9 $\pm$ 8.0	9.6 $\pm$ 6.1
Decreasing	63.0 $\pm$ 10.9	91.3 $\pm$ 5.9	6.3 $\pm$ 3.9

No statistically significant relationship was determined between swimming speed and river flow by applying the mixed linear model regression. However, plotting river flow versus swimming speed indicates that there is a threshold river flow of 44  $m^3s^{-1}$  (1554  $ft^3s^{-1}$ ), above which trout swimming speed and activity decreases (Figure 41). Mean value for swimming speed up to the threshold river flow is faster (mean  $\pm$  SE: 28.3  $\pm$  1.1 cm/s, range: 114.1 cm/s) than above the threshold (12.0  $\pm$  2.1 cm/s range: 35.9 cm/s). The threshold flow may indicate the point at which the rainbow trout sought refuge from flows. Rate of movement within the river was relatively constant oscillating around the 0 m/h, indicating that the changes in swimming speed were not explained by changes in movement patterns.

Some fish seemed to alter their behavior by increasing swimming speed when boats, including kayaks and whitewater rafts, came near their location. Increases in velocity could be a single elevated speed or a sustained increase over about a minute. Figure 42 depicts RTE 3's responses throughout a single day during three different data recording sessions. The graphs show that some of the highest swimming speeds recorded were associated with the close proximity of boaters.



**Figure 41.** Plot of river flow and swimming speed of electromyogram-tagged trout (top); plot of river flow and rate of fish movement while tracking (bottom).



**Figure 42. RTE 3 swimming speeds during tracking on August 29 2005. Open circles depict swimming speeds when boaters are near the fish, filled circles indicate swimming speeds when boaters are not near the fish.**

## 3.2. Laboratory Studies

### 3.2.1. Temperature Preference

The temperature gradient throughout the apparatus was recorded with the array of thermistors in the absence of a subject fish. This is apparent from the roughly symmetrically increasing lengths of the bars radiating outward from the center of the circular bar graph of the temperatures within the 32 chambers (Figure 43a). The temperatures in the chambers increased continuously from 12 °C at Position 1 to 24°C at

position 15 and decreased from 24°C at position 18 to 12°C at position 32 (b). The radially inward flowing water from the mixing chambers produced a velocity of 0.05 m/s with a Marsh-McBirney model 201D current meter (0.02 m/s detection limit).

