

**Third Annual Report  
To the Bureau of Reclamation  
April 4, 2007**

**Indexing Carrying Capacity of Salmonids on the Basis of  
Longitudinal Stream Temperatures**

**Principal Investigator**

**Dr. Hiram W. Li**

**Oregon Cooperative Fish and Wildlife Research Unit (USGS-BRD)**

**(541) 737-1963**

**[hiram.li@oregonstate.edu](mailto:hiram.li@oregonstate.edu)**

**Research Team**

**Dr. Guillermo Giannico**

**(541) 737-2479**

**[guillermo.giannico@oregonstate.edu](mailto:guillermo.giannico@oregonstate.edu)**

**Dr. Scott Heppell**

**(541) 737-1086**

**[scott.heppell@oregonstate.edu](mailto:scott.heppell@oregonstate.edu)**

**Dr. Peter Bayley**

**(541) 737-0569**

**[peter.bayley@oregonstate.edu](mailto:peter.bayley@oregonstate.edu)**

**Department of Fisheries and Wildlife**

**Oregon State University**

**Corvallis OR 97331-3803**

**and**

**Russ Faux**

**Watershed Sciences, Inc**

**230 SW 3rd St. Suite #202**

**Corvallis OR 97333**

**(541) 760-1835**

**[faux@watershedsciences.com](mailto:faux@watershedsciences.com)**

**[www.watershedsciences.com](http://www.watershedsciences.com)**

## Table of Contents

Chapter	Title
1	Executive Summary
2	Introduction
3	Longitudinal Patterns Of Stream Temperature As An Index Of Carrying Capacity For Redband Trout.
4	Habitat Classification of Redband Trout Using Physiological Criteria
5	Movement Patterns Of Redband Trout In Relation To Push-Up Dams And Site Productivity Over Different Seasons.
6	Effects of Community Interactions on Redband Trout IFD
7	Calibrating Capture Techniques for Population Estimation
8	Management implications
9	Future Efforts

## 1. Executive Summary

### Longitudinal Stream Temperature as An Index of Trout Carrying Capacity

- Our work supports the working hypothesis that longitudinal patterns stream temperature can index carrying capacities of high desert stream systems.
  - We found that 62% of the variance in standing crops of redband trout (*Oncorhynchus mykiss gairdneri*) standardized by habitat area is attributable to temperature.
- We used physiological criteria to classify the habitat quality of stream reaches of different stream temperatures.
  - Physiological criteria indicate that 21.1-23° C is the temperature point where heat shock protein (hsp)70 exceeds background levels and whole body lipids drop.
  - Physiological Habitat Quality defines four classes: <18 (optimal), 18.0-21° C (suboptimal), 21.1-23° C (marginal), > 23° C (poor).
  - Joseph Feldhaus has developed non-lethal assays to use in the field. This is a major breakthrough for future assessment of stream habitats for threatened and endangered species.
- Estimates of carrying capacity could then be calculated using a simple spreadsheet model based upon physiological criteria.
- This should be an effective tool for projecting carrying capacity of streams given different restoration scenarios simulated by the Heat Source Model and LIDAR estimates of habitat area.

### Movement Patterns and Production (Tagged Trout)

- Shifts in seasonal production (a product of growth and survival rates) are strongly associated with stream temperature.
  - Temperatures were uniform (7° C daily mean) across all reaches from fall to early winter (September-January).
  - Winter (December-March/April) growth rates were significantly higher in the downstream reaches than in the upstream most reach. This appears to be an exception to the general relationship between growth and temperature; however, with FLIR we detected anomalies suggesting groundwater inputs in the mid-and lower elevation reaches which would be metabolically beneficial for trout. These anomalies are difficult to detect with fixed temperature loggers.
  - The season of highest growth occurs during spring (March/April-June). Stream temperatures were ideal (9° C daily mean) and uniform across stream reaches. Likewise, growth was uniformly high across all valley segments.
  - During summer base flow (June-September), growth rates are lower in all valley segments than during spring. During summer, growth rates were significantly higher in upstream reaches (daily mean ~16° C) than in mid and downstream segments (daily mean ~18°-19°C).

- Survival rates were significantly higher in upstream reaches than in mid and lower reaches of the tributary basins.
- Standing crops were not significantly different among reaches during spring, but standing crops were significantly different among reaches at the end of summer.
  - During late summer the following gradient was observed: standing crops were lowest in the warmer, lower reaches, and higher in the cooler reaches upstream.
- During the fall, two phenotypes were observed. We observed both sedentary (stayed in summer habitat) and migrant (emigrated from summer habitat in tributaries into the South Fork John Day and John Day rivers) redband trout.
  - We found that migrants grew faster during the summer prior to emigration than did individuals which remained sedentary during fall.
  - Growth rates during summer were higher in Murderers Creek than Black Canyon Creek. Likewise, most fall migrants were from Murderers Creek.
  - In Murderers Creek, upper basin redband trout had higher survival rates than those from the mid-and lower valley segments. 91% of trout in the upstream reach remained sedentary during fall, and 9% emigrated during fall.
  - Greater proportions of redband trout emigrated from the middle and lower reaches of Murderers Creek.
    - 48% of the redband trout in the downstream reach emigrated during fall; whereas 32% of the redband trout in the middle reach emigrated during fall.
  - Densities were higher in upstream reaches, therefore the percentages do not necessarily reflect differences in the absolute number of emigrants.

### **Movement and Barriers**

- Nearly 9,000 juvenile redband trout were tagged with Passive Integrated Transponders.
- During the years of study, push-up dams were not significant barriers to current movement patterns of redband trout in the South Fork John Day.
- Trout tagged in the South Fork John Day River migrated into tributaries at a low rate (< 2%) during spring and summer.
  - Redband trout in the South Fork John Day Basin were sedentary during the summers of our study. Less than 3% of redband trout exhibited movement greater than 90m during summer low flow. This occurred when stream temperatures increased from 16<sup>o</sup>-23<sup>o</sup>C.
  - Shifts in distribution do occur between the period of declining discharge and summer base flow, but the movements are typically localized within valley reaches and not between valley reaches.

- We do not know whether historic application of push-up dams caused the extirpation of other life history types that existed in the past. It is possible that before water withdrawal for irrigation and push-up dam barriers, long-distance migration of redband trout during summer was more common than during the years of our study.
- The study years (2004, 2005, 2006) have been high discharge years throughout the South Fork John Day basin. Movement patterns may be different in low discharge years. We suspect that upstream migration during summer will occur at a higher rate during years when discharge is low, and hence stream temperatures are higher.
- Layflat stanchion dams (LFSD) do allow fish passage as we can track returning juveniles returning to their home pools. Having said that, our data is not as precise as planned because the spring floods of 2006 destroyed or incapacitated our PIT-tag detection arrays.

### **Is the Ideal Free Distribution a Good Concept for Stream Restoration?**

- The Ideal Free Distribution (IDF) is useful for conceptualizing how density-dependent factors influence carrying capacity of habitat patches or the distribution of organisms at small spatial scales.
  - Our observation matches its predictions that those habitats conferring more benefits will support more biomass.
    - Higher standing crops are found in habitats that require less physiological maintenance energy costs.
    - Likewise survival and growth rates are higher in these habitats.
    - Potamodromy confers growth benefits to those redband trout that migrate to mainstem river to overwinter.
- A primary assumption is that the individual has full knowledge of the array of available habitats. If this is so then the IDF only holds within its home range. Our support for the latter condition is
  - Less than 3% of redband trout move upstream to cooler habitats as stream temperatures increased downstream. Counter arguments are (a) density-dependent agonistic behaviors by resident fish discourages settlement, (b) quality habitat are already approaching carrying capacity, or (c) a combination of the above.
  - Over small spatial scales, we found that we could attract some trout into sites where we supplemented natural prey; whereas, no immigrants were detected in control sites.

### **Calibrating Capture Techniques for Population Estimation**

- In order to control for problems of bias in population estimation we have been conducting calibration trials. We found that
  - Snorkel efficiency calibrations are needed for each individual snorkeler.

- Estimates from SNERDing were superior in terms of less bias and error (and no difference among operators), but require more time and energy investment per sample unit.
- For most applications, many pools need to be sampled for sufficient precision.
- Unbiased juvenile *O. mykiss* population estimates are feasible from efficiency-corrected pool snorkel samples.
- To date our detection efficiencies are roughly
  - Snorkeling counts ~25% of the Mark & Recapture population estimates.
  - Snorkel-herding (SNERDing) captures ~35-40% of the Mark & Recapture population estimates.
  - Electrofisher-herding (ELECTROHERDing) captures ~50% of the Mark & Recapture population estimates.

### **Estimating Ecosystem Limiting Factors Using Principles of Landscape Ecology**

- By using landscape analysis, we can develop hypotheses concerning habitat limitations for salmonids. This involves ordinating data concerning the abundance and distribution of fishes within the basin in relation to its physical and biological features.
- Research designs are informed by the landscape analysis.
- It appears that spatial hierarchies reveal different aspects governing population limitations. Larger spatial scales may reveal cumulative impacts, but if the basin is composed of a high diversity of tributary basins confounding factors can mask issues that are in clearer focus when analyses are performed at smaller spatial scales.

### **Management Implications**

- We now have a fast and cost effective means to rapidly survey the high desert streams of the Columbia River Plateau. We have demonstrated that longitudinal profiles of stream temperatures can index stream carrying capacities. This tool can then
  - Be used to set stream basin restoration priorities in terms of habitat quality, needs of protection, and benefit/costs analyses for recovery.
  - Can be used as a monitoring tool. Population status can be indexed to changes in the stream temperature profile.
- We have developed a non-lethal assay to determine heat stress in salmonids as a means of determining habitat quality using physiological criteria. This may be a very useful tool, as many basins in the Mid-Columbia Plateau have problems of elevated temperatures and different populations of salmonids may have different temperature limits. We no longer need generic standards, but can be highly specific for each stock.

- Snorkeling counts are standard for agency inventories of fish populations. We show that interobserver variation is high and that constant calibration of divers is necessary.
- Interobserver variation for snorkel-herding (divers chasing fish into bag nets) is inconsequential. Moreover snorkel-herding is less stressful than electrofishing. It also has a higher efficiency of detection than snorkel counts 35-40% vs. 25%, respectively.
- Mark-and-recapture calibrations should be conducted in every basin to be sampled, as local environmental constraints (e.g., turbidity, cover, channel structure, predators) can affect detectability.

## 2. Introduction

The purpose of this introduction is to present an overview of the research problem, to provide the management context of theses to research goals of the contract, and to discuss research still in progress. More than one person was involved in each of the research component listed, but only the lead student or PI is listed. In general, the PIs were associated with the research components as follows:

Hiram Li: landscape ecology, community ecology and production ecology

Guillermo Giannico: landscape ecology, community ecology, fish behavior.

Scott Heppell: physiological ecology and production ecology

Peter Bayley: production ecology and calibration of population estimates.

Research Justification: Redband trout (*Oncorhynchus mykiss gairdneri*) are federally listed as a threatened species in the Mid-Columbia Basin. The purpose of our project was to determine whether or not replacing push-up dams with layflat stanchion dams would increase the production of redband trout from the South Fork John Day Basin. Push-up dams are used by irrigators to divert streamwater for irrigation. They often span the width of the stream channel thereby acting as a potential barrier to returning adults migrating upstream to spawn, prohibit passage of parr to summer rearing and overwintering grounds, and inhibit behavioral thermoregulation during the heat of the summer by blocking access to coldwater refugia. The best management practice is to construct the dams during middle to late June and to remove the dams by October, in the hopes that movement during the summer is inconsequential. Layflat stanchion dams have a slot that allows fish to negotiate passage through the dams and are being considered as alternatives to the traditional streambed-based irrigation dam.

Research Objectives and Hypotheses: We asked the following question, what are the factors limiting the production of redband trout in the basin? Do push-up dam barriers impose a significant barrier to trout movement? As South Fork John Day basin is listed as being out of compliance with the Oregon's 303(d) stream temperature requirements, is temperature the major problem?

We developed three main hypotheses to be tested:

1. Push-up dams are effective barriers to fish migration and reduce fish production.
2. Elevated stream temperatures reduce fish production
3. Barriers and temperature patterns interact to inhibit migratory behaviors of fish to regulate body temperatures.

Simply put, we postulated that if temperature was the most important driver governing the production of redband trout, we should observe the following:

- a. Most of the variance in the relation between stream temperature and standing crops in general linear models would be attributable to temperature.
- b. The highest relative biomass ( $\text{g}/\text{m}^2$ ) of redband trout would be in the coldest stream habitats.
- c. Production would be higher in the colder habitats due to greater growth, or greater survival, or some combination of both factors.
- d. The greatest smolt/parr ratio would be produced from the coldest stream habitats. This would be detected in the PIT-tag detector arrays in dams in the Columbia River.

*Research Approach:* Our approach was to examine the problem across levels of biological organization from the top down (Figure 1). Briefly, land and riverscape perspectives set the context of how factors acting at larger spatial scales influence constraints at smaller spatial scales. Responses to these constraints may be detectable at different levels of biological organization. In particular, we are interested in increasing the production of redband trout in the South Fork John Day Basin. Changes to the riverscape have effects cascading down to the population through ecosystem and community processes. However, physical influences will cascade up to the population and the community through enzymatic and physiological processes.

The overarching theme was that the version of the Ideal Free Distribution, or IFD that incorporates fish physiology into habitat modeling (Hughes and Grand 2000), By characterizing habitat quality in a hierarchical spatial pattern, we could capture the carrying capacity of the watershed as a whole. We defined the context of the problem by describing potential constraints of land and riverscape patterns.

Using remote sensing techniques, we mapped patterns of longitudinal stream temperature, geomorphic and vegetative structure, and land use across the catchment. These patterns were compared with patterns of standing crops of redband trout within the basin, through spatially continuous surveys conducted in different years and different seasons. This information enabled us to design our sampling scheme and quantify stream habitats by temperature classes. We also generated hypotheses concerning limiting factors affecting fishes, especially redband trout at different spatial scales from parsimonious linear models using Akaike Information Criteria.

Based on these observations, we established sampling strata for the study. A BACI design could not be employed because of logistical constraints. Grant SWCD and cooperating landowners chose the time and the place for push-up dam replacement.

Simply put, we could not find equivalent control sites for the treatments and did not have enough time for an adequate Before-After comparison. Instead we used virtual barriers (PIT-tag detection arrays) to estimate the percent of fish that would be blocked from upstream or downstream movements. In order to conduct this experiment, we had to (a) document intra- and interseasonal movements of fishes and then (b) identify detection sites to monitor movement patterns.

Landscape and Riverscape Ecology: Luis Francisco Madriñán (Ph D.research in progress) was assigned to examine how factors at the landscape-riverscape scale influenced the distribution of redband trout. This required ordination of physical characteristics of the catchment basin, patterns of human activity, and redband trout survey information. His research provided the sampling template for other studies, generated hypotheses concerning limiting factors, integrated findings from different organizational levels of biological response to provide a rich explanation of redband trout production in the South Fork John Day Basin. Luis Francisco Madriñán and Seth White took on the responsibility to examine movement patterns and changes in distribution of redband trout during the descending limb of the hydrograph and during summer base flow.

Habitat Classification based on Physiological Criteria: Joseph Feldhaus (M.S. 2006) As elevated stream temperatures appeared to be profound physical constraint, we needed to develop physiological criteria by which to judge habitat quality from stream temperature alone. We used two indicators: the induction of heat shock protein 70 and relative concentrations of whole body lipids. Heat shock proteins are induced when stress begins to deform other proteins within the fish. In essence, they help proteins to retain their integrity and therefore function in the face of environmental insults. However they are very expensive physiologically to produce and energetic limitations limit their production (Feldhaus 2006). Therefore, temperature can deplete whole body lipids through elevated metabolic demands as well as due to increasing costs of heat shock protein production. In effect, these are the physiological costs of inhabiting thermally-impacted habitats and therefore important to models of IFD. The end result is that we can now use a spreadsheet to calculate the stream miles in habitat categories ranging from optimal to poor.

Production Ecology of Redband Trout: Ian Tattam (M.S. 2007) documented patterns of growth and survival of redband trout in relation to fish movement through all seasons of the year. This addressed the issue of the impact of push-up dams upon trout production and the expected benefits of push-up dam replacement by layflat stanchion dams (LFSD). He also continues to follow our tagged fish both in the SFJD and through the Columbia River Hydro system (see hypothesis 4. above).

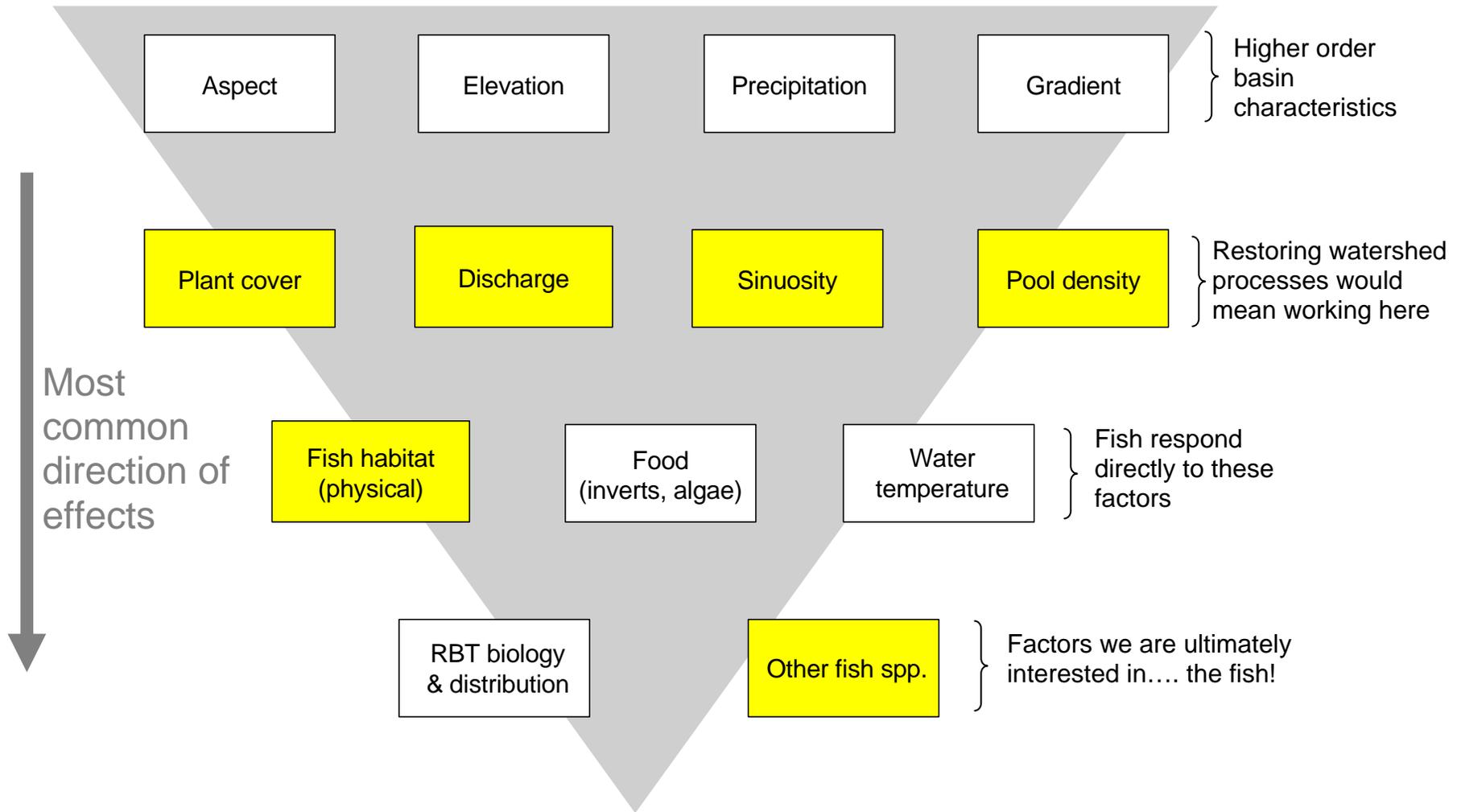
Community Ecology Effects on Redband Trout IFD: Seth White (Ph. D. research in progress) was assigned to expand the concept of the IFD to include community influences, namely how factors of interspecific and intraspecific competition influenced the carrying capacities of pools located in stream reaches of different valley segments. Factors such as stream temperature change or changes to habitat complexity can greatly

affect the economics of growth of species, often differentially. This may alter intensities of interactions among species, thereby changing the rules of community composition and affecting the population size of the species of interest, namely redband trout.

*Calibrating Detection Efficiencies of Different Enumeration Techniques:* Dr. Peter Bayley has taken the lead in calibrating detection efficiencies of different methods we have employed in counting fishes in different habitats. This allows us use different gears and approaches to population estimation because we can account for gear biases.

## Figure 1. Our Picture of the South Fork John Day

Based on current research, this is our simplified picture of how the basin works. In general, order of effects moves downwards. Influence arrows are omitted among and between levels for simplification, but one can imagine the complexity. We have data (at various levels of resolution) that inform each of these categories, and our approach has been to first detect signals at the landscape scale, then test specific hypothesis based on larger-scale patterns. Note the yellow boxes are characteristics that can be directly modified through restoration activities. Hypotheses are developed that explore how connections are broken.



### 3. **PROGRESS REPORT: Temperature as an Index of Trout Carrying Capacity** **Luis Francisco Madriñán (Lead Author)**

The study by Torgersen et al. (1999) suggested that the longitudinal distribution of stream temperatures would be a good index of the carrying capacity of salmonids for high desert stream systems. The objective was to validate the concept. This would require the following components: (1) Document standing crops of salmonids, *i.e.*, redband trout in relation to stream temperature; (2) Determine the statistical strength of the relationship; (3) devise a classification scheme for rating habitat according to physiological criteria; (3) assign different stream reaches into habitat quality categories based on the classification scheme; and (4) quantify the amount of habitat in the different categories and compare the amount of redband trout supported in each one. We combined the approaches of landscape ecology and fish physiology to validate the index. The combination of FLIR profiles and thermal image mosaics facilitated detection of patterns in stream temperature at reach and channel unit spatial scales across the entire basin. We principally focused in the reach-level thermal mosaics to examine the distribution patterns of Redband trout.

Forward-looking infrared (FLIR) images were used as a reference and when available the information from in-stream temperature loggers was retrieved and matched with the FLIR data. To compare the fish distribution patterns to the water temperature a 7 day maximum temperature was calculated for the seven days immediately prior to the snorkeling survey and averaged with the max temperature recorded from the FLIR images at the reach scale. The procedure of establishing physiological criteria for habitat quality will be described in a following chapter (Feldhaus MS Thesis 2006), but the methods of landscape ecology used for the study are described below.

#### **Materials and methods:**

Temperature Data: We used FLIR imagery to map the longitudinal temperature profiles in the study sites. FLIR was flown in late summer 2004 (August 20) over the main stem of the SFJD River. The SFJD, Black canyon creek and Murderers creek were also flown in late summer 2005 (September 12). Thermal infrared sensors measure TIR energy emitted back from the water surface. This technology accurately represents bulk water temperatures where the water column is thoroughly mixed (a mixed column of water was assumed given that the streams in the study area do not average greater than 1 m depth), providing a continuous, instantaneous snapshot of the superficial water temperature of the entire system. The temperature variability of this type of imagery is generally less than 0.6°C (Torgersen et al., 2001).

To understand the daily, monthly and seasonal variation in temperature we deployed 18 Optic Stowaway temperature loggers (HOBO<sup>®</sup>) to record temperature (in degrees °C) each hour, from early June to late September 2004 and 2005. The locations of these loggers were selected using the FLIR imagery, to track the areas with the biggest change in temperature across the basin. Discharge and temperature data were also available from US Bureau of Reclamation gauging stations at Murderers Creek (UTM = 11T 297905mE, 4910076mN; elevation = 908 meters, stream kilometer 0.6), and in the SFJD 10 kilometers upstream of Izee Falls (UTM = 11T 300646mE, 4888621mN; elevation = 1198 meters). Unless otherwise noted, all other temperature records were collected by our research group.

Reach classification: To standardize the extent of our sampling design we developed a method to classify valley river segments (reaches) using a combination of geomorphologic features and water temperature. We used a 10 m digital elevation model in which we divided the SFJD and the two tributaries using three metrics (elevation, slope of the stream channel and aspect). After this initial classification we overlapped the FLIR imagery and subdivided some of the reaches if the difference in temperature was greater than 3°C per each section.

Once the reach breaks were determined we used the temperature-hsp 70 classification developed by Feldhaus (2006) based on heat shock proteins (hsps). Based on these findings, we classified habitat in the system into 4 major classes: Optimal (<18 C), Suboptimal (18.1 to 21 C°), marginal (22.1 to 23 C°) and poor (>23 C°).

Snorkeling surveys: Information on the seasonal movement patterns of redband trout was gathered to determine the degree to which trout were moving while distributional surveys were being conducted. 16 Radio tags were surgically implanted (1.7 and 2.1 gram pulsed radio transmitters with external whip antennas, Advanced Telemetry Systems, Isanti, MN<sup>1</sup>) on trout bigger than 170 mm and 210 mm respectively, during the months of July through September 2004, in Murderers creek and the SFJD. These fish were tracked on a daily basis.

Contiguous snorkeling surveys were conducted in 2004 and 2005. Stream reaches were systematically surveyed for mapping purposes and we obtained exhaustive counts of juvenile Redband trout and other fish community members. During the surveys fish were located and counted visually using a two person crew consisting of a snorkeler and a data recorder on shore. In 2004 we snorkeled 30.4 river km from July 5 to 11 (465 units); in 2005 we increase our sampling to cover 41.6 river km from July 11 to 21 (820 Units).

A power analysis was applied to the data to decide how much of the stream needed to be subsampled in late August in order to identify possible shifts in fish distribution associated with lower water levels and higher summer temperatures. This analysis was performed with a 0.5 significance level and a power of 0.80 to detect any changes in the distribution (Peterman, 1990). From August 12 to 16 2004, 33% of the pools were subsampled and between August 20 and 26, 2005 31% of pools were subsampled. Geographic locations of habitat units (pools and riffles) were recorded using a portable Trimble Geoexplorer 3 global positioning system (GPS) with differential correction (accuracy of 10m).

Our sampling design included the main stem South Fork John Day and two tributaries (Murderers creek and Black canyon creek). We sampled fish between 0900 to 1700 hours to ensure maximum visibility. To avoid temporal variability we used 3 different snorkeling crews to cover the greatest spatial extent in the shortest time. The confidence in our observations was 31% for *O. mykiss*.

To compare habitat use by redband trout and the other fish species, stream habitat information in each habitat unit was collected at during the snorkeling surveys. Parameters collected included maximum and tail pool depths, tail, middle and head wetted width,thalweg length, dominant and subdominant substrate, embededness, % undercut banks and number of pieces of large woody debris.

#### GIS analysis:

Digital maps were created using LiDAR (Light detection and ranging) imagery. A total of 516,282,572 laser points were collected over the study area using an Optech ALTM 3100 LiDAR system set to acquire points at an average spacing of less than 0.5 m (>4 points per square meter).

Trimble 5700 ground GPS units were deployed and used to process kinematic solutions to the onboard GPS and inertial measurement unit (IMU) using PosPAC v4.1. Points were computed per flight line using the REALM Survey Suite v3.5. Microstation V8 and TeraScan were used to import the points into processing bins, remove pits and noise and compute the bare earth model. Terra Modeler was then used to create TINs and output to

ARCINFO ASCII lattice models, which were then imported into ArcMap(ESRI, Redlands, California, USA) to render one meter mosaics of first and ground models.

The map templates were loaded in a geographical information system (GIS) for spatial analyses of trout distribution, stream temperature, and aquatic habitat. The bankfull width was calculated for all the streams in the study area, and individual pools were identified and fish distribution and temperature data for each location were added.

Once all the fish information was collected, a database with all the information was created. A cluster and outlier analysis (Anselin Local Moran's I) was used to identify possible spatial autocorrelation among sets of clusters of fish distribution with values similar in magnitude and clusters of fish distribution with very heterogeneous values (Tiefelsdorf, 2002).

To identify higher-than-expected fish densities we employed the “Hot Spot Analysis” using ArcGIS® spatial statistic tools software to identify spatial clusters of statistically significant high or low attribute values. The rendering option was applied to calculate the Getis–Ord  $G_i^*$  statistic by rendering features quantitatively; (the point renderer displays potential patterns in the input count data). The  $G$ -statistic tells whether high values or low values (but not both) tend to cluster in a study area (Ord and Getis, 1995).

### ***Results:***

We combined information from two analyses of thermal imagery (FLIR) facilitated detection of warm and cold water point sources such as tributary junctions and flood irrigation canals. The thermal environment of the South Fork John Day was spatially heterogeneous but mostly dominated by temperatures between 22 to 23 C° with disjoint patches of relatively cooler water (18 – 22 C°) (Table 1, Figure 2). Continuously recording data loggers were used in each study section to progressively correct for elapsed flight time and associated stream temperature change. The duration of overflights across the SFJD and its tributaries did not exceed 1 h, during which time stream temperature increased by 0.8 – 1.2C°. The high resolution of the FLIR profiles (18 cm per pixel) allowed us to clean thermal anomalies associated with irrigation channels, tributary inputs and riparian vegetation.

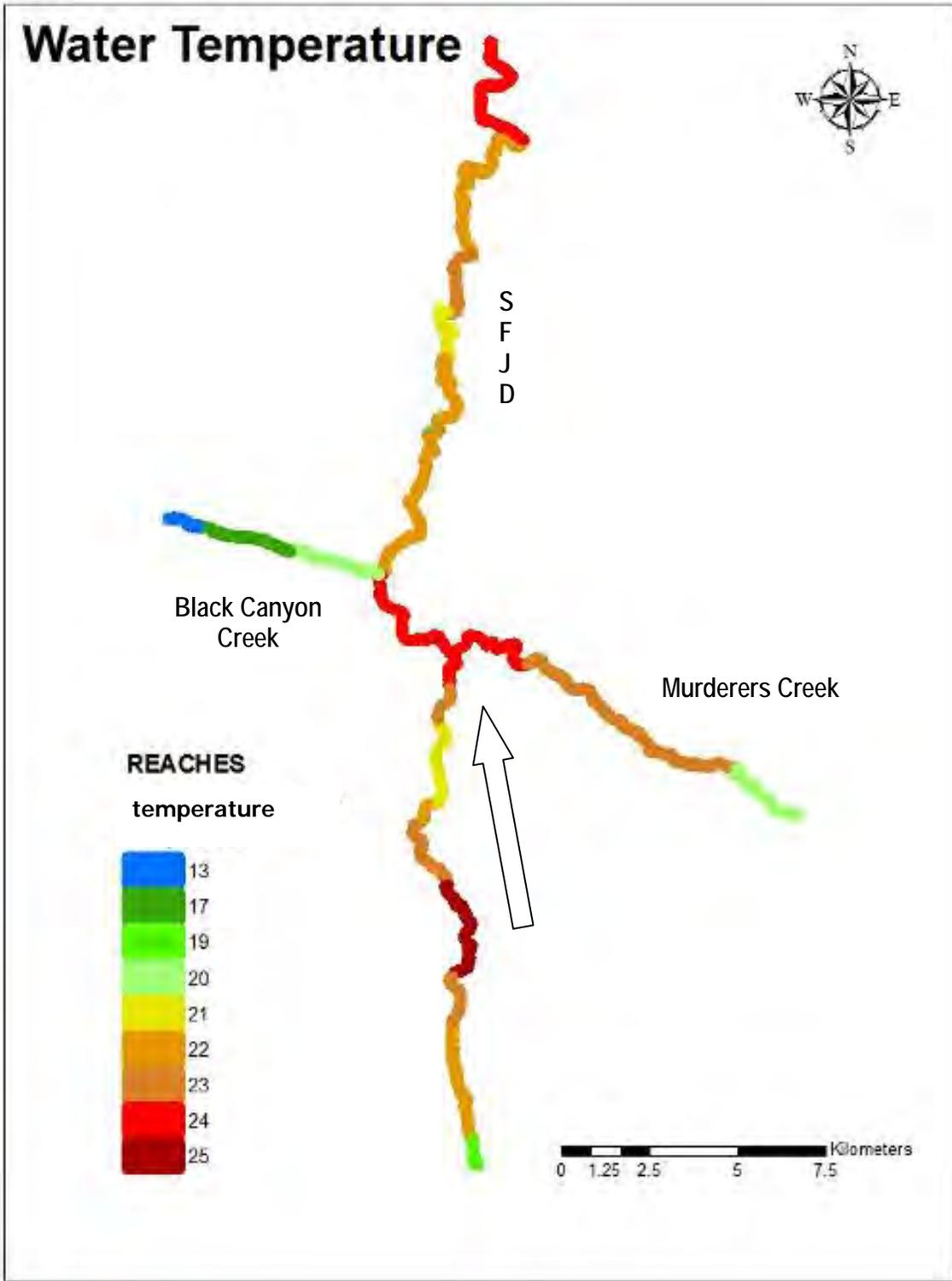


Figure 2. Longitudinal stream temperature patterns by reach in the SFJD Basin. Block arrow denotes the direction of flow.

### *Fish distribution*

The snorkeling survey was conducted at 860 sample sites (740 pools and 120 riffles), throughout all 24 reaches. In Murderers creek we stopped sampling where the habitat became too shallow to snorkel; the total extent of the South Fork John day was sampled up to a natural barrier (Izee falls) for anadromous forms. In 2004 we observed a total of 20,835 fish, 2940 of which were redband trout (RBT). In 2005 we observed a total 31,639 fish of which 5151 were RBT. Redband trout in the South Fork were widely distributed along the entire basin. However, young of the year and small Parr (65 – 150 mm) were principally associated with cooler temperatures and in most cases higher gradient ( $> 4^\circ$ ).

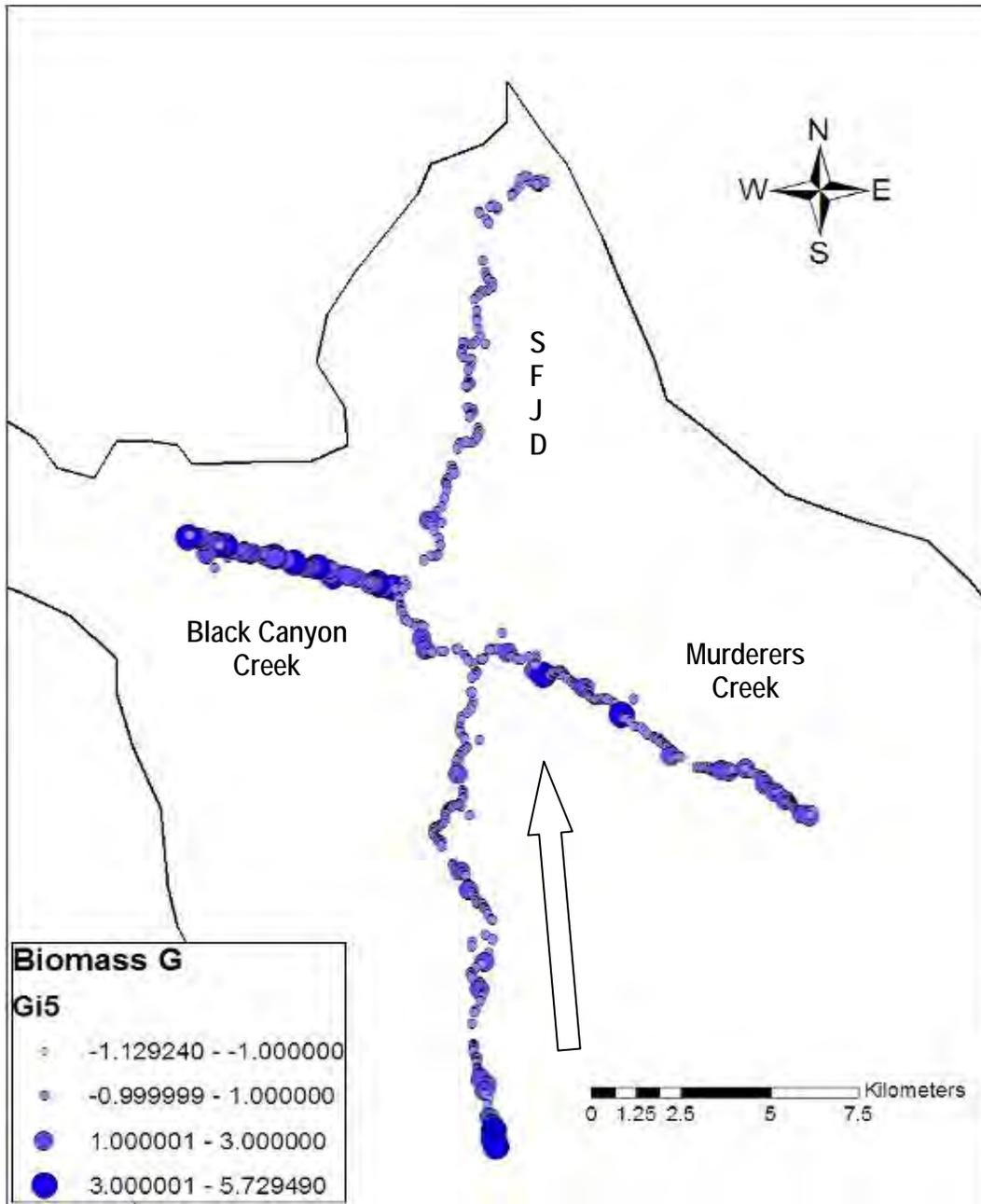
The G-statistic detected three “hot spots”, or areas of high density in the basin: the lower elevation reach in Black Canyon Creek, the highest reach in Murderers Creek and below Izee Falls in the South Fork John Day River proper (Figure 3). The G-statistic indicates that high values (values higher than the mean for the study area) tend to be found near each other. A low value for the G-statistic indicates that values lower than the mean tend to be found together. We applied a threshold of 95% as criteria for this analysis.

- $Gi^* = -1.19$  to  $-1.0$ , moderate spatial correlation of low values
- $Gi^* = -1.0$  to  $1.0$ , no significant spatial correlation of values
- $Gi^* = 1.0$  to  $3.0$ , moderate spatial correlation of high values
- $Gi^* > 3.0$ , significant spatial correlation of high values

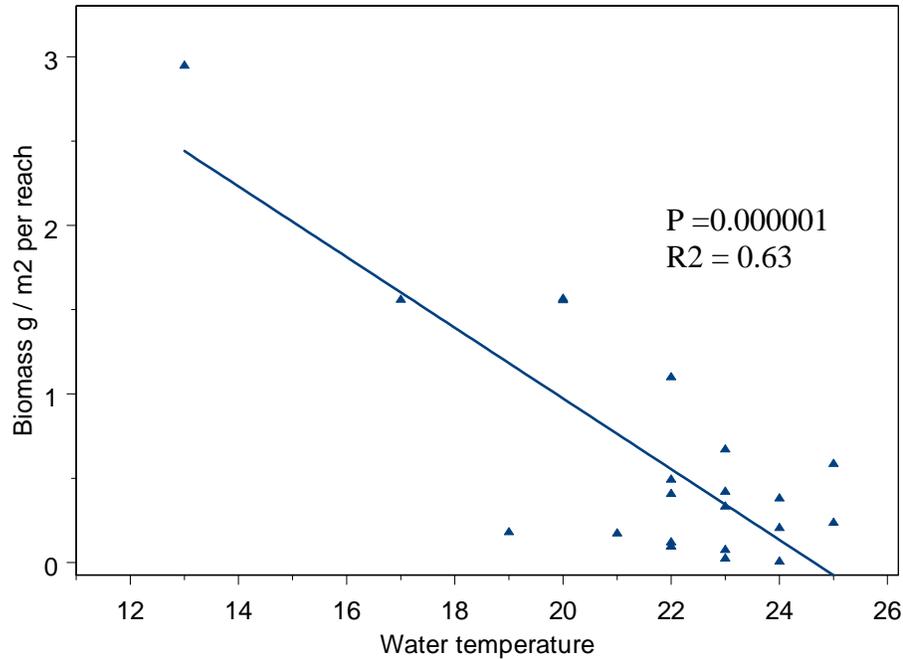
The influence of stream temperatures on carrying capacity was determined from (a) multiple regression analysis and (b) physiological study of the influence of temperature on the induction of hsp 70 and whole body lipids in trout exposed to different temperatures in laboratory experiments and in the field (see Feldhaus 2006) The regression of stream temperature and number of pools per reach on G scores of biomass was significant ( $p=0.000000001$ ). Both variables accounted for 90% of the variation. Temperature accounted for 60% of the variation (Figure 4). Carrying capacity was calculated by summing up standing crops of fishes in stream reaches in the following physiological classes: Optimal ( $<18$  ), Suboptimal (18.1 to 21  $C^\circ$ ), marginal (22.1 to 23  $C^\circ$ ) and poor ( $>23$   $C^\circ$ ).

**Table 1. Carrying capacity (Standing Crops) of redband trout in stream reaches of different physiological constraints.**

<b>Temperature range</b>	<b>&lt; 18 C°</b>	<b>18.1 - 21 C°</b>	<b>21.1 - 23 C°</b>	<b>&gt;23 C°</b>
<b>Total pool area (m<sup>2</sup>)</b>	356	1866.6	2482.2	5082.8
<b>Habitat % (pool area m<sup>2</sup>)</b>	3.64	13.96	25.36	51.93
<b># of Segments</b>	2	4	6	10
<b>Total biomass (kg)</b>	1560	3516	1719	8328
<b>Biomass g (Average / St. Dev )</b>	24.22 / 0.5	6.87 / 9.0	2.32 / 2.23	1.73 / 1.27



**Figure 3.** Identifying statistically distinctive differences in biomasses of redband trout in the mainstem SFJD river, and Black Canyon and Murderers creeks using “Hot Spot” Analysis. Block arrow denotes direction of mainstem flow. Explanation for the Biomass G score is explained in the text.



**Figure 4. The relation between maximum temperature and G-statistic for biomass. Explanation of the G statistic is presented in the text.**

### *Management Implications*

Temperature appears to be a strong indicator of redband trout carrying capacity in an arid basin. The concept has been tested by confirming cause-and-effect relations. In this case, we posed the idea that population behavior was largely determined by physiological tolerances of individual trout. We followed the concept that population distribution patterns were governed by mechanisms at lower levels of biological organization. In this case the levels of organization that provided the explanation were as follows: physiology of individuals down to the behavior of enzyme systems (see Feldhaus 2006).

Many, if not all habitat monitoring, assumes that standing crop information can be used to determine habitat quality, but recent debate questions that assumption (Bélanger & Rodriguez 2002). In practice, population patterns are correlated with physical environmental factors and then habitat quality is inferred from patterns of association. The risk is that confounding interactions, and thus spurious correlations, can make this messy, unless there is an independent process to test specific assumptions.

In our case, we found that the distribution of redband trout was highly correlated with both temperature and pool availability ( $R^2 = 0.90$ ) and that the preponderance of the variation was attributable to temperature alone ( $r^2 = 0.62$ ). However, we backed the field work with an independent study comprising physiological experiments in the laboratory, and validation with physiological assays taken from fishes in the field. Both studies were coordinated so the design and the conduct of each study was informed by the other. For example, field

validation tests of heat shock protein induction and whole body lipid content were taken from sites determined from the field study and the physiological study created the criteria for grouping field data in appropriate categories of physiological performance for redband trout.

This combination of complementary studies helps us pin-point habitat quality issues on a map, which can be seen (figure 2) in the SFJD River as the maroon color-coded reach that starts 5 stream segments upstream from the confluence with Murderers Creek. This map also shows that the hot-spots or centers of high trout density are associated with cold temperatures. We can partition the biomass supported within the basin by temperature classes and project how much biomass increase we might expect through restoration activities that reduce temperature. For instance, we may wish to examine the distribution and quantity of habitats of different quality (i.e., physiological temperature tolerance classes) that occur during high and low flow years. You can get a rough, but accurate picture of the dynamic nature of carrying capacity within a basin.

With this indicator, we can rapidly assess a large number of watersheds in the Columbia River Plateau and rank them in terms of potential productivity (or in need of restoration). The index can be used to monitor restoration effectiveness in temperature-limited streams. These surveys can be conducted rapidly and for relatively low cost through remote sensing devices (e.g. LiDAR and FLIR). The data will consist of a breakdown of stream area by physiological temperature tolerance classes, and indexed to biomass supported within the catchment. If specific quantified estimates of biomass and their distribution are needed, then the accounting model of habitat quality and quantity in relation to standing crops will need to be calibrated with a continuous sampling survey such as that which we designed and executed for the SFJD.

In this study, we reversed the order for which multi-tiered biological assessment and monitoring is usually conducted. As presented, Tier I monitoring is performed to identify population status, Tier II is then designed to determine recovery trends and the effectiveness of the recovery strategy. Stage III, Validation Monitoring, also known as Effectiveness Monitoring, is performed to identify cause-and-effect relationships. The purpose of establishing cause-effect relationships is to be able to extend findings from one case study to other potential habitat restoration locations. We argue that a good restoration plan is designed with specific hypotheses, and that implicit in the study design is a means to test the underlying assumptions (i.e., hypotheses) of the recovery plan in terms of presumed cause-and-effect relationships. If cause-and-effect can be demonstrated, then an index can be established for future use. Indices are valuable because they can save effort, time, and money, but many indices remain unproven. Few have tested the assumptions governing their function. For instance, Hilsenhoff's Index, the Index of Biotic Integrity, and the EPT indices are associative or correlative in nature. Explanations governing the observed patterns are ambiguous at best. Mechanisms have not been examined as has been done with our work.

**4. THESIS: Habitat Classification of Redband Trout Using Physiological Criteria**  
**Joseph Feldhaus**

AN ABSTRACT OF THE THESIS OF

Joseph W. Feldhaus for the degree of Master of Science in Fisheries Science presented on April 27, 2006.

Title: Heat Shock Proteins and Physiological Stress in Redband Rainbow Trout (*Oncorhynchus mykiss gairdneri*) in the South Fork John Day River, Oregon.