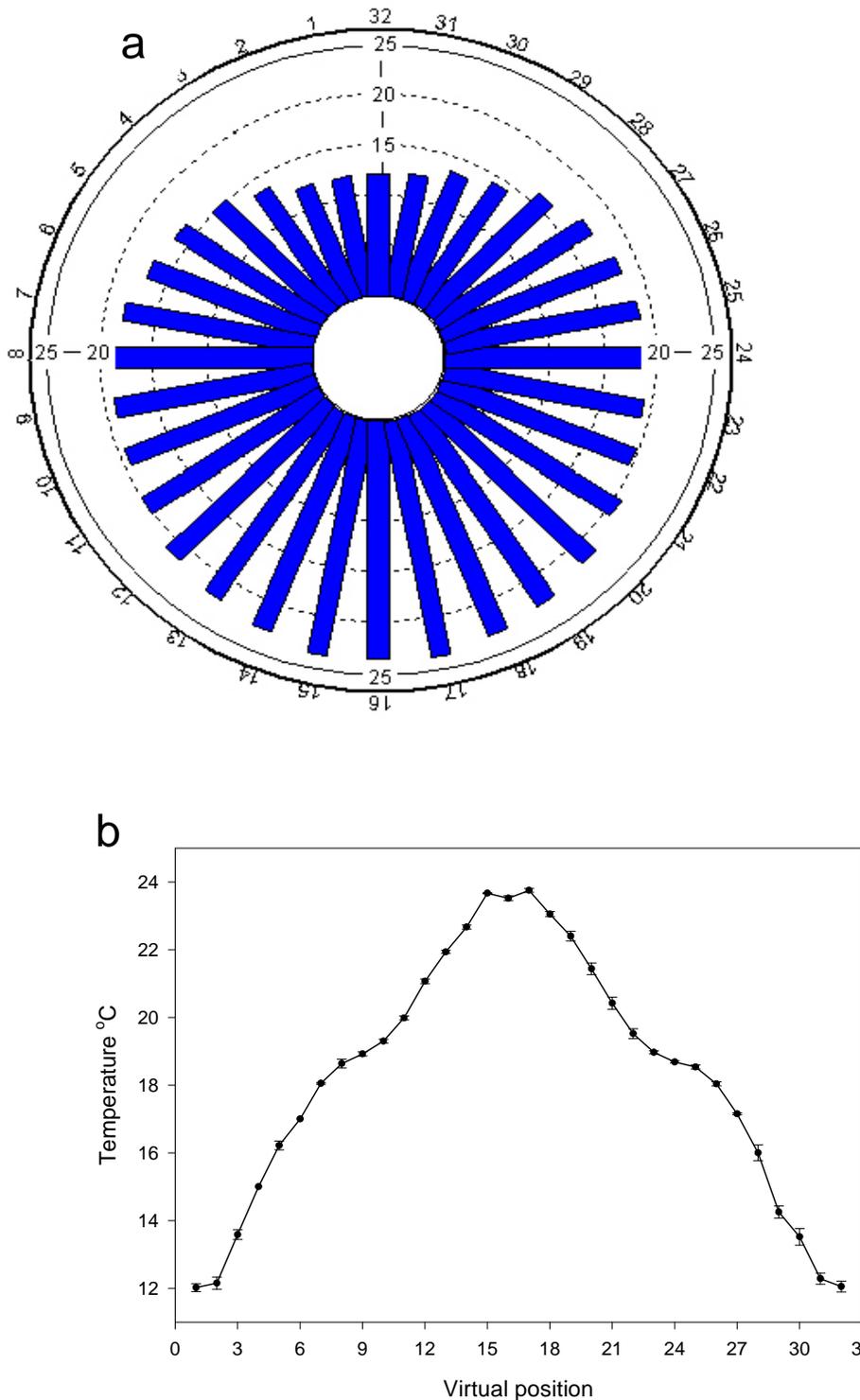
The rainbow trout acclimated to a temperature of 12°C (N=15) and 15°C (N=13), preferred similar temperatures of 16.0 and 16.2°C, whereas individuals acclimated to a higher temperature of 18°C (N=15) preferred a higher temperature of 18.4°C within the annular apparatus (Table 18). Hardhead acclimated to the three temperatures, 12°C (N=14), 15°C (N=13), and 18°C (N=13), preferred higher temperatures of 20.0, 21.0, and 19.6°C respectively. This is consistent with the rainbow trout preference for cold waters upstream and the hardhead's preference for warmer, waters downstream in the tributaries of the Sacramento and San Joaquin Rivers (Moyle 2002).

**Table 18. Rainbow trout and hardhead and preferred temperatures per acclimation group (Mean ± SE). Superscripts represent statistically significant groups (p<0.05).**