Abstract approved:

---

Hiram Li

Scott Heppell

The goal of this research was to characterize thermal habitat requirements for juvenile redband steelhead trout (*Oncorhynchus mykiss gairdneri*) in the South Fork John Day River (SFJD), Oregon using physiological indicators of fish condition. Physiological indices of fish condition measured were whole body lipid content and heat shock proteins, specifically hsp70. Hsp70 is a family of highly conserved molecular chaperones proteins that protect cellular function by preventing irreversible protein damage. Hsp70 levels were measured in liver, white muscle, and fin tissue.

This thesis includes a study of passive integrated transponder (PIT) tag handling stress in juvenile rainbow trout. PIT tags are used to identify individual fish. The objectives of the PIT tag study were to determine if there is a stress response, measured as a difference in plasma cortisol levels, between PIT tagged and non-PIT tagged rainbow trout (*O. mykiss*), to determine if PIT tag-related stress induces hepatic hsp70 synthesis in juvenile rainbow trout, and to examine the relation between cortisol and hsp70 levels. There was a difference in plasma cortisol six hours after tagging, with both treatment and tank effects. There were no differences detected after the 24h sampling period. Plasma cortisol levels were highly variable, but changes in plasma cortisol did not alter hepatic hsp70 levels.

A laboratory experiment demonstrated that hsp70 levels increase significantly between 19 and 22°C in both fin and liver tissue. The finding that hsp70 can be quantified in fin tissue is significant because it provides a non-lethal technique for assessing thermal stress in rare or endangered fish. The response of hsp70 in relation to temperature was sigmoid.

During the summers of 2004 and 2005, a field study in the SFJD was conducted. The objectives were to: (1) determine if SFJD redband rainbow trout experience thermal stress, (2) develop a non-lethal technique for measuring cellular hsp70 levels, (3) and characterize the relation between whole body lipids and hsp70 for fish in the SFJD. Maximum hsp70 levels in liver and white muscle tissue in field collected fish occurred when mean weekly maximum temperatures (MWMT) exceeded 22-23°C. Short and long term stream temperature averages of 15.6-18.2°C and temperature maximums of 18.8-21.6°C significantly increased hsp70 levels in liver tissue. Both the hsp72 and hsp73 isoforms were significantly elevated in white muscle tissue when long and short term average stream temperatures were 16-18.5°C and 19.6-23°C for temperature maximums. Lipid levels began to decrease when MWMT exceeded 23°C.

Results of this research suggest increased cellular hsp70 levels in juvenile redband rainbow trout in the SFJD River is symptomatic of cellular stress related to thermal conditions, as are decreasing lipid levels. Measurement of cellular hsp70 levels provides another tool that can be used to characterize physiological suitability of thermal habitat and potentially to define thermal limits, as differences of 1 or 2°C are sufficient to change expression of thermal stress proteins detected using these techniques. When using hsp70 as an index of thermal stress in different *O. mykiss* subpopulations, differences in hsp70 expression between tissues should be considered.

©Copyright by Joseph W. Feldhaus  
April 27, 2006  
All Rights Reserved

Heat Shock Proteins and Physiological Stress in Redband Rainbow Trout  
(*Oncorhynchus mykiss gairdneri*) in the South Fork John Day River, Oregon

by  
Joseph W. Feldhaus

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

Presented April 27, 2006  
Commencement June 2006

Master of Science thesis of Joseph W. Feldhaus  
presented on April 27, 2006.

APPROVED:

---

Co-Major Professor, representing Fisheries Science

---

Co-Major Professor, representing Fisheries Science

---

Head of the Department of Fisheries and Wildlife

---

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of the Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

---

Joseph W. Feldhaus, Author

## ACKNOWLEDGEMENTS

I would like to express sincere appreciation to Dr. Hiram Li and Dr. Scott Heppell for serving as co-major professors. Hiram and Scott are wonderful mentors and I appreciate their guidance and friendship. I would like to acknowledge Dr. Matt Messa and Dr. Lisa Madsen for serving on my committee, and Dr. Jimmy Yang for serving as the Graduate Council Representative. Financial support for this work was provided by the Bureau of Reclamation (BOR). Michael Newsom (BOR) and Chris Jordan (NOAA Fisheries) provided valuable assistance with efforts to acquire research funds.

I am grateful to Tim Unterwegner, the District Biologist in John Day. Without Tim's patience and oversight, fish collection efforts in the South Fork John Day River would not have been possible. Thanks to Mary Hansen (ODFW) and Laura Tesler (ODFW) for guiding me through the gauntlet of permitting regulations. For helping collect fish samples and proving to be great friends, I am grateful to Ian Tattam, Francisco Madrinan, Jeremiah Osborne-Gowey, George Boxall, Seth White, and Dr. Peter Bayley. Thanks for the memories. Jim Davis, Jens Lovtang, Virgil Mueller, Josh Togstad, Nick Weber, and Matt Webber also provided field and laboratory assistance. Thanks to Jeff Moss at the Prineville, OR Bureau of Land Management office for sharing temperature data.

For help with laboratory work and analysis, I would like to thank Jack Hotchkiss (USGS-CRRL), Cariska Anthony, Grant Feist, Morgan Packard, and Tracey Momoda. Morgan and Tracey, thanks for smiling and keeping my spirits up on the days nothing seemed to work right. I also thank Gene Shippentower for sharing juvenile rainbow trout from his own research. Thanks to Rob Chitwood for making sure all the work at the Smith Farm Fish Performance and Genetics Laboratory went smoothly and efficiently. I would also like to acknowledge Dr. Carl Schreck and Dr. Guillermo Giannico for constructive criticism through all stages of this work.

I would like to acknowledge several professors outside of Oregon State University. Dr. Lee Weber at the University of Nevada Reno showed kindness by letting me visit for a week, and shared lab space and equipment while I learned the basics of Western blotting. For sharing protocols and answering multiple e-mail

questions, I also thank Dr. Ingeborg Werner at the University of California, Davis. Each of these professors provided valuable input and guidance at critical times during the completion of this work and their cooperation is greatly appreciated.

These last two years have been challenging and I have been lucky to have the support of my family. Last, but not least, I need to acknowledge the love and support of my wife, Jodi. Thanks for tolerating the long work weeks, late nights, and the crazy schedule.

## TABLE OF CONTENTS

Chapter 1: General Introduction .....	1
Introduction .....	1
References .....	5
Chapter 2: Passive Integrated Transponders do not alter hepatic hsp70 or plasma cortisol levels .....	8
Introduction .....	8
Materials and methods .....	10
Tissue sample preparation for hsp70 Western blotting.....	12
Western blotting.....	12
Cortisol Assay .....	13
Statistics .....	13
Results .....	14
Plasma cortisol .....	14
Hepatic hsp70 levels .....	15
Discussion .....	16
References .....	21
Chapter 3: Heat Shock Proteins and Physiological Stress in Redband Steelhead Trout ( <i>Oncorhynchus mykiss gairdneri</i> ) in the South Fork John Day River, Oregon. ....	25
Introduction .....	25
Study Area.....	28
Materials and Methods.....	29
Fish Sampling .....	29
Temperature Data.....	30
Laboratory temperature exposure and experimental design .....	31
Tissue sample preparation for hsp70 determination .....	33
Western blotting.....	33
Lipid determination.....	35
Statistics and Data Analysis.....	35
Results .....	36
South Fork John Day River temperature data.....	36
Laboratory experiment.....	37
Fish size and whole body lipids .....	39
Liver hsp70 levels from June-September 2004.....	40
Liver hsp70 levels in field collected fish from 23-24 July 2005.....	41
Caudal fin hsp70 levels.....	42
White muscle hsp70 levels.....	42
Hsp70 levels and lipid content .....	43
Discussion .....	43
References .....	73
Chapter 4: General Discussion.....	79
BIBLIOGRAPHY .....	83
APPENDICES .....	91

## LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1- Log mean plasma cortisol levels (ng/ml) $\pm$ one SE prior to treatment (0 h), and at 2, 6, 24, and 120h after treatment..	19
2- Relative heat shock protein 70 (hsp70) levels prior to experimental treatment (0 h), and 24, and 120h after treatment..	20
3- Map of juvenile redband steelhead trout sampling in the South Fork John Day River catchment, Grant County, Oregon..	56
4- Maximum daily water temperatures ( $^{\circ}$ C) recorded by an instream temperature logger (dashed line) and the Bureau of Reclamation gauging stations (solid line) at Izee falls and Deer Creek from 1 July-15 September 2004..	57
5- Maximum daily water temperatures ( $^{\circ}$ C) recorded by an instream temperature logger at the Below Wind study location and the Bureau of Reclamation gauging station (solid line) at Izee falls from 1 to 23 July 2005..	58
6- Thermal profile of the 19, 22, and 25 $^{\circ}$ C temperature stressors..	59
7- Relative heat shock protein 70 (hsp70) content in liver tissue from rainbow trout ( <i>Oncorhynchus mykiss</i> ) acclimated to 13 $^{\circ}$ C and exposed to an acute temperature stressor of 19, 22, or 25 $^{\circ}$ C followed by a 12-hour recovery at 13 $^{\circ}$ C before sampling..	60
8- Relative heat shock protein 70 (hsp70) content in caudal fin and liver tissue from rainbow trout ( <i>Oncorhynchus mykiss</i> ) acclimated to ambient water temperature (13 $^{\circ}$ C) and exposed to an acute temperature stress of 19, 22, or 25 $^{\circ}$ C followed by a 12-hour recovery at 13 $^{\circ}$ C..	61
9- Whole body lipids (mean %; $\pm$ 1 standard error of the mean) in juvenile redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ) collected from the South Fork John Day River..	62
10- Comparison between years of whole body lipids (mean %; $\pm$ 1 standard error of the mean) in juvenile rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ) collected from the South Fork John Day River. ....	63
11- Mean weekly maximum temperature ( $^{\circ}$ C) compared to whole body lipids (mean %; $\pm$ 1 standard error of the mean) in juvenile redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ) collected from the South Fork John Day River on 24-25 July 2005.....	64

## LIST OF FIGURES (Continued)

<u>Figure</u>	<u>Page</u>
12- Representative Western blot of hsp70 protein detected in liver and fin tissue of juvenile redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ) collected on 23-24 July 2005.....	65
13- Relative heat shock protein 70 (hsp70) band density (mean densitometry value $\pm$ 1 standard error of the mean) in liver tissue from redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ). . . . .	66
14- Relation between relative heat shock protein (hsp70) band density (mean densitometry value $\pm$ 1 standard error of the mean) in liver tissue of redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ) and the maximum temperature within 5 days of capture.....	67
15- Relative heat shock protein 70 (hsp70) band density (mean densitometry value $\pm$ 1 standard error of the mean) in liver tissue from redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ). . . . .	68
16- Relative heat shock protein 70 (hsp70) band density (mean densitometry value $\pm$ 1 standard error of the mean) in liver (black bars) and fin (grey bars) from redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ). . . . .	69
17- Representative Western blot of hsp72 and hsp73 protein detected in white muscle tissue of juvenile redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ) collected on 23-24 July 2005. . . . .	70
18- Relative expression of (a) hsp72 (hsp70) and (b) hsp73 (hsc70) in white muscle tissue from redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ). . . . .	71
19- The mean weekly maximum temperature (MWMT) compared to whole body lipids (%) and relative hsp70 band density in liver tissue of juvenile redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ). . . . .	72

## LIST OF TABLES

<u>Table</u>	<u>Page</u>
1- Sampling locations and coordinates (UTM; NAD 83) for collection locations in the South Fork John Day Basin in 2004 and 2005. ....	53
2- Temperature data (°C) for sampling locations in the South Fork John Day River from 10-11 June 2004; 30-31 July 2004; 11 September 2004; and 23-24 July 2005 .....	54
3- Relation between heat shock protein 70 (hsp70) levels and water temperature measurements preceding fish collection.. ....	55

## LIST OF APPENDICES

<u>APPENDIX</u>	<u>Page</u>
A- Summary of mean whole body lipids (%) and water content (%) of juvenile redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ) collected from the South Fork John Day River..	92
B- Scatter plot of whole body lipids (%) compared to the mean weekly maximum temperature (°C) before capture for juvenile redband rainbow trout ( <i>Oncorhynchus mykiss gairdneri</i> ) collected on 23-24 July 2005..	93
C- Relation between liver tissue hsp70 protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the 24-hour average daily water temperature (°C) ..	94
D- Relation between liver tissue hsp70 protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the 24-hour maximum daily water temperature (°C)..	95
E- Relation between liver tissue hsp70 protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean weekly average daily water temperature (°C).....	96
F- Relation between liver tissue hsp70 protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean weekly maximum daily water temperature (°C).....	97
G- Relation between liver tissue hsp70 protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean monthly average daily water temperature (°C).....	98
H- Relation between liver tissue hsp70 protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean monthly maximum daily water temperature (°C).....	99
I- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the 24-hour average daily water temperature (°C).....	100
J- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the 24-hour maximum daily water temperature (°C).....	101

LIST OF APPENDICES (Continued)

<u>APPENDIX</u>	<u>Page</u>
K- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the maximum water temperature ( $^{\circ}$ C) within five days of capture.....	102
L- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean weekly daily average water temperature ( $^{\circ}$ C).....	103
M- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean weekly maximum daily water temperature ( $^{\circ}$ C).....	104
N- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean monthly average daily water temperature ( $^{\circ}$ C).....	105
O- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean monthly maximum daily water temperature ( $^{\circ}$ C).....	106
P- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the 24-hour average daily water temperature ( $^{\circ}$ C).....	107
Q- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the 24-hour maximum daily water temperature ( $^{\circ}$ C).....	108
R- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the maximum water temperature ( $^{\circ}$ C) within five days of capture.....	109

LIST OF APPENDICES (Continued)

<u>APPENDIX</u>	<u>Page</u>
S- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean weekly daily average water temperature ( $^{\circ}$ C).....	110
T- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean weekly daily maximum water temperature ( $^{\circ}$ C).....	111
U- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and the mean monthly average daily water temperature ( $^{\circ}$ C).....	112
V- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value $\pm$ 1 standard error of the mean) and mean monthly maximum daily water temperature ( $^{\circ}$ C).....	113

## Chapter 1: General Introduction

### Introduction

A Biological Opinion (BiOp) was issued by the US National Oceanic and Atmospheric Administration Fisheries (NOAA-Fisheries) in 2000 that described operations of the Federal Columbia River Power System. One result of the BiOp was a mandated federal research, monitoring, and evaluation program (RME) for the Columbia River Basin. The goal of the tributary Federal tributary RME program is to describe the “health” of fish population processes and their habitat so it can be determined if mitigation or changes to current management are required to ensure survival of Columbia River Basin salmon and steelhead listed under the Endangered Species Act (Bouwes 2005). Prior to wholesale application of the RME approach, it is first being tested through pilot projects in the Wenatchee, John Day, and Salmon river subbasins. These pilot projects will help identify and prioritize restoration actions that will increase freshwater salmonid production (Bouwes 2005).

Monitoring of longitudinal summer stream temperatures is one approach being tested as a method for rapidly assessing the carrying capacity of threatened and endangered salmonids. It has been hypothesized that fish will select habitat offering the best opportunities for growth and survival as outlined by the Ideal Free Distribution (IFD) concept (Li and Bowen, 2003). The ideal free distribution (IFD) theory proposed by Fretwell and Lucas (1970) predicts animals will distribute themselves in habitat of different availability so that animals will receive an equal proportion of available resources. Hughes and Grand (2000) modified the IFD concept and incorporated a physiological model of fish growth that predicts input-matching of fish to resource renewal rates is an exception and that temperature-based size-segregation is more common in nature. By linking summer temperatures with physiological performance indices, it should be possible define summer water temperatures as physiologically suitable, marginal, and unsuitable.

The South Fork John Day subbasin in Northeastern Oregon is on the Oregon 303d list for Water Quality Limited Streams<sup>1</sup>. Anthropogenic disturbances within catchment basins have increased summer stream temperatures, and are believed to be a contributing factor that limits juvenile steelhead trout production. The SFJD is an important rearing and spawning ground for a population of Middle Columbia River steelhead trout (*O. mykiss*) that were listed in 1999 by NOAA-Fisheries as a threatened Evolutionarily Significant Unit (ESU). Steelhead trout east of the Cascade Mountains are considered to be part of the redband trout complex (Behnke 1992) and are sometimes referred to as redband steelhead trout (*O. mykiss gairdneri*). Redband trout can tolerate stream temperatures in excess of 26°C (Behnke 1992; Zoellick 1999). One explanation for the persistence of inland populations of redband rainbow (resident population of *O. mykiss gairdneri*) trout in warm stream reaches is behavioral thermoregulation and not stock adaptation (Li et al. 1994). Another explanation is physiological adaptation to thermal history leading to temperature-dependent swimming performance and aerobic metabolism that allow for short term (< 2h) exposure to temperatures greater than 24°C (Gamperl et al. 2002).

One technique that can be used to examine and document thermal stress in salmonids is measurement of cellular heat shock proteins (Lund et al. 2002, Lund et al. 2003, Werner et al. 2005). Heat shock proteins (hsps), or stress proteins, are highly conserved cellular chaperone proteins (Feder and Hofmann 1999). The genes encoding hsps have been found in every species examined (Feder and Hofmann 1999). Molecular chaperones can be synthesized constitutively or in response to a stressor (Hochachka and Somero 2002). Heat shock proteins assist proper protein folding and are involved with the cellular immune response (Zugel and Kaufmann 1999). Heat shock proteins are also involved in cellular signalling that regulate growth and development and have been hypothesized to alter physiological signals in response to stress or disease states (Nollen and Morimoto 2002). The increase in molecular chaperones in response to heat stress is called the heat shock response (Parsell and Lidquist 1994, Hochachka and Somero 2002). This heat shock, or cellular stress

---

<sup>1</sup> A complete list of 303(d) listed streams can be found at <http://www.deq.state.or.us/wq/303dlist/303dpage.htm>.

response, protects vital cellular functions by binding denatured proteins and preventing inappropriate associations between proteins (Parsell and Lidquist 1994). The cellular stress response can be elicited from a wide range of stressors; include extreme temperature, heavy metals, ultraviolet light, gases, hypoxia, hyperoxia, and exposure to alcohols (Feder and Hofmann 1999; Hochachka and Somero 2002, Sanders 1993). Heat shock proteins have also been identified as potential biomarkers for environmental stress in fish (Iwama et al. 1998).

Before heat shock proteins can be used as an indicator of cellular stress, it is important to establish that the response is not induced by handling the organism during collection (Sanders, 1993). In rainbow trout (*O. mykiss*), handling stress did not alter levels of hepatic hsp70 (Vijayan et al., 1997), and hepatic hp60 levels were not altered in muscle, gill, or heart tissue (Washburn et al., 2002). Common forms of hatchery related stress, including anaesthesia, formalin exposure, hypoxia, hyperoxia, capture stress, crowding, feed deprivation, and cold stress do not alter levels of gill hsp30, hsp70, or hsp90 (Zarate and Bradley, 2003) in Atlantic salmon (*Salmo salar*). The effect on hepatic hsp70 of passive integrated transponder (PIT) tags, commonly used in the Columbia river system to track salmonid movement, levels has not been explicitly studied.

Before hepatic hsp70 levels can be reliably measured in PIT tagged fish, it must first be determined if PIT tags alter hepatic hsp70 levels. PIT technology has been used to estimate survival and travel time of juvenile salmonids between hydroelectric dams equipped with PIT tag readers (Prentice et al. 1990b, Muir et al. 2001, Peterson et al. 1994, Hockersmith et al. 2003), and is being used in the SFJD River to assess the effect of lay flat panel stanchion irrigation dams on seasonal movement, habitat use, growth, and migration timing of juvenile steelhead trout (*Oncorhynchus mykiss*). Using PIT tags to track movement of *O. mykiss* in relation to thermal patterns would be useful.

However, there is evidence that cortisol, the primary stress hormone, plays a role in mediating hsp70 levels in fish tissue following physiological stress (Basu et al. 2001). Chronic increases in plasma cortisol levels can increase disease susceptibility, decrease growth rates, and inhibit reproduction (Hazon and Balment 1998, Schreck

2000, Schreck et al. 2001). No study has directly measured physiological indicators of stress related to PIT tagging, including cortisol and heat shock proteins. If increases in cortisol associated with PIT tagging impair the heat shock response, it would indicate PIT tagging fish in temperature impaired streams (e.g., SFJD River) has unforeseen physiological consequences impacting thermal tolerance not detected in laboratory studies of growth and survival.

The goal of this research is to examine the utility of using heat shock proteins, specifically hsp70, to measure thermal stress in wild populations of redband steelhead trout in the South Fork John Day River. The specific objectives addressed in Chapter 2 are to: (1) determine if there is a stress response, measured as a difference in plasma cortisol levels between PIT tagged and non-PIT tagged rainbow trout (*O. mykiss*), (2) determine if PIT tagging induces hepatic hsp70 synthesis in juvenile rainbow trout, and (3) examine the relation between cortisol and hsp70 levels in juvenile rainbow trout. In Chapter 3, the specific objectives are to (1) determine if redband rainbow trout in the SFJD River are experiencing thermal stress, (2) determine the temperature that increases cellular hsp70 levels, (3) develop a nonlethal technique for measuring cellular hsp70 levels, and (4) characterize the relation between whole body lipids and hsp70 for fish in the SFJD.

Information from this research will increase knowledge about the physiological impacts of elevated stream temperatures on juvenile redband steelhead trout production in the SFJD River. This information will also broaden understanding of how summer water temperatures relate to early life history strategies, growth, survival, distribution patterns, and the carrying capacity and production of summer trout habitat. Physiological criteria for suitability of juvenile redband steelhead trout habitat will also provide a useful index of habitat quality. A physiological index of habitat quality can be used to meet the goals of the Federal tributary RME program.

## References

- Basu, N., T. Nakano, E.G. Grau & G.K. Iwama. 2001. The effects of cortisol on heat shock protein 70 levels in two fish species. *General and Comparative Endocrinology* 124: 97-105.
- Behnke, R.J. & American Fisheries Society. 1992. Native trout of western North America. American Fisheries Society, Bethesda, Md. 275 pp.
- Bouwes, N. ed. 2005. Integrated status and effectiveness monitoring John Day pilot program, 2005 draft annual report. Compiled and edited by Eco Logical Research, Inc. Providence, UT for NOAA Fisheries Service and the Bonneville Power Administration.
- Feder, M.E. & G.E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243-282.
- Fretwell, S.D. and H.L. Lucas. 1970. On territorial behavior and other factors influencing habitat distribution in birds. I. Theoretical development. *Acta. Biotheor.* 19:16-36.
- Gamperl, A.K., K.J. Rodnick, H.A. Faust, E.C. Venn, M.T. Bennett, L.I. Crawshaw, E.R. Keeley, M.S. Powell & H.W. Li. 2002. Metabolism, swimming performance, and tissue biochemistry of high desert redband trout (*Oncorhynchus mykiss ssp.*): Evidence for phenotypic differences in physiological function. *Physiological and Biochemical Zoology* 75: 413-431.
- Hazon, N. & R. Balment. 1998. Endocrinology. pp. 519. *In*: D.H. Evans (ed.) *The physiology of fishes*, CRC Press, Boca Raton
- Hockersmith, E.E., W.D. Muir, S.G. Smith, B.P. Sandford, R.W. Perry, N.S. Adams & D.W. Rondorf. 2003. Comparison of migration rate and survival between radio-tagged and PIT-tagged migrant yearling chinook salmon in the snake and Columbia rivers. *North American Journal of Fisheries Management* 23: 404-413.
- Hochachka, P.W. & G.N. Somero. 2002. *Biochemical adaptation: mechanism and process in physiological evolution*. Oxford University Press, New York. xi, 466 pp.
- Hughes, N.F. & T.C. Grand. 2000. Physiological ecology meets the ideal-free distribution: Predicting the distribution of size-structured fish populations across temperature gradients. *Environmental Biology of Fishes* 59: 285-298.