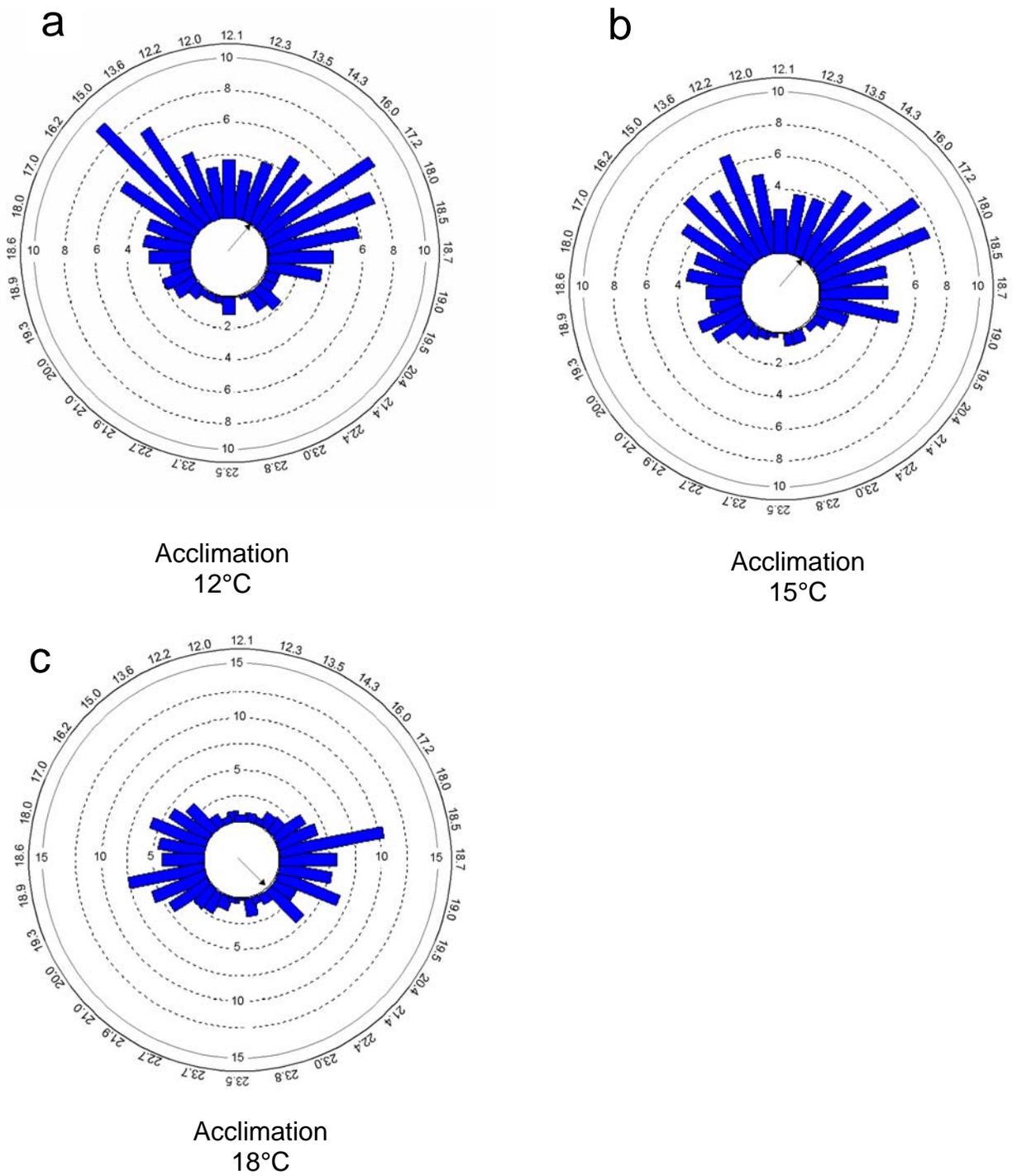
	Acclimation Temperature		
	12°C	15°C	18°C
Rainbow trout	16.0 <sup>a</sup> (± 0.12)	16.2 <sup>a</sup> (± 0.14)	18.4 <sup>b</sup> (± 0.10)
Hardhead	20.0 <sup>c</sup> (± 0.17)	21.0 <sup>d</sup> (± 0.15)	19.6 <sup>c</sup> (± 0.18)

The distribution of the frequencies of the two species from the three temperature groups can be displayed in circular plots, which provide additional evidence for their selection of a particular thermal environment. The water temperature around the annular apparatus is symmetrically distributed with regard to the 32 position. Thus, the temperature is roughly the same at positions 3 and 29, 6 and 26, and so on. When the water temperature was a maximum of 24°C at positions 15 and 17 (see Figure 43b), the temperatures were 13.5 and 13.7°C at positions 3 and 30, and 18.5 and 16.7°C at positions 6 and 26, and so on. One would then expect the distributions of responses to be bimodal, with the peaks corresponding to the same temperatures on either side of the chamber.

The modes in the frequency distributions of the responses of the rainbow trout were consistent with the above-mentioned averages of the response. Trout that were acclimated to a water temperature of 12°C were most frequently recorded in the annular channel in water between temperatures of 15.0° and 16.2°C on one side of the apparatus in water of 17.2°C (see the longest radial bars in Figure 44a). The circular frequency distribution of the responses of rainbow trout acclimated to the higher water temperature of 15°C was also bimodal, with peak frequencies of responses at 12.2° and



**Figure 43. a) Circular plot of the mean temperatures inside the 32 positions of the annular apparatus. b) Linear plot of the mean temperatures. The means are based on three replicates. Error bars represent the SE.**



**Figure 44.** Circular histograms showing the distributions of the behavioral responses of rainbow trout to gradients of temperature in annular apparatus for fish acclimated to 12°C (a), 15°C (b) and 18°C (c). Mean thermal preference is indicated by an arrow.

17.2°C (Figure 44b). Finally, modes in the distribution of the preferences of the trout acclimated to 18°C occurred at 18.9° and 18.5°C (Figure 44c). The mean thermal preferences (determined by deriving the circular mean of the circular frequency distribution and comparing to the mean temperature plots in Figure 43 a and b), of 14.3°, 14.3°, and 21.4°C have little value, because they are averages of two symmetrical modes, each with similar temperatures. The 12° and 15°C trout actively avoided water > 19°C, whereas the 18°C fish showed a pronounced avoidance of water < 16° and > 20°C.

The maximum temperature in the chamber was not symmetrical in its distribution; hence, the distributions of responses to the highest temperatures should be unimodal. Such was the case with the hardheads (Figure 45). The responses of hardhead, which were acclimated to water of 12° and 15°C, had a single mode at 23.8°C. The peak in the distribution of responses for fish acclimated to 18 °C occurred at 23.0°C. The mean responses to varying temperatures, now more valid due to the unimodal nature of the distributions, were 23.5°, 23.5°, and 22.4°C (determined by deriving the circular mean of the circular frequency distribution and comparing to the mean temperature plots in Figure 43 a and b),. Hence, these temperatures were considerably higher than those determined on the basis of a linear averaging of the responses. All the hardhead acclimation groups avoided water < 17°C.

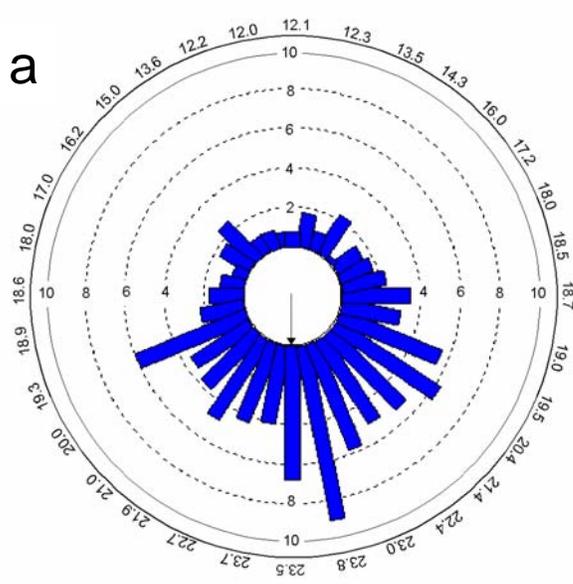
The rainbow trout displayed a preference for cooler water, typical of fast moving Sierra streams and river sections. Schurmann et al. (1991) and Myrick (2004) found similar results for 18°C acclimated rainbow trout, which preferred temperatures of 18.1°C (SE = ± 0.10) and 16.1°C (SE = ± 1.1), respectively. The preferred temperature of the 12° and 15°C acclimation rainbow trout groups coincides with an optimal temperature of 16°C for Chinook salmon (*Oncorhynchus tshawytscha*) (Salinger and Anderson 2006). Below the optimal 16°C temperature salmon swimming speed increased, and above the optimal temperature swimming speed decreased. Baltz et al (1987) observed rainbow trout in the Pit River in 16°C water except in July when they were in 18°C water.

The water temperatures selected by the adult hardhead were within in a narrow thermal range (1.4°C). Knight (1985) observed hardhead during the summer in water between 15° and 28°C in a river along the western edge of the Sierra Nevada mountain range. Knight determined in the laboratory the acute temperature preference juvenile hardhead (< 100 mm TL), using a horizontal temperature gradient. Our data fit those of Knight (Table 19), hardhead tended to choose water temperatures above their acclimation temperature until reaching a thermal preferred maximum near 30°C. Myrick (1996 and 2000) found no significant difference in  $U_{crit}$  for hardhead acclimated to 10°, 15°, and 18°C. It appears that temperature is not the limiting factor for hardhead distribution in the South Fork American River. While capturing hardhead in Slab Creek Reservoir the water temperature was noted on several occasions at 8°C. This contrasts the species' experimentally observed preference for warmer water. Furthermore, very few hardhead were trapped or observed in the warmer lower reach of the South Fork American River during Year 1 of this study (Klimley et al. 2007). This wide distribution of the species