- Iwama, G.K., P.T. Thomas, R.H.B. Forsyth & M.M. Vijayan. 1998. Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries* 8: 35-56.
- Li, H. W., and M. D. Bowen. 2003. Indexing carrying capacity of salmonids on the basis of longitudinal stream temperatures. United States Bureau of Reclamation, Denver, Colorado.
- Li, H.W., G.A. Lamberti, T.N. Pearsons, C.K. Tait, J.L. Li & J.C. Buckhouse. 1994. Cumulative Effects of Riparian Disturbances along High Desert Trout Streams of the John Day Basin, Oregon. *Transactions of the American Fisheries Society* 123: 627-640.
- Lund, S.G., D. Caissie, R.A. Cunjak, M.M. Vijayan & B.L. Tufts. 2002. The effects of environmental heat stress on heat-shock mRNA and protein expression in Miramichi Atlantic salmon (*Salmo salar*) parr. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1553-1562.
- Lund, S.G., M.E.A. Lund & B.L. Tufts. 2003. Red blood cell Hsp 70 mRNA and protein as bioindicators of temperature stress in the brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* 60: 460-470.
- Muir, W.D., S.G. Smith, J.G. Williams, E.E. Hockersmith & J.R. Skalski. 2001. Survival Estimates for Migrant Yearling Chinook Salmon and Steelhead Tagged with Passive Integrated Transponders in the Lower Snake and Lower Columbia Rivers, 1993-1998. *North American Journal of Fisheries Management* 21: 269-282.
- Nollen, E.A.A. & R.I. Morimoto. 2002. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J Cell Sci* 115: 2809-2816.
- Oregon Department of Environmental Quality. 811 SW Sixth Avenue, Portland, OR 97204-1390. Site accessed on 11 July, 2005.  
<<http://www.deq.state.or.us/wq/303dlist/303dpage.htm>>
- Parsell, D.A. & S. Lindquist. 1994. Heat Shock Proteins and Stress Tolerance. pp. 457-494. In: R.I. Morimoto, A. Tissières & C. Georgopoulos (ed.) *The Biology of heat shock proteins and molecular chaperones*, Cold Spring Harbor Laboratory Press, Plainview, N.Y.
- Peterson, N.P., E.F. Prentice & T.P. Quinn. 1994. Comparison of Sequential Coded Wire and Passive Integrated Transponder Tags for Assessing Overwinter Growth and Survival of Juvenile Coho Salmon. *North American Journal of Fisheries Management* 14: 879-873.

- Prentice, E.F., T.A. Flagg, C.S. McCutcheon & D.F. Brastow. 1990a. PIT-tag Monitoring Systems for Hydroelectric Dams and Fish Hatcheries. American Fisheries Society Symposium 7: 323-334.
- Sanders, B.M. 1993. Stress Proteins in Aquatic Organisms: An Environmental Perspective. *Critical Reviews in Toxicology* 23: 49-75.
- Schreck, C.B. 2000. Accumulation and long-term effects of stress in fish. pp. 147-158. *In: G.P. Moberg & J.A. Mench (ed.) The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*, CABI Publishing, Walingford, UK.
- Schreck, C.B., W. Contreras-Sanchez & M.S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* 197: 3-24.
- Vijayan, M.M., C. Pereira, R.B. Forsyth, C.J. Kennedy & G.K. Iwama. 1997. Handling stress does not affect the expression of hepatic heat shock protein 70 and conjugation enzymes in rainbow trout treated with beta-naphthoflavone. *Life Sciences* 61: 117-127.
- Washburn, B.S., J.J. Moreland, A.M. Slaughter, I. Werner, D.E. Hinton & B.M. Sanders. 2002. Effects of handling on heat shock protein expression in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* 21: 557-560.
- Werner, I., T.B. Smith, J. Feliciano & M.L. Johnson. 2005. Heat shock proteins in juvenile steelhead reflect thermal conditions in the Navarro River watershed, California. *Transactions of the American Fisheries Society* 134: 399-410.
- Zarate, J. & T.M. Bradley. 2003. Heat shock proteins are not sensitive indicators of hatchery stress in salmon. *Aquaculture* 223: 175-187.
- Zoellick, B.W. 2004. Density and biomass of redband trout relative to stream shading and temperature in southwestern Idaho. *Western North American Naturalist* 64: 18-26.
- Zugel, U. & S.H.E. Kaufmann. 1999. Role of Heat Shock Proteins in Protection from and Pathogenesis of Infectious Diseases. *Clin. Microbiol. Rev.* 12: 19-39.

## Chapter 2: Passive Integrated Transponders do not alter hepatic hsp70 or plasma cortisol levels

### Introduction

Passive integrated transponder (PIT) tags provide a method to uniquely identify (Prentice et al. 1990a) and track individual fish movement in streams (Ombredane et al. 1998, Roussel et al. 2000 and 2004; Cucherousset et al. 2005). In the Columbia River Basin, USA, this technology has been used to estimate survival and travel time of juvenile salmonids between hydroelectric dams equipped with PIT tag readers (Prentice et al. 1990b, Muir et al. 2001, Peterson et al. 1994, Hockersmith et al. 2003). Studies examining survival and tag retention of PIT tagged salmonids (Prentice et al. 1990a, Peterson et al. 1994, Gries and Letcher 2002, Dare 2003, Hockersmith et al. 2003) have found high survival and tag retention rates in juvenile salmonids.

In the South Fork John Day (SFJD) River in Northeastern, Oregon, PIT tags are being used to assess the seasonal movement, habitat use, growth, and migration timing of juvenile redband steelhead trout (*Oncorhynchus mykiss gairdneri*) as it relates to longitudinal temperature patterns and barriers to movement, including lay flat panel stanchion irrigation dams. The SFJD is on the Oregon 303d list for Water Quality Limited Streams<sup>2</sup>, and elevated stream temperatures are believed to be a limiting factor in juvenile steelhead trout production. The ability to uniquely identify juvenile trout with PIT tags provides an opportunity to monitor movement and habitat use of individual salmonids in thermally impaired streams such as the SFJD.

No study has directly measured physiological indicators of stress related to PIT tagging, including cortisol and heat shock proteins (hsps). Physiological stress (e.g., handling) results in an elevation in plasma cortisol, the primary stress hormone, that can increase disease susceptibility, decrease growth rates, and inhibit reproduction (Hazon and Balment 1998, Schreck 2000, Schreck et al. 2001). Plasma cortisol also is important for energy metabolism, ion regulation, and can interact with other hormonal

---

<sup>2</sup> A complete list of 303(d) listed streams can be found at <http://www.deq.state.or.us/wq/303dlist/303dpage.htm>.

systems (Hazon and Balment). Heat shock proteins assist proper protein folding, are involved with the cellular immune response (Zugel and Kaufmann 1999), and play important roles in cellular signalling that regulate growth and development and have been hypothesized to alter physiological signals in response to stress or disease states (Nollen and Morimoto 2002).

Heat shock proteins, also called stress proteins, are molecular chaperone proteins that are classified into families based on molecular mass (kilodaltons; kDa). Constitutive hsp isoforms are synthesized under normal cellular conditions and inducible isoforms can be synthesized in response to both biotic and abiotic stressors (Hochacka and Somero 2002). Heat shock proteins are potential biomarkers for environmental stress in fish (Iwama et al. 1998) and have been used to evaluate thermal stress in juvenile salmonids (Lund et al. 2002, Werner et al. 2005).

The hsp70 protein family is highly conserved, correlated with thermal tolerance (Basu et al. 2002, Feder and Hofmann 1999), and highly temperature responsive (Sanders 1993, Parsell and Lindquist 1993). Measuring heat shock protein levels has advantages over measuring stress hormones (e.g., plasma cortisol) because levels of hsp70 in rainbow trout are not altered by handling stress (Vijayan et al. 1997, Washburn et al. 2002). Electrofishing has been reported to have no effect on heat shock protein levels (Lund et al. 2002, Werner et al. 2005). Common forms of hatchery stress including anaesthesia, hypoxia, hyperoxia, capture stress, crowding, feed deprivation, and cold stress were reported to have no effect on gill hsp70 levels in Atlantic salmon (*Salmo salar*; Zarate and Bradley 2003).

There is evidence that cortisol plays a role in mediating hsp70 levels in fish tissue following physiological stress (Basu et al. 2001), and may attenuate gill hsp30 (Ackerman et al. 2000) and hsp90 mRNA expression (Sathiyaa et al. 2001). If PIT tags increase plasma cortisol levels, this increase could adversely affect the ability of trout cells to initiate a heat shock protein response to subsequent thermal stress and may alter normal cellular responses to physiological signals. Therefore, the role between cortisol and hsp's should be investigated prior to using hsp levels as an indicator of physiological stress in PIT tagged fish, and the direct effect of PIT tagging on changes in plasma cortisol should be determined. If increases in cortisol levels

associated with PIT tagging impair the heat shock response, it would indicate PIT tagging fish has unforeseen physiological consequences that compromise thermal tolerance.

Given this background, the objectives of this study were to: (1) determine if there is a difference in plasma cortisol levels between PIT tagged and non PIT tagged rainbow trout (*O. mykiss*), (2) determine if PIT tagging induces hepatic (liver) hsp70 synthesis in juvenile rainbow trout, and (3) examine the relation between cortisol and hsp70 levels in juvenile rainbow trout.

### Materials and methods

#### *Fish and rearing conditions*

The experiment was carried out at the Fish Performance and Genetics Laboratory of the Oregon State University in Corvallis, OR, between August 12 and 14 September 2004. Fish were zero-age hatchery steelhead trout (*Oncorhynchus mykiss*) from the Roaring River Fish Hatchery on the McKenzie River, Oregon. On 12 August 2004 a total of 180 rainbow trout were divided between four outdoor, 1-meter diameter circular fiberglass tanks (45 fish/tank) and exposed to natural photoperiod. Tanks were supplied with 13-14°C flow through well water adjusted to 1 liter/minute flow. The fish were acclimated to experimental tanks for 21 days prior to beginning the experiment. During the acclimation period, fish were fed by hand to satiation twice per day during the week and once per day on weekends with BioDiet Grower, a semi-moist commercial pellet manufactured by Bio-Oregon. Fish were considered satiated when feeding slowed and uneaten pellets remained on the tank bottom. The fish were fasted for 48h before the start of the experiment. For three days after the start of the experiment, all fish were fed 9-10 g of food per tank each day, and then fed to satiation one time each day for the duration of the study. This study was approved by the Institutional Animal Care and Use Committee at Oregon State University under permit #3055.

### *Experimental design and sampling procedures*

After random assignment, Tanks 1 and 3 were assigned to handling control treatments, and Tanks 2 and 4 were assigned as PIT tagging treatments. Before processing each tank of fish, a fresh solution of buffered tricaine methanesulfonate (MS-222) was prepared in 5 liters of water for both light anesthesia (50 mg/l MS-222, 125 mg/l NaHCO<sub>3</sub>) and lethal dose solutions (200 mg/l MS-222, 500 mg/l NaHCO<sub>3</sub>). The light anesthesia was used during tagging and handling. At 0h, six fish were rapidly netted and killed from each tank. These fish provided baseline values for cortisol and hsp70 values prior to handling. Fish were lethally sampled at 2, 6, 24, and 120h post handling. A total of six fish were lethally sampled from each tank during each time period.

Immediately following the initial lethal sampling of a tank, the remaining fish were carefully and rapidly netted from that tank and handled according to the treatment (i.e., PIT tagging or handling control) assigned to the tank. Fish were considered anaesthetized and ready for handling after equilibrium was lost and opercular movements became irregular. It took between eight and ten minutes to process each tank of fish ( $N = 39/\text{tank}$ ).

PIT tagging each fish took approximately three to five seconds per fish. PIT tagging involved inserting a 124.2-kHz PIT tag (11.5 x 2.1 mm; Biomark, ID) into the body cavity using a modified hand held syringe following the methods described in Prentice et al. (1990b). The fish in the non-PIT tag tanks were anesthetized in a manner identical to fish from the PIT tagged tanks and each individual fish was held out of the water for three to five seconds to serve as handling controls. Following handling, all fish were carefully placed back in the experimental tank and allowed to recover.

Length (mm) and mass (g) were recorded for all fish, and blood was collected into ammonium-heparinized capillary tubes by severing the caudal peduncle with a razor blade. Blood was transferred into microcentrifuge tubes, centrifuged, and plasma was separated and stored on ice. Immediately following blood collection, liver samples were removed, wrapped in aluminum foil, and flash frozen in liquid nitrogen. Plasma and liver samples were stored at -80°C for later analysis.

*Tissue sample preparation for hsp70 Western blotting*

Liver samples were lysed in ice-cold lysis buffer (50 mM, 20 mM NaCl, 5 mM EDTA, pH 7.5) containing protease inhibitors (0.5 mg/ml leupeptin, 2 mM phenylmethylsulfonyl fluoride (PMSF), 1 mg/ml aprotinin, and 0.7 mg/mL pepstatin). Liver samples were homogenized with a tissue tearor (Model 985-370; Biospec Products, Inc.) and tissue lysates were centrifuged for 30 minutes at 2700 x g at 0°C, and the resulting supernatant was aliquoted and stored at -80°C. Protein concentrations in lysates were assayed with the bicinchoninic acid (BCA) protein assay method (Sigma-Aldrich, Saint Louis, MO). Plates were read on a Molecular Devices Vmax Kinetic Microplate reader at 570 nm.

*Western blotting*

Western blot analyses were performed based on methods described by Towbin et al (1979). Briefly, protein samples were mixed with an equal amount of SDS sample buffer (Laemmli 1970), and then heated at 95°C for 3 minutes. Equivalent amounts of protein (25 µg) were separated by gel electrophoresis on 8% Tris-Glycine gels (Invitrogen corporation) for 2 hours at 125 volts. A calibrated molecular weight marker (Biorad) and 52-ng recombinant chinook salmon hsp70 protein (StressGen Biotechnologies Corp., Victoria, British Columbia; SPP-763) were applied to each gel to serve as internal standards for molecular weight determination and blotting efficiency. Proteins were transferred to PVDF membrane (Millipore Corp., Billerica, MA, USA) at 100 volts for 1 hr, then blocked overnight at 4°C in blocking solution (5% non-fat dry milk, 20 mM tris buffer, and 0.01% Tween-20). Membranes were incubated at room temperature for 1h with a polyclonal primary antibody for hsp70 (StressGen, SPA-758) at a 1:5000 dilution; then washed three times (10 minutes per wash), in tris-buffered saline solution (TBS), TBS with 0.5% Tween-20 (TBS/Tween), and TBS. According to the manufacturer, this antibody detects proteins of the molecular mass of 70 kDa and 73 kDa, corresponding to the apparent molecular mass of constitutive hsc70 (hsp73) and inducible hsp70 (hsp72) isoforms. In liver tissue from this study, only one band was detected of approximately 70-75 kDa. With one-dimensional gel electrophoresis, it is possible that hsp bands assigned to a size-class

have more than one hsp homologue. Blots were incubated at room temperature for 1h with a 1:5000 dilution of alkaline phosphatase conjugated goat-anti rabbit IgG (StressGen, SAB-301). Blots were rinsed as previously described, and proteins were visualized colorimetrically using an alkaline phosphatase conjugate substrate kit (Biorad, 170-6432) according to manufacturer instructions. Blots were developed for 15 minutes and the reaction was stopped by rinsing with distilled water for 10 minutes. Relative hsp70 band density was quantified by densitometry. Each stained blot was scanned at 600 dpi and 256 shades of grey using a Hewlet Packard 3970 desktop scanner. Band density was measured using ImageQuant 6.1 (Amersham Biosciences). Relative protein band density for each sample is expressed by subtracting background and dividing by the hsp70 protein standard band density.

#### *Cortisol Assay*

Plasma cortisol levels were analyzed using a radioimmunoassay as described in Foster and Dunn (1974) and adapted by Redding et al. (1984). All values below the lowest standard (3.9 ng/ml) were designated to contain 1.95 ng/ml cortisol.

#### Statistics

All heat shock protein and plasma cortisol data were tested for normality and equality of variance. Data for cortisol were log transformed to increase homogeneity of variance. Significance was set at  $P < 0.05$ . Results are reported as the mean  $\pm$  one standard error of the mean. Variation in plasma cortisol levels among tanks at Time 0h suggested tank effects were a potential confounding factor. To account for tank effects in analysis of plasma cortisol data, a nested factorial model was used and tank was nested within treatment. Across time, one-way ANOVA was used to examine changes in fish plasma cortisol levels within individual tanks. Analysis of liver hsp70 levels did not show significant difference in fish liver hsp70 band density among tanks at Time 0h. Therefore, tank effect was not included in statistical models. Hsp70 data was analyzed with one and two-way ANOVA. If significant differences were found, a

Tukey-Kramer multiple comparison test was used. Analyses were conducted with the statistical software packages S-Plus 6.2 (Insightful Corp., Seattle, WA) and SAS (SAS Institute 2003).

## Results

PIT tag retention during this experiment was 100%. The average fork length (mm) of fish sampled in this experiment was 110 mm and ranged from 79 to 142 mm. The average mass (g) was 16.7 g with a range of 7.1-24.6 g.

### *Plasma cortisol*

Within each time block, there was no difference in fish length among the four experimental tanks (ANOVA,  $P > 0.05$ ). There was no evidence that log plasma cortisol levels were explained by fish length at Time 0h ( $F_{1,22} = 0.97$ ,  $P = 0.336$ ), Time 2h ( $F_{1,22} = 3.79$ ,  $P = 0.0645$ ), Time 6h ( $F_{1,22} = 0.18$ ,  $P = 0.678$ ), Time 24h ( $F_{1,22} = 1.21$ ,  $P = 0.283$ ), or Time 120h ( $F_{1,22} = 0.45$ ,  $P = 0.510$ ). Since the length of fish sampled among tanks within a time block was not significantly different and because length was not a significant explanatory variable of log plasma cortisol levels, fish length was not included as an explanatory variable in subsequent analysis.

Prior to the handling stress (Time 0h), there was a significant difference in log plasma cortisol among fish sampled from each of the four experimental tanks ( $F_{3,20} = 4.11$ ,  $P = 0.02$ ) suggesting a tank effect (Figure 1). For this reason, ANOVA models with log plasma cortisol as the response include treatment and tank in analysis for each of the four post treatment time blocks (Time 2, 6, 24, and 120h). At 0h, the only significant difference in log mean plasma cortisol level among tanks was measured in fish sampled from Tank 2 and 3 (log cortisol values = 0.002 to 1.320; Tukey-Kramer  $P = 0.05$ ; Figure 1).

Between 0 and 2h, plasma cortisol levels more than doubled in all tanks (Figure 1). This increase is significant in Tank 3 and 4 (Tukey-Kramer,  $P < 0.0001$ ) but not Tank 2 (Tukey-Kramer,  $P = 0.126$ ). There is suggestive, but inconclusive evidence that plasma cortisol levels from fish measured in Tank 1 (Tukey-Kramer,  $P$

= 0.047) increased between Time 0h and 2h. At Time 2h, treatment effect depends on tank ( $F_{2, 20} = 3.84, P = 0.039$ ). After accounting for the tank by treatment interaction, the effect of treatment at 2h was not significant ( $F_{1, 20} = 1.81, P = 0.19$ ). A comparison of mean log plasma cortisol levels suggests no significant difference between Tank 1, 2, and 3 (Tukey-Kramer,  $P > 0.12$ ). At 2h, there is suggestive, but inconclusive evidence of a difference in log mean plasma cortisol measured in fish sampled from Tank 2 and 4 (Tukey-Kramer,  $P = 0.053$ ).

Between 2h and 6h, mean plasma cortisol levels measured in fish decreased in all tanks (Figure 1). This decrease is significant in the two control tanks (T1 and T3; Tukey-Kramer,  $P < 0.05$ ) but not the PIT tag tanks (T2 and T4; Tukey-Kramer,  $P > 0.10$ ).

At 6h, both the interaction (treatment\*tank;  $F_{2, 20} = 3.81, P = 0.040$ ) and treatment effects were significant ( $F_{1, 20} = 12.85, P = 0.002$ ; Figure 1). Within the 6h time block, there was no significant difference in plasma cortisol levels detected between the two control tanks (T1 and T3; Tukey-Kramer,  $P = 0.095$ ) or the two treatment tanks (T2 and T4; Tukey-Kramer,  $P = 0.617$ ). The log plasma cortisol levels from fish in Tank 1 were significantly different than values measured in Tank 2 (Tukey-Kramer;  $P = 0.024$ ) and Tank 4 (Tukey-Kramer;  $P = 0.0015$ ). Log plasma cortisol levels for fish sampled from control Tank 3 were similar to plasma cortisol levels for fish sampled from both treatment tanks (Tukey-Kramer,  $P > 0.25$ ; Figure 1).

There was no treatment by tank effect at Time 24 or 120h (ANOVA,  $P > 0.20$ ). There were no significant differences in log plasma cortisol levels between treatment and control fish at Time 24h ( $F_{1, 22} = 0.65, P = 0.43$ ) or Time 120h ( $F_{1, 22} = 1.76, P = 0.1985$ ).

#### *Hepatic hsp70 levels*

Hepatic hsp70 levels were measured at Time 0, 24, and 120h (Figure 2). Hepatic hsp70 levels were similar in all tanks at Time 0h ( $F_{3, 20} = 0.68, P = 0.57$ ). Because hepatic hsp70 levels in fish livers were not significantly different at Time 0h among tanks, tank effect was not included in analysis at Time 24 or 120h. There is no evidence that PIT tagging significantly altered hsp70 levels between control and

treatment fish sampled at 24 ( $F_{1, 22} = 0.148$ ,  $P = 0.70$ ) or 120h ( $F_{1, 22} = 0.028$ ,  $P = 0.87$ ). Hepatic hsp70 levels were not correlated with fish length ( $F_{1, 70} = 0.86$ ,  $P = 0.356$ ,  $r^2 = 0.046$ ) or mass ( $F_{1, 70} = 0.837$ ,  $P = 0.36$ ,  $r^2 = 0.012$ ). Through time, hepatic hsp70 levels did not change ( $F_{2, 69} = 2.41$ ,  $P = 0.0974$ ).