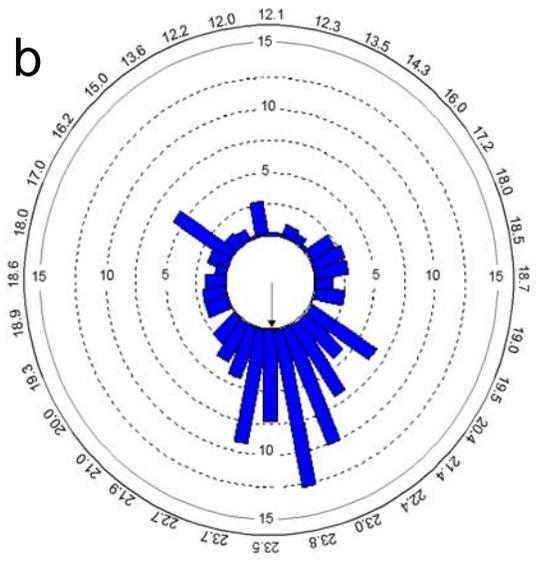
contrasts with the typical partitioning of species in aquatic environments with respect to temperature (Coutant 1987).

**Table 19. Comparison of hardhead laboratory temperature preference (mean  $\pm$  SE) between the present study and Knight (1985).**

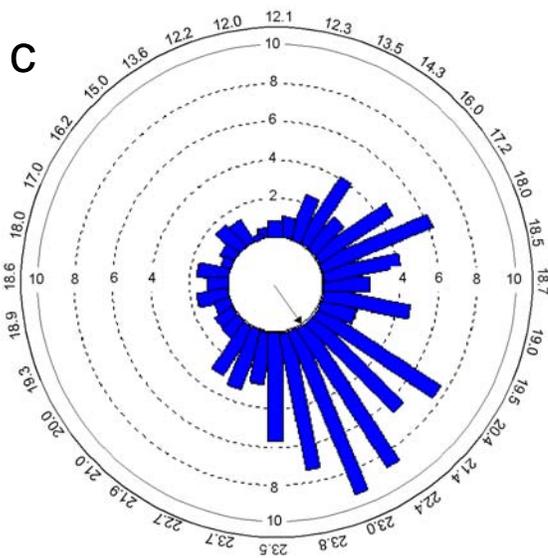
Species	Acclimation temperature ( $^{\circ}$ C)							Source
	10	12	15	18	20	25	30	
Juvenile	15.26		19.27		24	26.28	28.63	Knight (1985)
Hardhead	( $\pm$ 0.96)	--	( $\pm$ 2.35)	--	( $\pm$ 1.96)	( $\pm$ 0.98)	( $\pm$ 0.45)	
Adult Hardhead	--	20	21	19.6	--	--	--	Present Study
		( $\pm$ 0.17)	( $\pm$ 0.15)	( $\pm$ 0.18)				



Acclimation  
12°C



Acclimation  
15°C



Acclimation  
18°C

**Figure 45. Circular histograms showing the distributions of the behavioral responses of hardhead minnows to gradients of temperature in annular apparatus for fish acclimated to 12°C (a), 15°C (b) and 18°C (c). Mean thermal preference is indicated by an arrow.**