### Discussion

Both handling and PIT tagging were stressful events, as indicated by increased plasma cortisol levels. An increase in plasma cortisol levels at 2h post treatment followed by a decrease at 6h indicates a quick response to handling stress followed by rapid recovery. This type of response to handling has been documented by others (Sharp et al. 1998). Jepsen and co-workers (2001) in a radio tagging study with juvenile chinook salmon (*O. tshawytscha*) found that 3h after tagging, radio tagged fish had increased plasma cortisol levels that remained elevated for 24-48 hours after tagging, returning to levels equal to control fish after 7 days. If radio tags, which are larger than PIT tags and involve a much more invasive implantation procedure than PIT tagging did not elicit a chronic increase in plasma cortisol, then it could be expected that PIT tags should not significantly alter cortisol levels relative to fish that were handled as controls.

The cortisol data was more variable than expected. There is evidence PIT tagged fish have increased plasma cortisol levels 6h after tagging compared to non-tagged fish, but these results are inconclusive and may be confounded by tank effects (Figure 1). The absence of a significant decrease in plasma cortisol levels from Time 2h to Time 6h among the two PIT tagging tanks and a significant decrease in plasma cortisol levels among the control tanks offers evidence that PIT tagged fish might have greater plasma cortisol levels than control fish for up to 6 hours post tagging. However, this conclusion is conservative because there is still substantial variation among tanks at Time 6h (Figure 1). If PIT tagging was more stressful on these fish than handling, it could be expected that plasma cortisol levels would be higher (e.g., more than doubled) as observed in this study. In this experiment, a sample size of six fish per tank with two replicates for each treatment may not be sufficient to conclude

PIT tagged fish have higher plasma cortisol levels 6h post tagging compared to a non-PIT tagged fish. Collectively, these data suggests PIT tagging does not cause chronic (> 24h) increases in plasma cortisol levels, but there might be short term effects ( $\leq$  24h). Further investigation is needed to examine short term ( $\leq$  24h) effects of PIT tagging on plasma cortisol levels. To account for possible tank effects, subsequent studies should increase the number of replicate treatment tanks and increase the sample size at each time period.

Unlike the cortisol data, there was less variability in the liver hsp70 data and neither tank nor treatment effects were found. There was no relation between fish length and hepatic hsp70 levels, and there was no correlation between hsp70 and plasma cortisol levels. The conclusion that PIT tag implantation does not alter hepatic hsp70 levels supports other research that shows no effect of handling on hsp70 levels (Vijayan et al. 1997, Washburn et al. 2002, Zarate and Bradley 2003).

Once cellular hsp70 protein production is induced, levels will remain elevated for extended periods of time (> 24h). In brook trout (*Salvelinus fontinalis*), peak hsp70 proteins in both red blood cells and white muscle tissue reached peak levels 12h following an acute temperature stress, and these levels remained elevated for over 48h (Lund et al. 2003). With juvenile chinook (*O. tshawytscha*), a single exposure to 26°C for 10-15 minutes was sufficient to produce significantly elevated hepatic hsp70 levels for 14 days (Mesa et al. 2002). Therefore, we focused our analysis of hsp70 protein levels at 24 and 120h post-treatment. If PIT tagging caused an increase in hsp70 levels, this response would have been expected to be detected in both the 24h and 120h sampling periods.

There was no evidence in this study that plasma cortisol levels altered hepatic hsp70 levels. This is important because elevated cortisol levels can suppress hepatic hsp70 levels in juvenile rainbow trout subjected to an acute heat stress (Basu et al. 2001), and chronically stressed fish may have decreased ability to produce hepatic hsp70 (Basu et al. 2002). If PIT tags increased plasma cortisol levels in a chronic fashion, it could decrease a fish's ability to mount a heat shock response to an hsp70 inducing stressor (e.g., temperature, disease, toxins) which could compromise survival. Alternatively, the increase in plasma cortisol observed at 2h post-handling

may have suppressed synthesis of hsp70 proteins. Also, one-dimensional gel electrophoresis and the antibodies used in this study may not be sensitive enough to detect changes in different hsp70 isoforms.

In conclusion, PIT tagging and the associated handling does not alter liver hsp70 levels. Furthermore, PIT tags have little or no effect on short or long term plasma cortisol levels in juvenile rainbow trout (*O. mykiss*). Since PIT tagging does not alter hepatic hsp70 levels, it should be possible to use PIT tags in combination with lab or field manipulations to examine the effect of specific or multiple stressors on hepatic hsp70 levels. Therefore, PIT tagging fish as a method to study long term effects of stressors on survival, growth, and reproduction of fish looks promising. Together, these results support a growing body of literature documenting the suitability of PIT tags as a low impact tagging procedure for juvenile salmonids.

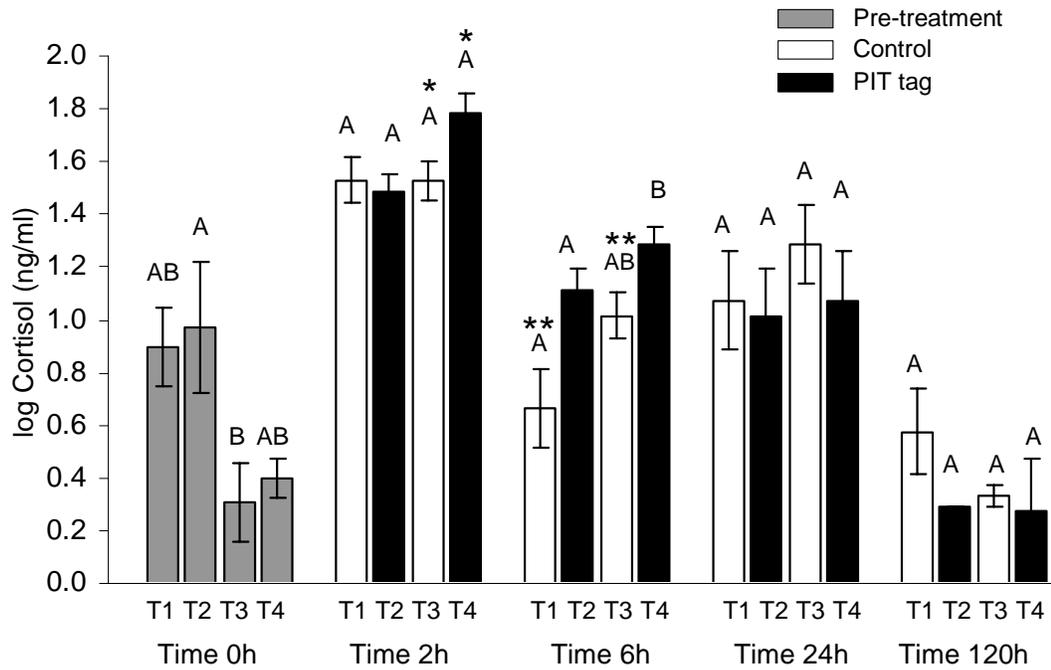


Figure 1- Log mean plasma cortisol levels (ng/ml)  $\pm$  one SE prior to treatment (0 h), and at 2, 6, 24, and 120h after treatment. Open bars represent fish from control tanks (T1, T3) and dark bars represent PIT tag tanks (T2, T4). Shared letters within a time block represent non-significant differences ( $P > 0.05$ ) at that time interval. A single asterisk indicates a significant difference ( $P < 0.05$ ) in a tank from Time 0h to Time 2h. A double asterisk indicates a significant difference in a tank from Time 2h to Time 6h;  $n = 6$  for each bar.

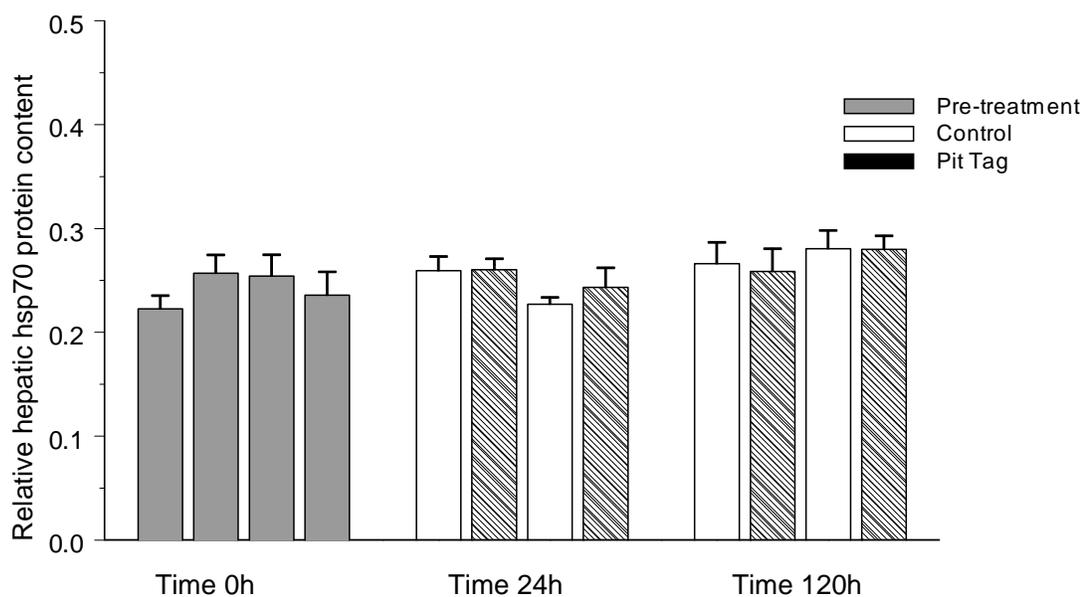


Figure 2- Relative heat shock protein 70 (hsp70) levels prior to experimental treatment (0h), and 24, and 120h after treatment. Open bars represent fish from handling control tanks, dark bars represent fish that were pit tagged. Values represent mean densitometry values of protein bands expressed as density relative to the positive control in the western blot ( $\pm 1$  standard error of the mean) band density;  $n = 6$  for each bar.

### References

- Ackerman, P.A., R.B. Forsyth, C.F. Mazur & G.K. Iwama. 2000. Stress hormones and the cellular stress response in salmonids. *Fish Physiology and Biochemistry* 23: 327-336.
- Basu, N., T. Nakano, E.G. Grau & G.K. Iwama. 2001. The effects of cortisol on heat shock protein 70 levels in two fish species. *General and Comparative Endocrinology* 124: 97-105.
- Cucherousset, J., J.M. Roussel, R. Keeler, R.A. Cunjak & R. Stump. 2005. The use of two new portable 12-mm PIT tag detectors to track small fish in shallow streams. *North American Journal of Fisheries Management* 25: 270-274.
- Dare, M.R. 2003. Mortality and Long-Term Retention of Passive Integrated Transponder Tags by Spring Chinook Salmon. *North American Journal of Fisheries Management* 23: 1015-1019.
- Feder, M.E. & G.E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243-282.
- Foster, L.B., and R.T. Dunn. 1974. Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clinical Chemistry* 20: 365-368.
- Gries, G. & B.H. Letcher. 2002. Tag Retention and Survival of Age-0 Atlantic Salmon following Surgical Implantation with Passive Integrated Transponder Tags. *North American Journal of Fisheries Management* 22: 219-222.
- Hazon, N. & R. Balment. 1998. Endocrinology. pp. 519. *In: D.H. Evans (ed.) The physiology of fishes*, CRC Press, Boca Raton
- Hockersmith, E.E., W.D. Muir, S.G. Smith, B.P. Sandford, R.W. Perry, N.S. Adams & D.W. Rondorf. 2003. Comparison of migration rate and survival between radio-tagged and PIT-tagged migrant yearling chinook salmon in the snake and Columbia rivers. *North American Journal of Fisheries Management* 23: 404-413.
- Hochachka, P.W. & G.N. Somero. 2002. *Biochemical adaptation: mechanism and process in physiological evolution*. Oxford University Press, New York. xi, 466 pp.
- Iwama, G.K., P.T. Thomas, R.H.B. Forsyth & M.M. Vijayan. 1998. Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries* 8: 35-56.

- Jepsen, N., L.E. Davis, C.B. Schreck & B. Siddens. 2001. The Physiological Response of Chinook Salmon Smolts to Two Methods of Radio-Tagging. *Transactions of the American Fisheries Society* 130: 495-500.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Lund, S.G., D. Caissie, R.A. Cunjak, M.M. Vijayan & B.L. Tufts. 2002. The effects of environmental heat stress on heat-shock mRNA and protein expression in Miramichi Atlantic salmon (*Salmo salar*) parr. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1553-1562.
- Mesa, M.G., L.K. Weiland & P. Wagner. 2002. Effects of acute thermal stress on the survival, predator avoidance, and physiology of juvenile fall chinook salmon. *Northwest Science* 76: 118-128.
- Muir, W.D., S.G. Smith, J.G. Williams, E.E. Hockersmith & J.R. Skalski. 2001. Survival Estimates for Migrant Yearling Chinook Salmon and Steelhead Tagged with Passive Integrated Transponders in the Lower Snake and Lower Columbia Rivers, 1993-1998. *North American Journal of Fisheries Management* 21: 269-282.
- Nollen, E.A.A. & R.I. Morimoto. 2002. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J Cell Sci* 115: 2809-2816.
- Ombredane, D., J.L. Bagliniere & F. Marchand. 1998. The effects of Passive Integrated Transponder tags on survival and growth of juvenile brown trout (*Salmo trutta L.*) and their use for studying movement in a small river. *Hydrobiologia* 372: 99-106.
- Oregon Department of Environmental Quality. 811 SW Sixth Avenue  
Portland, OR 97204-1390. Site accessed on 11 July, 2005.  
<<http://www.deq.state.or.us/wq/303dlist/303dpage.htm>>
- Parsell, D.A., and Lindquist, S. 1993. The function of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annual Review of Genetics* 27: 437-496.
- Peterson, N.P., E.F. Prentice & T.P. Quinn. 1994. Comparison of Sequential Coded Wire and Passive Integrated Transponder Tags for Assessing Overwinter Growth and Survival of Juvenile Coho Salmon. *North American Journal of Fisheries Management* 14: 879-873.
- Prentice, E.F., T.A. Flagg, C.S. McCutcheon & D.F. Brastow. 1990a. PIT-tag Monitoring Systems for Hydroelectric Dams and Fish Hatcheries. *American Fisheries Society Symposium* 7: 323-334.

- Prentice, E.F., T.A. Flagg & C.S. McCutcheon. 1990b. Feasibility of Using Implantable Passive Integrated Transponder (PIT) Tags in Salmonids. American Fisheries Society Symposium 7: 317-322.
- Redding, J.M., Schreck, C.B., Birks, E.K., and Ewing, R.D. 1984. Cortisol and Its Effects on Plasma Thyroid Hormone and Electrolyte Concentrations in Fresh Water and during seawater Acclimation in Yearling Coho Salmon, *Oncorhynchus kisutch*. General and Comparative Endocrinology 56: 146-155.
- Roussel, J.M., R.A. Cunjak, R. Newbury, D. Caissie & A. Haro. 2004. Movements and habitat use by PIT-tagged Atlantic salmon parr in early winter: the influence of anchor ice. Freshwater Biology 49: 1026-1035.
- Roussel, J.M., A. Haro & R.A. Cunjak. 2000. Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology. Canadian Journal of Fisheries and Aquatic Sciences 57: 1326-1329.
- Sanders, B.M. 1993. Stress Proteins in Aquatic Organisms: An Environmental Perspective. Critical Reviews in Toxicology 23: 49-75.
- Sathiyaa, R., T. Campbell & M.M. Vijayan. 2001. Cortisol modulates HSP90 mRNA expression in primary cultures of trout hepatocytes. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 129: 679-685.
- Schreck, C.B. 2000. Accumulation and long-term effects of stress in fish. pp. 147-158. *In*: G.P. Moberg & J.A. Mench (ed.) The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare, CABI Publishing, Walingford, UK.
- Schreck, C.B., B.L. Olla & M.W. Davis. 1997. Behavioural responses to stress. *In*: G.W. Iwama, J. Sumpter, A.D. Pickering & C.B. Schreck (Eds.), Fish Stress and Health in Aquaculture, Cambridge University Press, Cambridge, pp. 145-180.
- Schreck, C.B., W. Contreras-Sanchez & M.S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197: 3-24.
- S-Plus version 6.2 for windows, Insightful Corporation, Seattle, Washington USA.
- Towbin, H., T. Staehelin, J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Sciences of the United States of America, Vol 16 (9): 4340-4354.

- Vijayan, M.M., C. Pereira, R.B. Forsyth, C.J. Kennedy & G.K. Iwama. 1997. Handling stress does not affect the expression of hepatic heat shock protein 70 and conjugation enzymes in rainbow trout treated with beta-naphthoflavone. *Life Sciences* 61: 117-127.
- Washburn, B.S., J.J. Moreland, A.M. Slaughter, I. Werner, D.E. Hinton & B.M. Sanders. 2002. Effects of handling on heat shock protein expression in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* 21: 557-560.
- Werner, I., T.B. Smith, J. Feliciano & M.L. Johnson. 2005. Heat shock proteins in juvenile steelhead reflect thermal conditions in the Navarro River watershed, California. *Transactions of the American Fisheries Society* 134: 399-410.
- Zarate, J. & T.M. Bradley. 2003. Heat shock proteins are not sensitive indicators of hatchery stress in salmon. *Aquaculture* 223: 175-187.
- Zugel, U. & S.H.E. Kaufmann. 1999. Role of Heat Shock Proteins in Protection from and Pathogenesis of Infectious Diseases. *Clin. Microbiol. Rev.* 12: 19-39.

### **Chapter 3: Heat Shock Proteins and Physiological Stress in Redband Steelhead Trout (*Oncorhynchus mykiss gairdneri*) in the South Fork John Day River, Oregon.**

#### Introduction

A challenge for fisheries ecologists is choosing the correct scale to study aquatic habitats (Feist et al. 2003). Studying aquatic habitat at the wrong scale can lead to a lack of information and tools for managers to effectively conserve and protect stream fish populations (Fausch et al. 2002). A promising approach for bridging the gap between conservation and research is studying systems using a top-down approach that is capable of integrating information from the bottom up (i.e., riverscape, Fausch et al. 2002). A well studied top-down controller of aquatic ecosystems is temperature. Temperature influences all levels of biological organization, and for this reason, temperature has been called the “abiotic master” (Beitinger et al. 2000).

Water temperature is a criterion that can be used for different purposes. For example, as an indicator of habitat quality it can be manipulated to enhance, protect, and restore aquatic ecosystems. Water temperature can also have community level effects that alter food webs (Tait et al. 1994). At the population level, water temperature has also been found to correspond with distribution patterns of spawning adult chinook salmon (*Oncorhynchus tshawytscha*; Torgersen et al. 1999). Research suggests the biomass of redband rainbow trout (*O. mykiss gairdneri*) is negatively correlated with solar insolation and maximum stream temperatures in streams of the John Day River basin in eastern Oregon (Li et al. 1994) and southwestern Idaho (Zoellick 2004). Moreover, it is hypothesized that temperature is the dominant environmental force driving salmonid evolution and life history strategies (Brannon et al. 2004). Physiological performance and bioenergetics studies on various strains of *O. mykiss* have also pointed to minor differences in growth above 22°C (Myrick and Cech 2000). Water temperature can also influence individual fish microhabitat choice (Baltz et al. 1987), behavior (Cunjak and Green 1986), and suppress appetite and growth (Linton et al. 1998, Railsback and Rose 1999). At the cellular level,

temperature affects virtually all components of cellular processes including protein stability and enzymatic rates (Hochachka and Somero 2002). Within this context, the uncertainties of global climate change effects on natural ecosystems demands a better understanding of how temperature affects the physiological limits of juvenile salmonids, especially as it is transmitted from the individual to the population.

Cellular thermal stress in wild salmonid populations can be measured using heat shock proteins (Lund et al. 2002, Lund et al. 2003, Werner et al. 2005). Heat shock proteins (hsps), or stress proteins, are highly conserved cellular chaperone proteins (Feder and Hofmann 1999). The genes encoding hsps have been found in every species examined (Feder and Hofmann 1999). Molecular chaperones can be synthesized constitutively or in response to a stressor (Hochachka and Somero 2002). Heat shock proteins assist proper protein folding and are involved with the cellular immune response (Zugel and Kaufmann 1999). Heat shock proteins are also involved in cellular signalling that regulate growth and development and have been hypothesized to alter physiological signals in response to stress or disease states (Nollen and Morimoto 2002). The increase in molecular chaperones in response to heat stress is called the heat shock response (Parsell and Lidquist 1994, Hochachka and Somero 2002). This heat shock, or cellular stress response, protects vital cellular functions by binding denatured proteins and preventing inappropriate associations between proteins (Parsell and Lidquist 1994). Because the cellular stress response can be elicited from a wide range of stressors, heat shock proteins have been identified as potential biomarkers for environmental stress in fish (Iwama et al. 1998). Examples of stressors that induce stress proteins include extreme temperature, heavy metals, ultraviolet light, gases, hypoxia, hyperoxia, and exposure to alcohols (Feder and Hofmann 1999; Hochachka and Somero 2002, Sanders 1993).

A particularly well studied heat shock protein is the highly conserved hsp70 family (Sanders 1993). The hsp70 family is also correlated with acquired thermal tolerance (Parsell and Lindquist 1994). However, synthesis of proteins is energetically costly, and the energy required to synthesize additional stress proteins may constitute a significant proportion of an organism's energy budget (Hochachka and Somero 2002). Hargis (1998) determined the induction temperatures for hsp30 and hsp70 to be

between 20 and 21°C in juvenile chinook salmon (*O. tshawytscha*). The induction temperature correlated with nearly complete inhibition of growth and was found to be an indicator of poor fitness, reduced growth rates, and decreased survival. Basu and co-workers (2001) reported unpublished work that suggests the maximal hsp70 induction in trout occurs at 21°C regardless of season, magnitude of heat stress, or acclimation temperature. In a laboratory experiment with juvenile steelhead parr (*O. mykiss*), chronic exposure to 20°C lead to hsp72 synthesis and decreased liver and muscle levels of the high-energy compounds ATP, phosphocreatine, and glycogen (Viant et al. 2003). In brook trout (*Salvelinus fontinalis*), the threshold for hsp synthesis was found to correspond to temperatures shown to influence the distribution of this species (Lund et al. 2003). Recently, Werner et al. (2005) measured expression of hsp72 in white muscle tissue of juvenile steelhead parr (*O. mykiss*) in the Navarro River watershed, California, and concluded these steelhead parr were experiencing thermal stress when maximum daily water temperatures reached 20-22.5°C. Furthermore, they found higher basal levels of hsp72 in fish collected from inland sites than coastal sites but were unable to determine if this was due to acclimatization or genetic adaptation of the subpopulations to thermal conditions. Together, these findings demonstrate that hsps are involved with individual growth, survival, and thermal tolerance. These findings have implications for understanding distribution limits of salmonid subpopulations, and for guiding management actions relating to summer water temperatures.

The South Fork John Day subbasin in Northeastern Oregon is on the Oregon 303d list for Water Quality Limited Streams<sup>3</sup>. Anthropogenic disturbances within catchment basins have increased summer stream temperatures. Elevated stream temperatures are believed to be a contributing factor that limits juvenile steelhead trout production. The South Fork John Day River (SFJD) is an important rearing and spawning location for a population of Middle Columbia River Steelhead trout (*O. mykiss*) that were listed in 1999 by the National Marine Fisheries Service as a threatened Evolutionarily Significant Unit (ESU). Steelhead trout east of the Cascade

---

<sup>3</sup> A complete list of 303(d) listed streams can be found at <http://www.deq.state.or.us/wq/303dlist/303dpage.htm>.