## 4.0 Conclusions and Recommendations

### 4.1. Field Studies

#### 4.1.1. Radio Tracking

##### **Conclusions**

The authors tracked the movements of ten small (SL 25.5–31.0 cm) and ten large (SL 32.0–38.5 cm) radio-tagged rainbow trout in response to frequent pulsed flow releases in the South Fork American River (California) from July to October 2005. During this period the river had base flows of  $5 \text{ m}^3\text{s}^{-1}$ , with 4-hour midday releases typically of  $40 \text{ m}^3\text{s}^{-1}$  on most days for whitewater rafting, plus larger releases on several days with peaks up to  $110 \text{ m}^3\text{s}^{-1}$ . Fish were released into the river 12.9 and 16.1 km upstream of Folsom Lake and re-located weekly. In week 1, the small trout dispersed within 1 km upstream or downstream of their release sites. Eight of ten small trout moved little in the following 8 weeks. Between weeks 4 and 7, one small trout moved 2.0 km upstream, while between weeks 5 and 7, another small trout moved 2.0 km downstream. In weeks 1 to 3, eight of ten large trout moved from 1.0 to 4.5 km downstream. Between weeks 5 and 6, one large trout moved from a position 1 km downstream to a position approximately 3.5 km downstream, and then moved to a position 8.0 km downstream of the release site between weeks 7 and 8. Large trout spent most of their time in runs (41%), followed by pools (30%), and rapids (29%). Small trout were most often observed in runs (42%), followed by rapids (30%), and pools (28%). Repeated measures ANOVA analyses showed no significant relationships between fish movement and water flow variables, release site, location within river, fish size, or fish condition ( $p > 0.05$  for all variables). Our results suggest that rainbow trout with  $\text{SL} > 25 \text{ cm}$  are not forced downstream by daily pulsed flow increases from 5 to over  $40 \text{ m}^3\text{s}^{-1}$ .

##### **Recommendations**

The rainbow trout in this study did not appear to be displaced downstream by the pulsed flow regime of the South Fork American River in the summer of 2005, in spite of over 20-fold daily flow fluctuations. The fact that hatchery-reared rainbow trout were used for this study may have influenced the results. These fish would not have experienced natural pulsed flood flows during their development. Yet they did not permit themselves to be swept downstream during the increased flow velocities. It would be informative to repeat this study in a river reach that contained adequate numbers of wild trout of a size suitable for radio-tagging.

Over the time intervals that we tracked fish, they did not seem to respond to increased flows by moving longitudinally upstream or downstream. However, the largest movements upstream were accomplished during weeks of lower pulsed flows (weeks 11 and 12). This suggests fish may move upstream more readily when pulsed flow peaks are less extreme, and high pulsed flows may limit the degree to which trout will move upstream. River regulators may wish to limit the magnitude of summer pulsed flow peaks at times when trout are expected to moving upstream in search of rearing habitat.

Initially after their release, the large trout in the study moved downstream, but in subsequent weeks, they tended to remain in approximately the same location. The smaller trout did not show this same initial downstream displacement. The larger fish would have had higher caloric

requirements relative to the smaller trout. If the larger trout were unable to find habitat with an adequate food supply, they may have traveled downstream in search of areas with greater food abundance, thus spacing themselves out relative to the locally available food supply. It is possible that the pulsed flow regime of the river has indirect effects on trout feeding, through impacts on the species composition and abundance of benthic macroinvertebrates. It would be beneficial to study the impact of pulsed flows on this community composition and biomass in conjunction with fish movement and fish diet in order to separate the direct (i.e., velocity) and indirect (i.e., food depletion) impacts of pulsed flows on fish in regulated systems. Also field tracking studies on the movements of juvenile rainbow trout (15–25 cm TL) would help the understanding of how pulse flows impact fish through various life stages.

#### **4.1.2. Electromyogram Telemetry**

##### **Conclusions**

The authors used radio telemetry with EMG sensors to study the movement patterns of rainbow trout in response to pulsed flows. Previous year's investigations suggested that the fish within the watershed exhibit minimal directional movements. Nine rainbow trout ( $\geq 30$  cm SL) were implanted with these sensors to investigate movement patterns, swimming speed, and oxygen consumption of hatchery fish experiencing pulsed flows. Swimming activity was calibrated in a Brett-type respirometer, and fish were released into the river with EMG sensors. Each individual's EMG outputs were recorded through a pulsed flow event on three separate occasions. EMG measurements were converted to swimming speeds using laboratory calibrations. The factors potentially related to median swimming speeds, such as river discharge, time, sex, location, and pulse stage, were analyzed using a mixed linear model. Pulse stage was found to be statistically significant; increasing pulse stage was correlated with increasing swimming speeds. In addition, above a river flow of  $44 \text{ m}^3\text{s}^{-1}$ , swimming activity decreased. These results indicate that the rainbow trout's ability to respond to pulsed flows without being displaced incurs other costs such as increased energy expenditure and decreased foraging opportunities at high flows.