Mountains are considered to be part of the redband trout complex (Behnke 1992) and are sometimes referred to as redband steelhead trout (*O. mykiss gairdneri*). Redband trout have been observed to tolerate stream temperatures in excess of 26°C (Behnke 1992; Zoellick 1999). One explanation for the persistence of inland populations of redband rainbow (resident population of *O. mykiss gairdneri*) trout in warm stream reaches is behavioral thermoregulation and not stock adaptation (Li et al. 1994). Another explanation is physiological adaptation to thermal history leading to temperature-dependent swimming performance and aerobic metabolism that allow for short term (< 2h) exposure to temperatures greater than 24°C (Gamperl et al. 2002).

Given this background, the objectives of this study were to (1) determine if redband rainbow trout in the SFJD River are experiencing thermal stress, (2) determine the temperature that increases cellular hsp70 levels, (3) develop a nonlethal technique for measuring cellular hsp70 levels, and (4) characterize the relation between whole body lipids and hsp70 for fish in the SFJD. Results from this study will increase knowledge about the physiological ecology of juvenile redband steelhead trout in the SFJD River. Information from this research will improve understanding about the effects of the physiological impacts of elevated stream temperatures on juvenile redband steelhead trout production. In turn, this knowledge will improve awareness of how temperature relates to early life history strategies, growth, survival, distribution patterns, and the carrying capacity and production of summer habitat. Physiological criteria for suitability of juvenile redband steelhead trout habitat will also provide a useful index of habitat quality.

### Study Area

The John Day River and its tributaries, the North, Middle, and South forks comprise >800 km of free-flowing river (Torgersen et al. 1999). The John Day River is one of only 42 streams in the contiguous United States exceeding 200 km that remains unpounded (Benke 1990). It is also one of the few drainage systems in the Columbia River basin that still supports wild runs of juvenile fall and spring chinook

salmon (*O. tshawytscha*) and summer steelhead (anadromous redband rainbow trout *O. mykiss gairdneri*; Li et al. 1994).

The SFJD River is located in Grant County, Oregon, and flows northward from the Ochoco and Aldrich mountains. The SFJD River enters the mainstem John Day River at Dayville, Oregon, drains an area of approximately 1637 square kilometers (Leitzinger 1993), and ranges in elevation from 710 to 1646 meters above sea level. The SFJD River is also considered the driest and most sparsely populated area of the John Day subbasins (The Upper John Day River Local Advisory Committee 2002).

Within the SFJD catchment, four tributaries provide spawning habitat for adult summer steelhead. These tributaries, between Dayville and the upstream anadromous fish barrier, Izee falls at river kilometer (RKM) 46.7, are Black Canyon Creek (RKM 22.7), Murderers Creek (RKM 26.6), Wind Creek (RKM 33.8), and Deer Creek (RKM 45.1). Murderers Creek is a 4<sup>th</sup> order stream and Deer, Wind, and Black Canyon Creek are 3<sup>rd</sup> order streams. Both Black Canyon and Wind Creek flow west to east, and Black Canyon drains from the Black Canyon Wilderness. Deer and Murderers Creek flow east to west and the lower section of Murderers Creek flows through the Murderers Creek wildlife preserve. The primary commercial activity in the SJFD catchment is cattle ranching, and most of this occurs within the lower 22.5 kilometers and upstream of Izee falls. Limited cattle ranching activities occur upper and lower Murderers Creek and the lower section of Wind Creek. A dirt logging road follows almost the entire length of Deer Creek, but logging in this catchment is limited.

## Materials and Methods

### *Fish Sampling*

Sampling was completed during the summers of 2004 and 2005 in the South Fork John Day River (SFJD). In 2004, ten fish were collected from each of four different locations on 10-11 June, 30-31 July, and 11 September. In 2005, ten fish were collected from each of eight different study locations from 23-24 July (Table 1, Figure 3). Four of the eight locations visited in 2005 were identical to 2004, and fish

were collected from both riffle and pool habitat. During 2004, all fish were collected by electrofishing using a Smith-Root Model 12B backpack electrofisher. In 2005, fish were collected by beach seine and electrofishing. Collections occurred before water temperatures reached 18°C and were completed within 2-3 h at each study location. Collected fish were held in 5 gallon buckets and water was refreshed with stream water every 10-15 minutes. Fish were removed from the collection bucket, individually anesthetized with a lethal solution of tricaine methanesulfonate (200 mg/l MS-222, 500 mg/l NaHCO<sub>3</sub>) and measured for fork length (mm) and weight (g), and visually examined for signs of parasites or disease. In 2004, 21 of the sampled fish carcasses were donated to the Oregon Department of Fish and Wildlife (ODFW) for disease profiling and these carcasses were not analyzed for lipid content.

For each individual fish, all tissues were removed and frozen on dry ice within five minutes following death. In 2004, only liver and caudal fin tissue was collected. In 2005, liver, lower caudal fin, and white muscle tissue from the caudal peduncle region was collected. Liver and white muscle tissue was quickly excised and wrapped in aluminum foil. Caudal fin tissue was placed in a labeled 0.7 mL eppendorf tube. Fish carcasses were individually packaged, placed in labeled plastic bags, kept on ice, and transported to Oregon State University (Corvallis, OR). Tissue samples were stored at -80°C for later analysis of heat shock proteins. Fish carcasses were stored at -20°C. This study followed the Institutional Animal Care and Use Committee (IACUC) guidelines at Oregon State University under permit #3080.

### *Temperature Data*

Optic Stowaway temperature loggers set to record temperature (°C) each hour were placed at all study locations in June and early July 2004. In 2005, Optic Stowaway and I-button temperature loggers were placed at study locations in May and early June. The logger at the Izee falls and Deer Creek study locations were not launched until the 2<sup>nd</sup> and 12<sup>th</sup> of July, respectively. Discharge and temperature data were also available from gauging stations operated by the US Bureau of Reclamation located in Deer Creek (UTM = 11T 302735mE, 4896710mN; elevation = 1222 meters, stream kilometer 5.6), Murderers Creek (UTM = 11T 297905mE, 4910076mN;

elevation = 908 meters, stream kilometer 0.6), and in the SFJD 10 kilometers upstream of Izee Falls (UTM = 11T 300646mE, 4888621mN; elevation = 1198 meters).

In 2004 and 2005, some temperature records were lost because of logger failure or exposure of the logger to air because of drought conditions. For this reason, temperature records from gauging stations were compared with nearby temperature loggers and data from the gauging stations were used to supplement missing temperature records. Data from the Deer Creek and Izee falls gauging stations were substituted for missing temperature records in the Deer Creek and Izee falls study locations from 1 May through 11 July 2004. The Izee falls gauging station is 10 kilometers upstream of the IZ study location. The Deer Creek gauging station is three kilometers upstream of the Deer Creek study location. In July 2005, temperature data from the Izee falls gauging station were used for the Izee falls study location. The 2004 temperature records for lower Black Canyon were collected by the U.S. Bureau of Land Management. Unless otherwise noted, all other temperature records were collected under the auspices of this project.

#### *Laboratory temperature exposure and experimental design*

The temperature experiment was carried out at the Fish Performance and Genetics Laboratory of the Oregon State University in Corvallis, OR, between 25 October and 20 November 2004. Fish were first generation yearling steelhead trout (*Oncorhynchus mykiss*) spawned from wild parents captured from the Umatilla River, Oregon near the Umatilla Fish Hatchery. Adult steelhead were transported to the Fish Performance and Genetics Laboratory, and spawned in spring 2003. A total of 144 yearling fish were evenly and randomly divided between 9 indoor, 1-meter circular fiberglass tanks (16 fish/tank) in an indoor facility. Ambient water entering the tanks was 13.0°C flow through well water and water depth in all tanks was 55 centimeters. Fish were acclimated to experimental tanks for 10 days. During acclimation, fish were fed by hand once per day to satiation with BioDiet Grower, a semi-moist commercial pellet manufactured by Bio-Oregon. Fish in each individual tank were fasted for 24 h before the start of the experiment.

Flow through well water was heated in a 387 liter insulated tank by a Hayward Electric Spa Heater (Model CSPA XII1). Heated water was gravity fed into experimental tanks at 2.0 liters/minute. The experiment was a block design with three blocks. Each block represented three days. Within each block were three tanks, and each tank was randomly assigned a temperature stressor of 19 (stressor 1), 22 (stressor 2), or 25°C (stressor 3). The order for completing each temperature treatment within a block was randomly determined and one treatment was completed each day. In each tank, between six and nine Onset Optic Stowaway temperature loggers recorded temperature (°C) once every minute. These temperature records were used to calculate rate of heating in each tank and were used to determine the length of time fish were exposed to the respective temperature stressor. Temperature loggers were placed on the bottom of the tank, as well as suspended from the standpipe 18 and 36 centimeters below the water surface. After 24 h of food restriction, and immediately before introducing warm water to the tank, six control fish were netted and placed in a lethal solution of buffered tricaine methanesulfonate at a concentration of 200 mg/l with 500 mg/l NaHCO<sub>3</sub>. Next, warm water was introduced to the experimental tank at a rate of 2 L/minute. During the experiment, water temperature was manually monitored with a thermometer. Temperatures in each tank were not allowed to exceed target temperatures (i.e., 19.0, 22.0, or 25.0 °C) by more than 0.5°C. The time at which the target temperature was first achieved for each experimental tank was recorded. Cold ambient water (13°C) was reintroduced to each experimental tank at a rate greater than 5 L/min 90 minutes after the target temperature for the experimental treatment was first recorded. Fish remained in the experimental tank for 12 hours following the end of the temperature stress (i.e., reintroduction of 13°C water) and were lethally sampled in buffered MS-222.

Length (mm) and weight (g) were recorded for all lethally sampled trout. For each fish, tissue was removed from the lower caudal fin and placed in a labeled 0.7 mL eppendorf tube, and flash frozen in liquid nitrogen. Fish livers were quickly excised, wrapped in aluminum foil, and flash frozen in liquid nitrogen. Tissue samples were transported to Oregon State University (Corvallis, OR) and stored at -80°C for later analysis.

Liver hsp70 levels were determined for all fish sampled prior to the temperature stressor (pretreatment; control) from each tank. Following the temperature stressor, six fish from each tank were randomly selected for further analysis. The remaining samples were not analyzed. To compare fin and liver hsp70 levels, a total of eight fish were randomly selected from the entire collection of control samples, and eight fish were randomly chosen from each of the 19, 22, and 25°C temperature stressors.

#### *Tissue sample preparation for hsp70 determination*

Liver samples were lysed in ice-cold lysis buffer (50 mM Tris base, 20 mM NaCl, 5 mM EDTA, pH 7.5) containing protease inhibitors (0.5 mg/ml leupeptin, 2 mM phenylmethylsulfonyl fluoride (PMSF), 1 mg/ml aprotinin, and 0.7 mg/ml pepstatin). Liver samples were homogenized with a tissue tearor (Model 985-370; Biospec Products, Inc.) and lysates were centrifuged for 5-10 minutes at 13000 x g at 1°C or 4500 x g for 30 minutes at 1°C, and the resulting supernatant was aliquoted and stored at -80°C. Fin tissue was weighed on an analytical balance ( $\pm 0.1$  mg), placed in a 1.5 mL eppendorf tube, frozen with liquid nitrogen and crushed into small pieces with a Teflon pestle. Lysis buffer and protease inhibitors (volume adjusted for fin mass) were added and the tissue further homogenized by hand. Fin samples were spun at 4500 x g at 1°C for 30 minutes, aliquoted, and frozen at -80°C. Protein concentrations in lysates were assayed with the bicinchoninic acid (BCA) protein assay method (Sigma-Aldrich, Saint Louis, MO). Plates were read on a Molecular Devices Vmax Kinetic Microplate reader at 570 nm.

#### *Western blotting*

Western blot analyses were performed based on methods described by Towbin et al (1979). Briefly, protein samples were mixed with an equal amount of SDS sample buffer (Laemmli 1970), and then heated (95°C) for 3 minutes. Equivalent amounts of protein for liver (25  $\mu$ g) and fin (25  $\mu$ g) were separated by gel electrophoresis on 8% Tris-Glycine gels (Invitrogen corporation) for 2 hours at 125

volts. A calibrated molecular weight marker (Biorad) and 52-ng recombinant chinook salmon hsp70 protein (StressGen Biotechnologies Corp., Victoria, British Columbia; SPP-763) were applied to each gel to serve as internal standards for molecular weight determination and blotting efficiency. Proteins were transferred to PVDF membrane (Millipore Corp., Billerica, MA, USA) at 100 volts for 1h, then blocked overnight at 4°C in blocking solution (5% non-fat dry milk, 20 mM tris buffer, and 0.01% Tween-20). Membranes were incubated at room temperature for 1 hour with a polyclonal primary antibody for hsp70 (StressGen, SPA-758) at a 1:5000 dilution; then washed three times (10 minutes per wash) in tris-buffered saline solution (TBS), TBS with 0.5% Tween-20 (TBS/Tween), and TBS. According to the manufacturer, this antibody detects proteins of the molecular mass of 70 and 73 kDa, corresponding to the apparent molecular mass of constitutive hsc70 (hsp73) and inducible hsp70 (hsp72) isoforms. Two bands were detected in white muscle tissue that apparently correspond with hsp72 and hsp73, but one band of approximately 70-75 kDa was detected in liver and fin tissue. With one-dimensional gel electrophoresis, it is possible that hsp bands assigned to a size-class have more than one hsp homologue. Blots were then incubated at room temperature for 1 hour with a 1:5000 dilution of alkaline phosphatase conjugated goat-anti rabbit IgG (StressGen, SAB-301). Blots were rinsed as previously described, and proteins were visualized colorimetrically using an alkaline phosphatase conjugate substrate kit (Biorad, 170-6432) according to manufacturer instructions. Blots were developed for 15 minutes and the reaction was stopped by rinsing with distilled water for 10 minutes. Relative hsp70 band density was quantified by densitometry. Each stained blot was scanned at 600 dpi and 256 shades of grey using a Hewlet Packard 3970 desktop scanner. Band density was measured using ImageQuant 6.1 (Amersham Biosciences). Protein band density is expressed by subtracting background and dividing by the hsp70 protein standard band density.

It is unknown if the single band of hsp proteins detected in the liver and fin tissue are homologous to the apparent hsp72 and hsp73 protein bands detected in white muscle tissue. Fin and liver tissue samples run side by side on the same gel with the same quantity of protein (25 µg) had bands develop on the same horizontal plane

at approximately 70-75 kDa. Fin and liver hsp70 results are compared using paired t-tests, but it should be noted that these may be different isoforms.

#### *Lipid determination*

Total lipid content for each fish was determined following the methods described in Anthony et al. (2000) and Reynolds and Kunz (2001). Briefly, to determine laboratory wet mass, fish were thawed and weighed on an analytical balance ( $\pm 0.1$  mg). Total body water (TBW, g) was determined by drying fish to a constant mass (i.e.,  $\pm 0.01$  g of previous 24hr mass) in a convection oven set at 60°C and TBW was calculated by subtracting the difference between the laboratory wet mass and the dry mass. Fish were then thoroughly homogenized with mortar and pestle. Lipids were extracted from dried samples with a Soxhlet apparatus and a 7:2 (v/v) hexane/isopropyl alcohol solvent system. Fat mass was determined by subtracting the mass of dried homogenized fish before Soxhlet extraction from the mass of lean dry fish mass after fat extraction. Whole body lipid (WBL) content was calculated by dividing the fat mass by lean dry mass. Fish wet mass recorded at the time of capture was not used to calculate fat content because internal organs and tissue were removed from each fish and subsequent variation in WBL based on field wet mass would be increased. Percent water (PW) was calculated by dividing TBW by laboratory wet mass.

#### Statistics and Data Analysis

All data were tested for normality and equality of variance. Significance was determined at  $P < 0.05$ . Results are reported as  $\pm$  one standard error of the mean. In the laboratory experiment, block (time) by treatment effects were tested using two-way ANOVA. There was no effect of time on control fish so data were subsequently pooled, and the three temperature stressors were compared using one-way ANOVA and multiple linear regression. Comparisons of fin and liver hsp70 data in the laboratory experiment were done with one-way ANOVA followed by a Tukey-Kramer multiple comparison test. For fish collected from the SFJD River in 2004, one-way

ANOVA was used to test for differences among locations within months and in a location between months. A two-way ANOVA was used to test for differences between year and location at lower Black Canyon, Below Wind Creek, Izee Falls, and Deer Creek. A Tukey-Kramer multiple comparison test was applied when significant differences were found (SAS Institute 2003). The estimated induction temperature (i.e., increased protein synthesis over basal levels), and associated 95% confidence intervals, were determined with sigmoid curves constructed with the statistical software package GraphPad Prism (San Diego, CA). All other analyses were completed with the statistical software package S-Plus 6.2 (Insightful Corp., Seattle, WA).

## Results

### *South Fork John Day River temperature data*

When using gauging station data for supplemental temperature records for 2004, temperature data from the Deer Creek (DC) and Izee gauging stations were compared with nearby instream temperature loggers. There were few differences between the maximum daily temperatures recorded at Izee falls gauging station and the instream temperatures recorded by temperature loggers at the Izee falls (IZ) study location (Figure 4). From 2 July-15 September 2004, the Izee gauging station recorded max daily water temperatures that were an average of 0.3°C lower than the downstream temperature logger at the IZ study location. In 2004, the maximum water temperature recorded at IZ and the Izee falls gauging station were 23.5°C and 23.7°C, on 25 July. During 2004, the DC temperature logger recorded maximum daily water temperatures that were an average of 0.2°C higher than temperatures recorded at the DC gauging station from 12 July-15 September 2004. In 2004, the maximum water temperature (19.4°C) at the DC study location occurred on 25 July. On the same day, the DC gauging station recorded a temperature maximum of 19.2°C. The coldest study location in 2004 was DC.

In 2005, the location with the lowest maximum daily water temperature recorded prior to fish capture was Upper Black Canyon Creek (18.4°C), while maximum daily water temperatures approached 26°C at Lower Murderers Creek (25.8°C) and in the South Fork John Day River at river kilometer 24 (25.4°C; Table 2). Additional temperature measurements are summarized in Table 2. From 1 July to 23 July 2005, a similar temperature profile is displayed at the Izee falls gauging station and the Below Wind Creeks study location (

Figure 5). The similarity in temperature records between BW and the IZ gauge in 2005 suggests that in 2004, temperatures at the Below Wind study location were equal to or slightly higher than the temperatures recorded at the Izee Falls gauging station in 2004.

#### *Laboratory experiment*

The average fork length of fish in this experiment was 144 mm (range = 115-170 mm) and average mass was 32.1g (range = 15.9-56.5 g). The temperatures reported for this experiment are the average of 6 or 9 Optic Stowaway temperature loggers. The vertical thermal profile indicates that temperatures were uniform throughout the tanks without any obvious stratification.

Peak temperatures recorded for trials 1-3 were 19.5, 22.7, and 25.7°C. For stressor 1, temperatures were maintained above 19°C for an average of 119 minutes (range = 111-126 minutes). On average it took 246 minutes to change the water temperature from 13 to 19°C (stressor 1) with an average heating rate of 1.5°C/hr (range = 1.2-1.7°C/hr). For stressor 2, temperatures were maintained above 22°C for an average of 105 minutes (range = 102-111 minutes). It took an average of 286 minutes to change the water temperature from 13 to 22°C, with an average heating rate of 1.9°C/hr (range = 1.7-2.1°C/hr). Water temperature was maintained above 25°C (stressor 3) for an average of 91 minutes (range = 76-107 minutes). On average, it took 333 minutes to change the water temperature from 13 to 25°C, and the average heating rate was 2.2°C/hr (range = 1.9-2.3°C/hr). Fish were lethally sampled 12 hours after reintroduction of 13°C (ambient) well water. A representative temperature profile for each temperature stressor is shown in Figure 6.

There was no interaction between block and treatment ( $F_{4, 99} = 0.385$ ,  $P = 0.887$ ), but there was a significant treatment effect ( $F_{2, 99} = 20.43$ ,  $P < 0.0001$ ). After accounting for block, there was no effect of the 19°C temperature stressor on liver hsp70 band density ( $F_{1, 102} = 0.05$ ,  $P = 0.815$ ). There was a significant difference in relative hsp70 band density between control and treatment fish following the 22°C ( $F_{1, 102} = 46.79$ ,  $P < 0.0001$ ) and the 25°C temperature stressors ( $F_{1, 102} = 560.45$ ,  $P < 0.0001$ ). The mean relative hsp70 band density in fish exposed to 22°C was 0.164 units greater than control fish, and this difference was significant ( $t_{102} = 6.84$ ,  $P < 0.0001$ ). Treatment fish exposed to 25°C had significantly greater hsp70 band density measurements than treatment fish exposed to 22°C ( $t_{102} = 13.745$ ,  $P < 0.0001$ ; Figure 7). Based on this analysis, the induction temperature for hsp70 protein in the livers of rainbow trout tested in this experiment is between 19 and 22°C, with a significantly elevated response occurring at 25°C (Figure 7).

The relative hsp70 band density measured in fin tissue was similar to liver tissue. Band density in both tissues increased with increasing temperature. The greatest band density measured in fin and liver tissue was from fish exposed to the 25°C treatment (Figure 8). Temperature had a significant effect on relative hsp70 band density in fin tissue ( $F_{3, 28} = 352.5$ ,  $P < 0.0001$ ). There was no difference in fin hsp70 band density following 19°C exposure when compared to controls ( $F_{1, 28} = 0.67$ ,  $P = 0.42$ ), but there was a significant effect of the 22°C ( $F_{1, 28} = 286.5$ ,  $P < 0.0001$ ) and 25°C ( $F_{1, 28} = 751.4$ ,  $P < 0.0001$ ) temperature stressors (Figure 8). Treatment fish exposed to 22°C had significantly elevated levels of hsp70 in fin tissue compared to those exposed to 19°C ( $F_{1, 28} = 10.53$ ,  $P < 0.0001$ ). Treatment fish exposed to 25°C had significantly greater fin hsp70 levels than fish exposed to 22°C ( $F_{1, 28} = 109.96$ ,  $P < 0.0001$ ). There is evidence that mean hsp70 band density measurements in liver and fin tissue are not the same at each temperature treatment ( $F_{3, 56} = 3.19$ ,  $P = 0.031$ ). Hsp70 band density measurements are different between liver and fin tissue at 22°C (see Figure 8; paired t-test,  $P = 0.043$ ), but not at any other temperature ( $P > 0.05$ ). Based on this analysis, the induction temperature for hsp70 protein in the fin tissue of rainbow trout tested in this experiment is between 19 and 22°C. The difference in

hsp70 levels in fin and liver tissue at 22°C suggests there is a difference in the cellular hsp70 response between these two tissues (Figure 8).

#### *Fish size and whole body lipids*

The fork length (mm) of fish handled in June 2004 was  $125.4 \pm 20.7$  (average  $\pm$  SD, range 95-125 mm),  $145.6 \pm 19.2$  mm (114-181 mm) in July 2004,  $145.5 \pm 19.4$  mm (113-198 mm) in September 2004, and  $126.3 \pm 19.5$  (94-180 mm) in July 2005. Whole body lipids (WBL) were determined on 99 juvenile rainbow trout in 2004 and 80 in 2005.

In 2004, there were no differences in WBL between locations for fish sampled at lower Black Canyon (LBC), Below Wind (BW), Deer Creek (DC), and Izee Falls (IZ) on 10-11 June ( $F_{3, 24} = 1.04$ ,  $P = 0.394$ ) or 11 September 2004 ( $F_{3, 32} = 1.98$ ,  $P = 0.136$ ; Figure 9). There was a significant difference in WBL among locations for fish sampled at LBC, BW, DC, and IZ on 30-31 July 2004 ( $F_{3, 31} = 7.36$ ,  $P < 0.001$ ). In July 2004, fish collected at BW had significantly lower WBL than fish collected at DC (Tukey-Kramer,  $P = 0.001$ ), LBC (Tukey-Kramer,  $P = 0.0164$ ), and IZ (Tukey-Kramer,  $P = 0.003$ ), (Figure 9). In general, WBL levels increased from June to July (Figure 9). In 2004, the interaction between month and location did not explain WBL levels ( $F_{6, 87} = 0.893$ ,  $P = 0.504$ ), but the effect of location ( $F_{3, 87} = 8.25$ ,  $P < 0.0001$ ) and month were highly significant ( $F_{2, 87} = 13.49$ ,  $P < 0.0001$ ). After accounting for location and month, the addition of mean weekly maximum temperature (MWMT) prior to fish capture at each location did not explain WBL ( $F_{4, 89} = 0.66$ ,  $P = 0.62$ ).

From June through September, there was a significant change in WBL from fish sampled in DC ( $F_{2, 24} = 9.73$ ,  $P = 0.0009$ ) and LBC ( $F_{2, 22} = 3.966$ ,  $P = 0.034$ ), but not IZ ( $F_{2, 22} = 3.04$ ,  $P = 0.068$ ) or BW ( $F_{2, 23} = 1.62$ ,  $P = 0.22$ ). In Deer Creek, there was a significant increase in mean WBL from June to July (Tukey-Kramer,  $P = 0.003$ ) and a significant decrease from July to September (Tukey-Kramer,  $P = 0.003$ ; Figure 9). At lower Black Canyon, there was a significant increase in WBL from June to July (Tukey-Kramer,  $P = 0.029$ ) but not July to September (Tukey-Kramer,  $P = 0.16$ ; Figure 9).

The same pattern for WBL determined for July 2004 was observed in July 2005 at DC, LBC, BW, and IZ (Figure 10). In 2005, there was a significant difference in WBL among all eight study location on 24-25 July ( $F_{7,72} = 5.27$ ,  $P < 0.0001$ , Figure 11) In 2005, mean WBL levels ranged from 13.9% for fish collected from lower Murderers Creek (LMC) to 21.7% for fish collected at upper Murderers Creek (UMC). Arranging the study locations from the lowest mean weekly average temperature recorded at upper Black Canyon (UBC) to the highest maximum daily temperature recorded at LMC suggests a pattern where mean WBL increase from UBC to IZ and then begins to decrease as temperature increases at BW, SF, and LMC (Figure 11). A second order polynomial regression of MWMT on WBL explains 19% of the variation between WBL and MWMT.

#### *Liver hsp70 levels from June-September 2004*

One protein band was detected on western blots of liver tissue and this band contains proteins of approximately 70-75 kDa (Figure 12). On 10-11 June 2004, there were no significant differences in liver hsp70 band density measured at DC, LBC, IZ, or BW ( $F_{3,36} = 2.56$ ,  $P = 0.070$ ; Figure 13). Significant differences in hsp70 band density were detected among locations on 30-31 July ( $F_{3,34} = 30.56$ ,  $P < 0.0001$ ) and 11 September ( $F_{3,35} = 21.51$ ,  $P < 0.0001$ ).

In July 2004, liver hsp70 band density increased with increasing water temperature among locations ( $r^2 = 0.729$ ). The liver hsp70 band density measured in fish collected from DC and LBC were similar (Tukey-Kramer,  $P = 0.06$ ; Figure 13). There was a significant difference in liver hsp70 band density between LBC and IZ (Tukey-Kramer,  $p < 0.0001$ ). There was no difference in hsp70 band density between BW and IZ (Tukey-Kramer,  $P = 0.09$ ; Figure 13).

In September, fish from DC had the lowest hsp70 band density measurement and this was similar to IZ (Tukey-Kramer,  $P = 0.137$ ), but not LBC or BW (Tukey-Kramer,  $P > 0.05$ , Figure 13). There was no significant difference in hsp70 band density between LBC and IZ in September (Tukey-Kramer,  $P = 0.208$ ) and all locations were significantly different than BW.

Across months, there was a significant difference in hsp70 band density in fish collected at LBC ( $F_{2, 26} = 4.85$ ,  $P = 0.016$ ), BW ( $F_{2, 27} = 116.5$ ,  $P < 0.0001$ ) and IZ ( $F_{2, 27} = 25.72$ ,  $P < 0.001$ ) but not at DC ( $F_{2, 27} = 2.26$ ,  $P = 0.123$ ). From June to July, there was a significant increase in hsp70 band density measured in fish collected at LBC, BW, and IZ (Tukey-Kramer,  $P < 0.05$ ). From July to September, a significant decrease in hsp70 band density was measured in fish collected from DC, IZ, and BW (Tukey-Kramer,  $P < 0.05$ ) but not LBC (Tukey-Kramer,  $P = 0.093$ ; Figure 13).

*Liver hsp70 levels in field collected fish from 23-24 July 2005*

In July 2005, there were significant differences in liver hsp70 levels between study locations ( $F_{7, 68} = 35.04$ ,  $P < 0.0001$ ). In 2005, the lowest hsp70 levels were measured in fish collected from UBC and DC. Liver hsp70 levels measured in fish collected from LBC were significantly different than liver hsp70 levels measured in fish collected from DC (Tukey-Kramer,  $P = 0.0313$ ) and UBC (Tukey-Kramer,  $P < 0.0001$ ). Liver hsp70 band density measured in fish collected from UMC, IZ, BW, and the SF at river kilometer 24 were similar, and these levels were significantly greater than band density measured in fish collected from LBC (Tukey-Kramer,  $P < 0.0001$ ).

The July 2005 hsp70 levels in fish liver demonstrated a sigmoid (threshold) relation with the maximum pre-visit water temperature (Figure 14). In liver tissue, a sigmoid curve was fit using mean monthly maximum temperature ( $MMT_{max}$ ), mean weekly maximum temperature (MWMT), mean monthly average temperature (MMAT), and maximum, minimum, and average temperature (24-h max, 24-h min, 24-h avg) twenty four hours prior to capture (Table 3). The relation between mean weekly average temperature (MWAT) and hsp70 band density was not sigmoid (Appendix E) and for this reason, the estimated threshold temperature was not calculated (Table 3). Based on the sigmoid curve fits and 95% confidence intervals around the inflection point, the estimated induction (increased protein synthesis over basal levels) temperature in liver tissue is 15.6-16.2°C for MMAT and 16.9 to 18.2 °C for the 24 h average (Table 3). The estimated induction temperature for temperature

maximum (24-h max, pre-visit max, MWMT,  $MMT_{max}$ , Table 3) ranges from a low of 18.8°C ( $MMT_{max}$ ) to a high of 21.6°C for the pre-visit maximum temperature.

A comparison of liver hsp70 band density for fish collected in July 2004 and 2005 at DC, LBC, IZ, and BW shows the same general pattern (Figure 15). There was no difference in liver hsp70 band density explained by an interaction between year and location ( $F_{3, 69} = 1.70$ ,  $P = 0.176$ ), but there was a difference among locations ( $F_{3, 69} = 53.05$ ,  $P < 0.0001$ ). At these four study locations in 2005, there is a linear relation between liver hsp70 band density and location ( $r^2 = 0.68$ ), and band density among locations increases with water temperature.

#### *Caudal fin hsp70 levels*

Fin tissue was collected from the lower caudal fin of 80 fish in 2005. Sufficient tissue existed to conduct analysis on 66 samples. Similar to liver tissue, one band of approximately 70-75 kDa was detected in fin tissue (Figure 12). Fin hsp70 band density showed a similar pattern to liver hsp70 band density (Figure 16). There was a significant difference in caudal fin hsp70 levels among locations ( $F_{7, 65} = 31.93$ ,  $P < 0.0001$ ). After excluding samples that did not have values for both fin and liver tissues, hsp70 band density was compared across locations between these tissues. Within a location, there was a significant difference in hsp70 band density measurements between fin and liver tissue at UMC (paired t-test,  $P = 0.0384$ ) and IZ ( $P = 0.0266$ , Figure 16).

#### *White muscle hsp70 levels*

Two bands were detected in white muscle tissue and correspond to the apparent molecular mass of the constitutive hsc70 (hsp73) and inducible hsp70 (hsp72) isoforms (Figure 17). The overall pattern for hsp70 band density for both the constitutive and inducible isoforms is similar to the pattern observed in liver tissue where band density increases with increasing temperature. There were significant differences in band density measurements among locations in both the constitutive ( $F_7$ ,

78 = 21.68,  $p < 0.0001$ ) and inducible bands ( $F_{7, 78} = 78.89$ ,  $p < 0.0001$ ; Figure 18). The band density of both the constitutive and inducible hsp70 isoforms displayed a sigmoid (threshold) relation with the 24-hour average, 24-hour minimum, 24-hour maximum, maximum temperature within 5 days of capture, MWMT, MWAT, MMAT, and  $MMT_{max}$ . Based on sigmoid curve fits, the estimated induction temperature is on average, from 0.2 to 2.3 °C higher in white muscle tissue than liver tissue (Table 3). Temperature threshold in white muscle tissue are 16-18.5°C for temperature averages (24-h avg, MMAT, MWAT) and 19.6-23°C temperature maximum (24-h max, pre-visit max, MWMT,  $MMT_{max}$ , Table 3).

#### *Hsp70 levels and lipid content*

In July, but not June or September 2005, there was a significant inverse linear relation between hsp70 band density and lipid levels ( $F_{1,31} = 7.94$ ,  $p = 0.008$ ;  $r^2 = 0.204$ ). In 2005, the relation between hsp70 and lipids was not linear. Liver and white muscle hsp70 levels reached maximal synthesis levels when mean weekly maximum temperatures (MWMT) reached 22-23°C. Although hsp70 levels remained constant, lipid levels began to decrease when MWMT exceeded 23°C (Figure 19).

#### Discussion

Juvenile redband steelhead trout in the South Fork John Day River (SFJD), Oregon experience summertime temperatures sufficient to increase cellular quantities of hsp70 measured in liver, white muscle, and fin tissue. The finding that hsp70 can be measured in fin tissue is important because it provides a non-lethal procedure for measuring hsp70 levels. There was also a general pattern of decreased WBL with increasing temperature. The decrease in whole body lipids (WBL) was associated with maximal hsp70 levels, but it is likely that other factors besides increased hsp70 levels contribute to this general pattern.

In the laboratory experiment, the estimated induction temperature for hsp70 protein synthesis in both fin and liver tissue was between 19 and 22°C. The temperature range determined in the laboratory overlaps estimated induction, or

“threshold”, temperature values for sublethal protein damage (i.e., increased hsp70 synthesis) determined for field collected fish. The estimated induction temperature for field collected fish was variable and changed with different temperature summaries. For example, in liver tissue, the average estimated induction temperature using the pre-visit maximum temperature was 21.3°C (95% confidence interval = 20.99 to 21.62). In contrast, the estimated induction temperature in liver tissue using MMAT was 15.9°C (95% confidence interval = 15.63 to 16.17). There was also a difference in estimated induction temperature between tissues. Among the various temperature summaries for the estimated induction temperature, estimates for hsp70 induction were between 0.2 to 2.3 °C higher for white muscle tissue than liver tissue. In general, estimated threshold induction temperatures based on average temperatures were lower than estimates based on maximum temperatures. The variability in estimated induction temperature using different temperature summaries, and different tissues, is important because it demonstrates how different temperature summaries lead to different conclusions about sublethal temperatures causing protein damage.

There is a highly significant sigmoid “threshold” relation between water temperature and hsp70 in liver tissue (Figure 14), and in both the constitutive and inducible hsp70 bands from white muscle tissue (Figure 18) for fish collected in July 2005. Fish from Deer Creek and upper Black Canyon experienced the lowest maximum summertime water temperatures (< 19.4°C), and these fish expressed the lowest hsp70 content measured in all three tissues. Trout from lower Black Canyon (LBC) Creek had the greatest variability in band density measurements; and hsp70 expression was intermediate between those tested from Deer Creek and upper Murderers Creek. Upper Murderers Creek had maximum water temperatures about 2°C higher than LBC (Table 2). The LBC study location is within 300 meters of the mainstem SFJD river. Water temperatures in the SFJD River are on average, 3-5°C higher than water temperatures in Black canyon during the summer. If trout collected at LBC had resided in SFJD and then swam into Black canyon seeking thermal refuge shortly prior to collection, then we would expect increased variability in hsp70 protein levels measured in LBC. PIT tag tracking data in lower Black Canyon Creek detected 11 rainbow trout moving at least one kilometer upstream between 26 June and 21 July

2005 (Ian Tattam, Oregon State University, unpublished data), and one of these 11 fish had been previously tagged at the end of December in the SFJD 12 kilometers downstream of Black Canyon Creek. Therefore, it is reasonable to assume that several of the fish collected at lower Black Canyon in July 2005 had experienced previous thermal stress in the SFJD.

Estimated “threshold” temperature values for hsp70 protein synthesis in liver tissue for fish collected from the SFJD subbasin in 2005 was 15.6-18.2°C for temperature averages (based upon 24-h avg, MMAT) or 18.8-21.6°C for temperature maximum (based upon 24-h max, pre-visit max, MWMT, MMT<sub>max</sub>). In white muscle tissue, threshold temperatures were 16-18°C for temperature averages and 20-23°C for temperature maximum. Differences in estimated threshold values between liver and muscle tissue are slight and both maximum and average temperature based estimates of thresholds overlap. Werner and co-workers (2005) reported threshold values of 18-19°C for short and long term temperature averages, and 20-22.5°C for maximum temperature during the 24 hours preceding fish collection for fish collected from the Navarro River, CA. Fish from the SFJD had a slightly lower estimated threshold temperature for temperature averages (16-18°C) compared to fish from the Navarro river (18-19°C). However, estimated threshold temperatures between these two stocks of fish using temperature maximum were almost identical and are between 20 and 23°C. These findings suggest that the induction temperature for hsp70 synthesis in rainbow trout is similar among at least two subpopulations.

Unpublished data discussed by Basu et al. (2001) suggests that maximal hsp70 induction in trout occurs at 21°C regardless of season, magnitude of heat stress, or acclimation temperature. If the unpublished findings of Basu et al. (2001) are comparable to results for rainbow trout collected from the SFJD and the Navarro River, this would suggest there is no difference in the induction temperature for hsp70 expression across multiple rainbow trout subpopulations. Alternatively, techniques used to quantify hsp70 expression in response to temperature are not precise enough to detect differences. Differences in laboratory techniques and variability in temperature and hsp70 measurements may also increase variability and mask differences in induction temperature among different rainbow trout subpopulations. Another

possibility is that the induction temperature for rainbow trout populations is similar (20-23°C) but warm-water adapted subpopulations of rainbow trout, such as those from interior populations of eastern Oregon, synthesize a greater quantity of hsp70 and are able to respond more efficiently to the repair of denatured protein. This hypothesis is not completely satisfactory because other research suggests too much or too little hsp70 expression can result in developmental malformations and cell death (Nollen and Morimoto 2002). The strict regulation of all cellular chaperone proteins, not just hsp70, suggests that the cells ability to handle thermal stress has more to do with isoform efficiency than quantity.

Norris and co-workers (1995) studied outbred tropical topminnows, *Poeciliopsis gracilis*, and could not demonstrate any variation in the constitutive hsp70 isoforms and reported a high degree of polymorphism in the inducible isoforms. Furthermore, Norris et al. (1995) showed that *Poeciliopsis* adapted to desert environments had lower levels of inducible hsp70 isoforms than tropical species and hypothesized that the inducible and constitutive isoforms had different cellular functions that were under different evolutionary constraints. Variability in the constitutive and inducible hsp70 isoforms has also been shown in Antarctic notothenioid fishes and is highlighted by 1) the inability of one species to upregulate the inducible transcript (Place et al. 2004), and 2) the loss of the heat shock response (Hoffman et al. 2005). Collectively, these findings suggest there are probably differences in type and function of hsp70 isoforms expressed among different rainbow trout populations. Additional study is needed to determine if there are differences in hsp70 isoforms and function before further inference can be made into how hsp70's are related to the apparent thermal resistance among various salmonid populations.

In the laboratory experiment, difference between liver and fin tissue hsp70 band density were detected following the 22°C temperature stress. Differences in hsp70 band density between fin and liver tissues was also observed in tissues samples of fish collected from the SFJD River at upper Murderers Creek and Izee falls in July 2005, but in this instance, hsp70 levels were significantly higher in liver tissue than in fin tissue. Difference in hsp70 expression suggests cells within these tissues are responding differently to temperature stress. Also, hsp70 isoforms may be expressed

in different tissues so it is possible that when exposed to temperature maximums of 21-23°C, the hsp70 response is different because different isoforms are being expressed. To my knowledge, this is the first study to document the use of fin tissue to measure hsp70 as it relates to temperature stress in wild populations of rainbow trout. The utility of fin tissue for this purpose was briefly discussed by Hargis (1998), but no data were shown.

In this study, whole body lipid (WBL) content was used as an index of energy reserves, and analysis of WBL shows that WBL begins to decrease after hsp70 band density reaches maximal levels (i.e., asymptote of sigmoid curve; Figure 19). However, the decrease in WBL does not occur until MWMT exceeds 23°C. The estimated threshold level for hsp70 induction in liver and white muscle tissue was between 20 and 23°C. Collectively, this suggests increased synthesis of hsp70 proteins might provide a benefit by allowing for maintenance of body condition. Since the physiological consequences of maintaining elevated hsp70 levels for extended periods of time are unknown (Werner et al. 2005) and the synthesis of proteins is energetically costly (Hochachka and Somero 2002), it is reasonable to assume that prolonged synthesis of heat shock proteins would eventually cause a negative impact on energy reserves. Some support for this hypothesis was presented by Viant et al. (2003) who report that juvenile steelhead parr (*O. mykiss*) exposed to a chronic 20°C temperature stress had increased hsp72 synthesis in white muscle tissue and this was associated with decreased liver and muscle levels of ATP, phosphocreatine, and glycogen. The decrease in WBL observed in trout from the SFJD when MWMT exceeds 23°C might be an indication of this cost. Alternatively, the changes in WBL might be more strongly related to either 1) differences in food availability among study locations related to community level impacts of water temperature (Tait et al. 1994), or 2) appetite suppression (Linton et al. 1998; Myrick and Cech. 2000). However, previous work by Myrick and Cech (2000) reports that rainbow trout consume less food at 22°C and show a decline in growth as temperatures neared 25°C. Therefore, changes in WBL shown in trout from the SFJD are probably best explained by a combination of physiological costs (e.g., increased metabolic

rates), behavioral modification (e.g., appetite suppression), and the effect of temperature on food web dynamics.

In 2004, there were significant increases in liver hsp70 levels from June to July for fish examined from the LBC, BW, and IZ study locations. There were also significant differences in hsp70 levels among the fish collected from the study locations in September 2004. The change from June to July is likely a function of increased water temperature. However, it is uncertain if the differences in September are related to continued thermal stress, or are the legacy of thermal stress experienced in July. For example, following a single acute temperature stress where water temperatures were raised in experimental tanks from 12 to 26°C at a rate of 2°C/hr and maintained at 26°C for 10-15 minutes, hepatic hsp70 levels measured in juvenile chinook salmon (*Oncorhynchus tshawytscha*) were significantly elevated over controls for 14 days (Mesa et al. 2002). After a 15°C heat shock (7.4-22.4°C) for 2 hours, Mazur (1996) reported elevated hsp70 levels in the gills of cutthroat trout up to 3 weeks following the stressor. Fish at each of the four study locations experienced different thermal histories. Difference in thermal history and the uncertainty of how long it takes hsp70 levels to return to basal levels following thermal stress suggest differences in hsp70 levels for fish collected in September from LBC, BW, DC, and IZ are a function of past and not current thermal stress.

An alternative explanation for changes in hsp70 levels from June to July and from July to September is a seasonal difference. Fader and co-workers (1994) showed seasonal changes in heat shock proteins of approximately 70 kDa in four different fish species, with the lowest levels measured in the winter and the highest levels measured in the spring. In the current study, the highest hsp70 levels measured in 2004 were from fish collected at the end of July. With the exception of Deer Creek, the coldest location sampled in 2004, there was an increase in hsp70 levels across all locations from June to July. These findings are contrary to the pattern detected by Fader et al. (1994) which showed decreases in hsp70 expression from Spring to Summer and from Summer to Spring. In the SFJD study, there was a significant change in hsp70 expression through time at all study locations except Deer Creek. Contrary to what would be expected from results presented by Fader and co-workers, hsp70 levels in

fish sampled from Deer Creek did not change from June through September. One component that is missing from Fader et al. (1994) is a comparison of fish sampled on a single date from multiple locations. For example, for fish collected from SFJD River, there is greater variation in hsp70 levels among locations (Figure 13) and temperatures (Table 2) on 10-11 June 2004 (variation among locations was not statistically significant), than there is in Deer Creek from June to September. The variation among locations is even more dramatic in July and September. In contrast, Fader et al (1994) only collected *Salmo trutta* from one location in Valley Creek (Valley Forge, PA). Fader and co-workers are correct in cautioning against incorrectly interpreting changes in hsp70 that do not consider seasonal variation. However, researchers also need to consider 1) variation within a season and among different locations, and 2) the effects of past thermal history on hsp70 expression.

The cellular stress response is species, organ and stressor specific, and factors other than temperature can alter cellular hsp levels (Iwama et al. 2004). For example, hsp70 levels in liver and kidney tissue increased in coho salmon (*Oncorhynchus kisutch*) when infected with bacterial kidney disease (Forsyth et al. 1997). Other factors shown to increase hsp levels in fish include industrial effluents (Vijayan et al. 1998), heavy metals (Sanders 1993), pesticides (Hassanein et al. 1999), and certain chemicals (Bierkens 2000). There is evidence that the stress hormone cortisol is involved with mediating hsp70 levels in fish tissue following physiological stress (Basu et al. 2001), and may attenuate gill hsp30 (Ackerman et al. 2000) and hsp90 mRNA expression (Sathiyaa et al. 2001). In contrast, handling stress (Vijayan et al. 1997), insertion of passive integrated transponders (PIT tags) into the body cavity of rainbow trout (this study), and electrofishing (Lund et al. 2002, Werner et al. 2005) have no apparent effect on hsp70 levels in salmonids. Common forms of hatchery stress including anaesthesia, hypoxia, hyperoxia, capture stress, crowding, feed deprivation, and cold stress have been reported to have no effect on gill hsp70 levels in Atlantic salmon (*Salmo salar*; Zarate and Bradley 2003). Increased muscle activity induces hsp70 in muscle tissue and blood of mammals (Milne and Noble 2002), but a recent exhaustive exercise study in male and female rainbow trout found no upregulation of hsp70, hsp30, or hsp90 in heart or muscle tissue (Clarkson et al.