##### **Recommendations**

EMG telemetry results suggest that fish are increasing their energy their expenditure by increasing their swimming speed to maintain position when experiencing increasing flows. This increased energy output may alter the rainbow trout's metabolic balance, decreasing resources for growth and reproduction. The authors recommend that increases in flows be gradual, thereby allowing rainbow trout the time to continue foraging and to acclimate to these changes in flow. In addition, based on their decreased activity at river flows above  $44 \text{ m}^3\text{s}^{-1}$ , flows should not regularly exceed that level, due to presumably decreased foraging time. More studies should be conducted with different fish species that are common in that stretch of river such as the Sacramento sucker and Sacramento pikeminnow. Efforts should also be made to investigate fish behavior at night, especially because late night pulsed flows tended to be larger than pulses during the daylight hours. Temperature, turbidity and increased macroinvertebrate drift associated with pulsed flows may also have relevance in determining fish behavior and energetics during pulsed flows.

## **4.2. Experimental Studies**

### **4.2.1. Temperature Preference**

#### **Conclusions**

Temperature preference chambers used previously have had design limitations handicapping their usefulness in determining aquatic animal preferences. To effectively determine adult stream fishes' preferences, the authors constructed a 3-m diameter, annular chamber of acrylic plastic. A smaller version of this annular apparatus proved to be effective in recent studies. The annular design decreases possible confounding variables of differential light intensities, water depths, and cover found in other chambers. Our annular chamber presented uniform light intensities, constant water depths and velocities, and stable vertical and horizontal temperature gradients for the experimental fish. Hardhead (mean TL 36.2 cm) and rainbow trout (mean TL 35.4 cm) were acclimated to 12°, 15°, and 18°C and tested individually in an 12°–24°C bimodal annular gradient. Whereas the trout preferred temperatures in the 16.0–18.4°C range, the hardhead preferred a significantly warmer range: 19.6°–20.0°C. The trout acclimation groups of 12 and 15°C actively avoided water > 19°C, whereas the 18°C trout showed a pronounced avoidance of water < 16° and > 20°C. All hardhead acclimation groups avoided water < 17°C.

#### **Recommendations**

Consideration of the species' behavioral preferences is integral to explaining the distribution of native fishes in the South Fork American River. The temperature preferences of adult rainbow trout were elucidated in experiments conducted on three groups of fish, acclimated to different temperatures. The non-anadromous trout preferred a cool or an intermediate water temperature throughout the years' seasons. When ambient water temperatures are elevated, as is typical during California summers, the trout choose a narrower water temperature range of 15°–18°C. During this season trout avoid the coolest water temperatures in favor of intermediate temperatures. During the cooler parts of the year, the trout would presumably prefer cooler temperatures (<16°C). Because the 18°C-acclimated rainbow trout showed a bimodal locational preference in their seeking their 18.4°C preferred temperature, these results argue for temperature, rather than some other influence in the laboratory, to be the dominant behavioral cue in this apparatus.

Understanding the temperature preference of hardhead will require a greater understanding of their life history, swimming, metabolic, and growth performance throughout the South Fork. This ecological information will aid in determining why hardhead occurred in limited numbers at warmer temperatures in the South Fork, and occurred in large numbers in colder reaches of the river, while they preferred warmer temperatures in our laboratory experiments. Hardhead could seek refuge in colder river stretches and reservoirs due to: 1) resource competition from native or non-native fishes, 2) parasitic infections decreasing survival at warmer temperatures, or 3) lower quality forage in the warmer river. River water temperatures should be managed to simulate the natural temperature range throughout the year, which best suits the native species present.

### **4.3. Benefits to California**

Human-manufactured water flow increases (pulses) are common within California's Rivers. Although native stream species have evolved with seasonal fluctuations, the increased frequency (e.g., for peaking hydroelectric operations) and late-summer timing (for recreational purposes) represents significant deviations from the natural hydrograph. The effects of flow pulses on the community of species present within the streams are relatively unknown. The field and laboratory studies described in this report provide a description of the impacts of pulsed releases of water for recreational and commercial purposes on hardhead and rainbow trout. The knowledge resulting from these studies may help agencies to manage their pulsed flows to minimize their effects on the resident fish fauna.

## 5.0 References

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