2005). Clarkson and co-workers (2005) concluded that the maintenance of core body temperature and a lack of exercise-induced protein damage explained why hsps were not upregulated. Collectively this suggests that handling and exercise stress do not explain changes in hsp70 levels observed in fish collected from the SFJD or in the laboratory experiment.

It is not entirely possible to dismiss other factors, including the effects of pesticides or pollutants, on hsp70 levels in fish collected from the SFJD. However, the strong correlation between hsp70 and temperature found in both July 2004 and 2005 suggests temperature is the primary factor explaining alterations in hsp70 levels. If factors besides temperature affect hsp70 expression, the three mainstem study locations (SF, BW, and IZ falls) have the greatest potential to be impacted by confounding factors because of increased risk for exposure to agricultural runoff. Water quality measurements are not available to test this conclusion, but given the remoteness of the study locations and the relatively minor agricultural use, altered hsp70 expression because of agricultural pollutants or industrial waste is not a major concern.

Thermal tolerance is an important physiological trait that helps define suitable habitat and indices used to define this habitat should be chosen carefully (Rodnick et al. 2004). Although results of this research suggest juvenile redband rainbow trout in the SFJD River can tolerate summer daily maximum temperatures in excess of 22°C, it is important to recognize that (1) they are physiologically compromised as indicated by the induction of hsps, (2) little is known about how fish respond when exposed simultaneously to multiple or sequential stressors (Schreck 2000), and (3) there is a difference between “stress” and “distress.” For example, this research has shown an increase in hsps consistent with the *cellular stress* response, but it does not show that cellular stress has led to erratic behavior or physical deterioration associated with distress (see Schreck et al. 1997 and Schreck 2000 for further discussion of stress in fish). Moreover, Feder and Hofmann (1999) discuss the use of hsps as biomarkers in environmental toxicology and how it can be complicated because multiple simultaneous stressors can yield significant hsp70 expression even if no single

toxicant is at harmful levels. Collectively, this has led to the warning that the simple use of hsps as an indicator of *stress* in fish is premature (Iwama et al. 2004).

Measurement of hsps show promise for better defining physiological temperature requirements for juvenile rainbow trout in the natural environment, but this research needs to proceed with caution. Subsequent research should carefully define the scope of inference, and methods for investigating hsps need to expand beyond the use of one-dimensional gel electrophoresis (e.g., proteomics). The changes in hsps at the cellular level are dynamic. Positive or negative impacts of altering hsp levels and the subsequent impacts on the physiological status of fish are poorly understood. In the future, research on the physiological tolerance of fish to temperature would benefit from measurements of both hsps and ubiquitin. Ubiquitin is a highly conserved protein of 76 amino acids that covalently binds to denatured protein, and proteins bound by ubiquitin are marked for proteolysis by nonlysosomal proteases (Rechsteiner 1987, Ciechanover 1998). Therefore, ubiquitin conjugates provide information about the amount of irreversibly damaged protein. There are few examples of ubiquitin measurements in an ecologically and environmentally relevant context for aquatic organisms (but see Hofmann and Somero 1995, Hofmann and Somero 1996 for work on intertidal mussels). Ubiquitin was not measured in the current study.

Similar to Behnke (1992) and Zoellick (1999), findings from this work support observations that redband trout can tolerate stream temperatures near 26°C (Behnke 1992; Zoellick 1999). Measurements of heat shock proteins strongly suggest these fish are experiencing thermal stress in the summer, but this does not provide convincing evidence that the redband rainbow trout in the SFJD River have greater thermal tolerance than any other rainbow trout subpopulation. Therefore, it is likely that the ability of redband rainbow trout in the SFJD to tolerate summer temperatures in excess of 22°C is best explained by physiological adaptation to thermal history leading to temperature-dependent swimming performance and aerobic metabolism that allow for short term (< 2h) exposure to temperatures greater than 24°C (see Gamperl et al. 2002).

In summary, the management of thermal habitat requirements for juvenile salmonids requires an understanding of physiological temperature limits. For this reason, it is important for studies of physiological limits to incorporate both spatial and temporal factors. The next challenge is to integrate this information from “the top down” and “the bottom up” (e.g., riverscape; Fausch et al. 2002) and provide a biologically meaningful interpretation of the results. Ultimately, the definition of these physiological limits will be used to help guide management of local land and water use practices. In turn, an understanding of these limits will contribute to a better understanding of the impacts of global climate change on fish populations.

Table 1- Sampling locations and coordinates (UTM; NAD 83) for collection locations in the South Fork John Day Basin in 2004 and 2005.

Sample Location	2004 Sampling Dates <sup>a</sup>	2005 Sampling Dates	UTM Coordinates <sup>b</sup>	Elevation (M)
Black Canyon				
Lower Black Canyon	10 Jun, 31 Jul, 11 Sep	25 Jul	11T 294907, 4912196	894
Upper Black Canyon	NA	25 Jul	11T 291492, 4913200	1037
Murderers Creek				
Lower Murderers Creek	NA	24 Jul	11T 297932, 4910336	908
Upper Murderers Creek	NA	24 Jul	11T 305099, 4906826	1001
Deer Creek	10 Jun, 30 Jul, 11 Sep	24 Jul	11T 300210, 4896411	1158
South Fork John Day				
SF RKM 24	NA	25 Jul	11T 296081, 4910855	881
Below Wind Creek	11 Jun, 31 Jul, 11 Sep	25 Jul	11T 297168, 4906262	939
Izee Falls	11 Jun, 30 Jul, 11 Sep	24 Jul	11T 298082, 4896082	1050

<sup>a</sup>. NA = not sampled

<sup>b</sup>. The first coordinate is meters east longitude, the second value is meters north latitude.

Table 2- Temperature data (°C) for sampling locations in the South Fork John Day River from 10-11 June 2004; 30-31 July 2004; 11 September 2004; and 23-24 July 2005. June 2004 temperature records for Deer Creek and Izee Falls are from respective gauging stations operated by the U.S. Bureau of Reclamation. Weekly temperature averages are for the 7 days preceding the sampling date. In 2004, monthly temperature ranges are for the 25 days preceding fish collection in June and September and from 1 or 2 July to 30-31 July at Lower Black Canyon (LBC), and 12 July to 30 July at Deer Creek. Monthly temperature ranges for 2005 are from 1 July to the sampling date. SF = South Fork John Day River; MWAT = mean weekly average temperature; MWMT = mean weekly maximum temperature; MMAT = mean monthly average temperature; MMT<sub>max</sub> = mean monthly maximum temperature; MDTR = mean daily temperature range; NA = Not Applicable.

Sampling location	Sampling date	24-h Average	24-h Minimum	24-h Maximum	Pre-visit Max Temperature; Date	MWAT	MWMT	MMAT	MMT <sub>max</sub>	MDTR
Lower Black Canyon	10 June 04	10.2	9.4	11.7	17.6; 4 June 04	11.2	13.6	10.3 <sup>a</sup>	12.7 <sup>a</sup>	4.2 <sup>a</sup>
Lower Black Canyon	31 July 04	17.4	14.2	21.0	22.2; 24 July 04	17.3	21.3	16.5	20.5	7.5
Lower Black Canyon	11 Sept. 04	13.3	10.4	16.7	20.9; 18 Aug 04	12.9	16.0	14.4	17.2	5.3
Below Wind Creek	11 June 04	NA	NA	NA	NA	NA	NA	NA	NA	NA
Below Wind Creek	30 July 04	NA	NA	NA	NA	NA	NA	NA	NA	NA
Below Wind Creek	11 Sept. 04	NA	NA	NA	NA	NA	NA	NA	NA	NA
Izee Falls	10 June 04	12.3	11.6	13.4	21.3; 4 June 04	15.2	17.8	13.4	15.6	4.2
Izee Falls	30 July 04	18.6	15.1	22.2	23.7; 25 July 04	18.8	22.7	18.2	21.9	7.2
Izee Falls	11 Sept. 04	14.0	11.2	16.5	21.5; 17 Aug 04	13.3	15.8	15.3	17.7	4.9
Deer Creek	10 June 04	7.7	6.9	8.7	14.5; 4 June 04	9.9	11.9	8.8	10.6	3.4
Deer Creek	30 July 04	16.0	13.3	17.9	19.2; 25 July 04	15.9	18.2	15.8	17.8	4.4
Deer Creek	11 Sept. 04	11.8	9.6	14.2	18.7; 20 Aug 04	11.2	13.5	13.1	15.0	3.8
Upper Black Canyon	24 July 05	14.3	11.3	17.8	18.4; 19 July 05	14.4	17.9	13.8	17.2	6.3
Lower Black Canyon	24 July 05	16.3	13.4	20.4	21.1; 19 July 05	16.7	20.6	15.9	19.7	6.9
SF at river kilometer 24	24 July 05	21.6	17.9	25.4	25.4; 23 July 05	21.3	25.0	20.0	23.4	6.7
Upper Murderers Creek	23 July 05	18.8	16.3	22.9	22.9; 22 July 05	17.3	21.8	16.5	19.5	6.1
Lower Murderers Creek	23 July 05	22.4	19.9	25.8	25.8; 22 July 05	21.0	25.3	19.6	23.7	8.5
Below Wind Creek	24 July 05	20.2	16.4	24.0	24.6; 22 July 05	19.9	23.7	18.9	22.4	7.1
Izee Falls	23 July 05	21.3	19.3	23.7	23.7; 22 July 05	20.0	23.0	19.1	22.2	6.2
Deer Creek	23 July 05	16.8	15.0	19.4	19.4; 22 July 05	14.9	18.0	14.0	16.8	5.4

<sup>a</sup> Monthly temperatures = temperature records were only available for 23 days prior to fish collection.

Table 3- Relation between study location water temperature measurements preceding fish collection and estimated threshold temperatures (increased protein synthesis over basal levels) for heat shock protein 70 (hsp70). The 95% confidence interval for the estimated threshold temperature at LogEC<sub>50</sub> was determined with GraphPad Prism where EC<sub>50</sub> represents 50% of the hsp70 values greater than 0.5 in liver (hsp70) tissue, and hsp72(hsp70) and hsp73(hsc70) in white muscle (WM) tissue.. Juvenile redband steelhead (*Oncorhynchus mykiss gairdneri*) trout were collected from 23-24 July 2005. NA= Relation is a third order polynomial, not a sigmoid curve (see results section).

Tissue type	Temperature measurement	Dose response/ Polynomial equation	Estimated threshold temperature (°C)	r <sup>2</sup>	Standard error of curve		
					bottom	middle	top
Liver: hsp70	24-h average	$y=0.788/(1+10^{17.57-x})$	16.9 to 18.21	0.708	0.037	0.320	0.023
Liver: hsp70	24-h maximum	$y=0.788/(1+10^{20.59-x})$	20.28 to 20.91	0.775	0.033	0.159	0.019
Liver: hsp70	pre-visit max	$y=0.788/(1+10^{21.31-x})$ $y=-1.2 - 0.16x + 0.03x^2 - 0.00009x^3$	20.99 to 21.62	0.774	0.030	0.157	0.019
Liver: hsp70	MWAT	NA	NA	0.703	NA	NA	NA
Liver: hsp70	MWMT	$y=0.794/(1+10^{20.77-x})$	20.47 to 21.07	0.769	0.030	0.149	0.020
Liver: hsp70	MMAT	$y=0.796/(1+10^{15.90-x})$	15.63 to 16.17	0.744	0.032	0.137	0.022
Liver: hsp70	MMTmax	$y=0.772/(1+10^{19.18-x})$	18.81 to 19.55	0.640	0.038	0.187	0.026
WM: hsp73 (hsc70)	24-h average	$y=0.821/(1+10^{18.22-x})$	17.97 to 18.46	0.848	0.029	0.123	0.023
WM: hsp73 (hsc70)	24-h maximum	$y=0.857/(1+10^{22.81-x})$	22.59 to 23.03	0.862	0.026	0.109	0.026
WM: hsp73 (hsc70)	pre-visit max	$y=0.845/(1+10^{22.76-x})$	22.54 to 22.98	0.863	0.026	0.110	0.024
WM: hsp73 (hsc70)	MWAT	$y=0.821/(1+10^{17.12-x})$	16.94 to 17.29	0.867	0.031	0.089	0.022
WM: hsp73 (hsc70)	MWMT	$y=0.825/(1+10^{21.55-x})$	21.33 to 21.78	0.867	0.028	0.113	0.023
WM: hsp73 (hsc70)	MMAT	$y=0.821/(1+10^{16.32-x})$	16.14 to 16.49	0.867	0.031	0.089	0.022
WM: hsp73 (hsc70)	MMTmax	$y=0.8790/(1+10^{21.58-x})$	21.01 to 22.14	0.728	0.032	0.282	0.047
WM: hsp72(hsp70)	24-h average	$y=1.548/(1+10^{18.23-x})$	17.93 to 18.54	0.780	0.057	0.154	0.047
WM: hsp72(hsp70)	24-h maximum	$y=1.629/(1+10^{22.92-x})$	22.66 to 23.18	0.801	0.052	0.131	0.053
WM: hsp72(hsp70)	pre-visit max	$y=1.604/(1+10^{22.87-x})$	22.61 to 23.14	0.800	0.053	0.133	0.050
WM: hsp72(hsp70)	MWAT	$y=1.549/(1+10^{17.17-x})$	16.93 to 17.42	0.787	0.065	0.121	0.046
WM: hsp72(hsp70)	MWMT	$y=1.566/(1+10^{21.56-x})$	21.37 to 21.94	0.795	0.057	0.143	0.047
WM: hsp72(hsp70)	MMAT	$y=1.550/(1+10^{16.38-x})$	16.13 to 16.62	0.787	0.065	0.121	0.046
WM: hsp72(hsp70)	MMTmax	$y=1.542/(1+10^{19.85-x})$	19.57 to 20.14	0.712	0.077	0.145	0.053

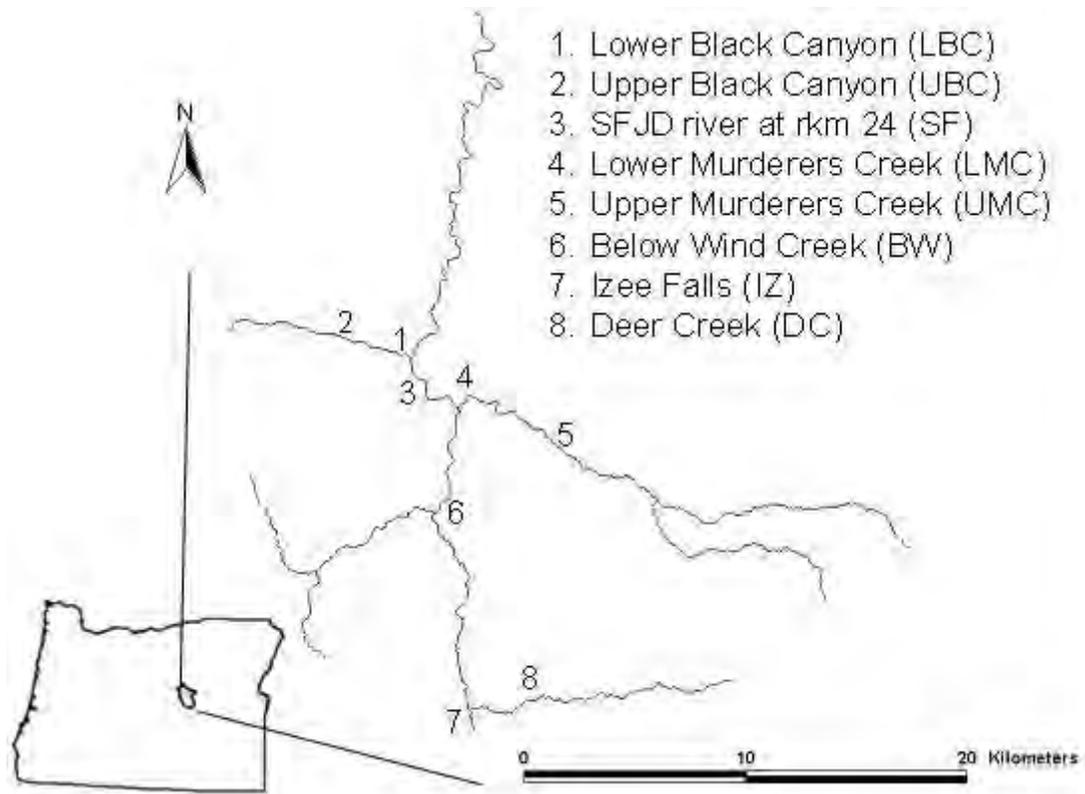


Figure 3- Map of juvenile redband steelhead trout sampling in the South Fork John Day River catchment, Grant County, Oregon.

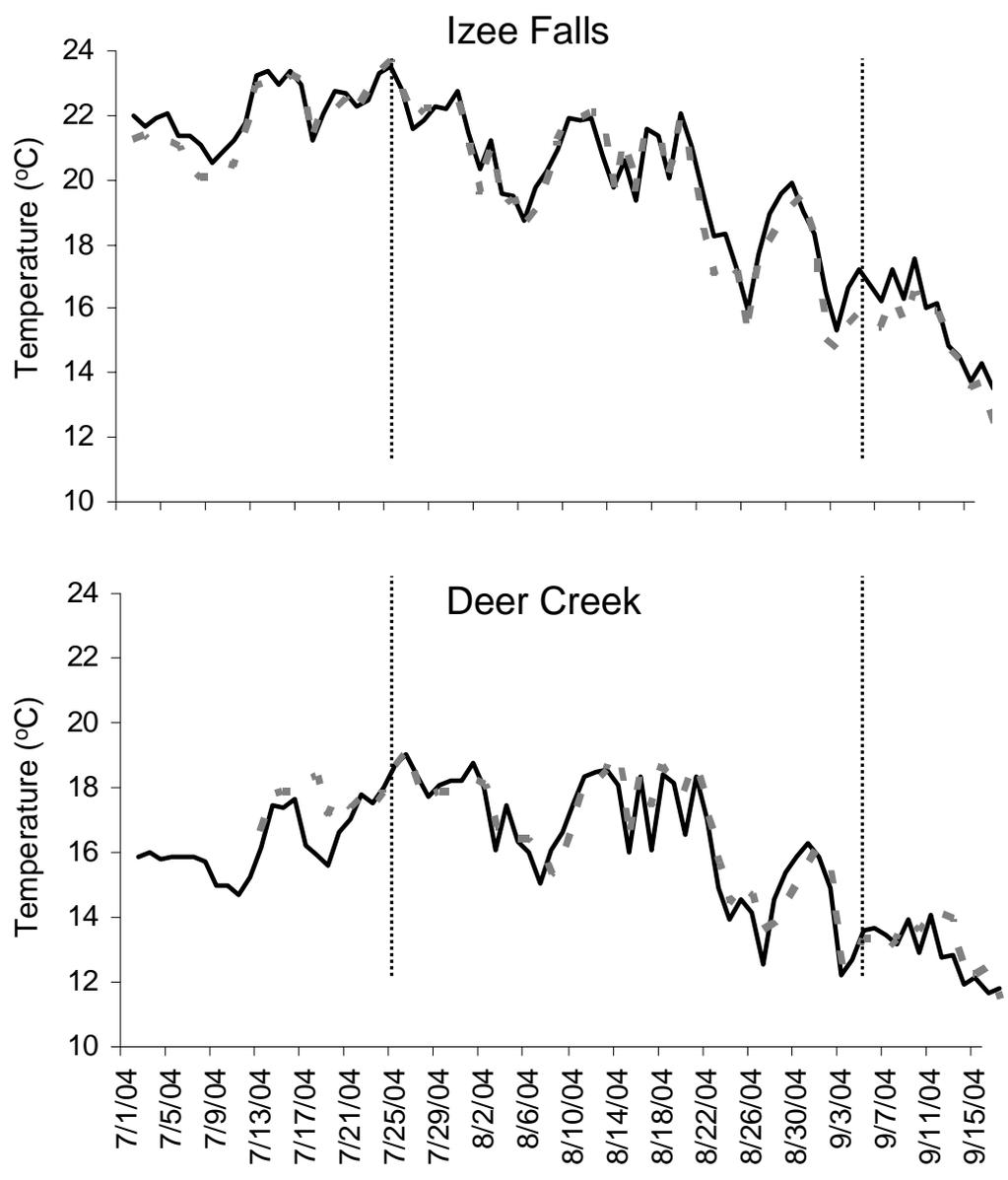


Figure 4- Maximum daily water temperatures (°C) recorded by an in-stream temperature logger (dashed line) and the Bureau of Reclamation gauging stations (solid line) at Izee falls and Deer Creek from 1 July-15 September 2004. Vertical lines indicate the 29 July and 11 September 2004 sampling dates.

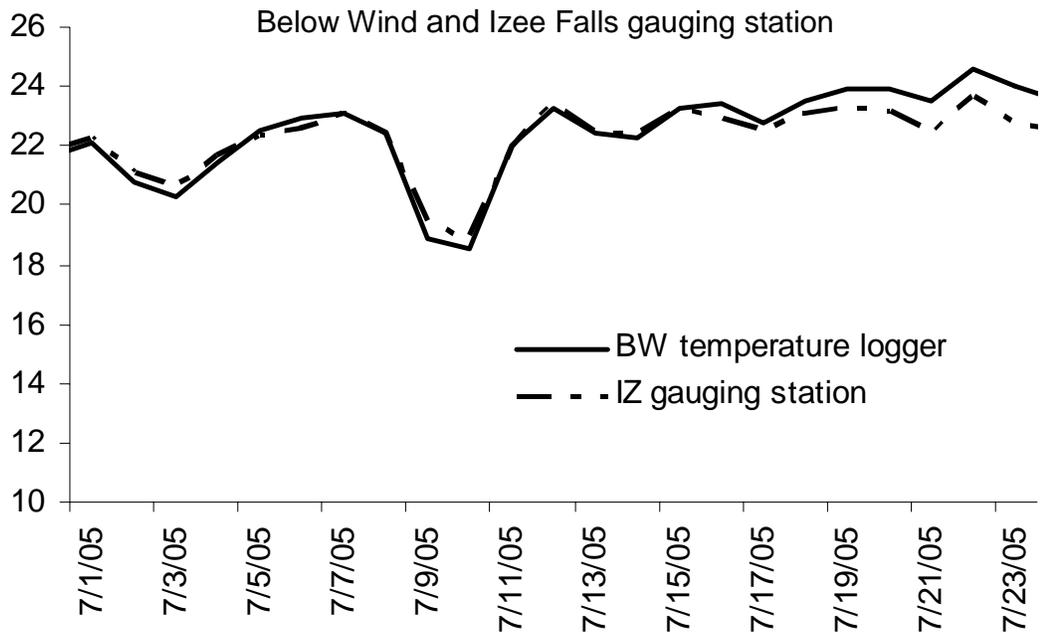


Figure 5- Maximum daily water temperatures (°C) recorded by an instream temperature logger at the Below Wind study location and the Bureau of Reclamation gauging station (solid line) at Izee falls from 1 to 23 July 2005.

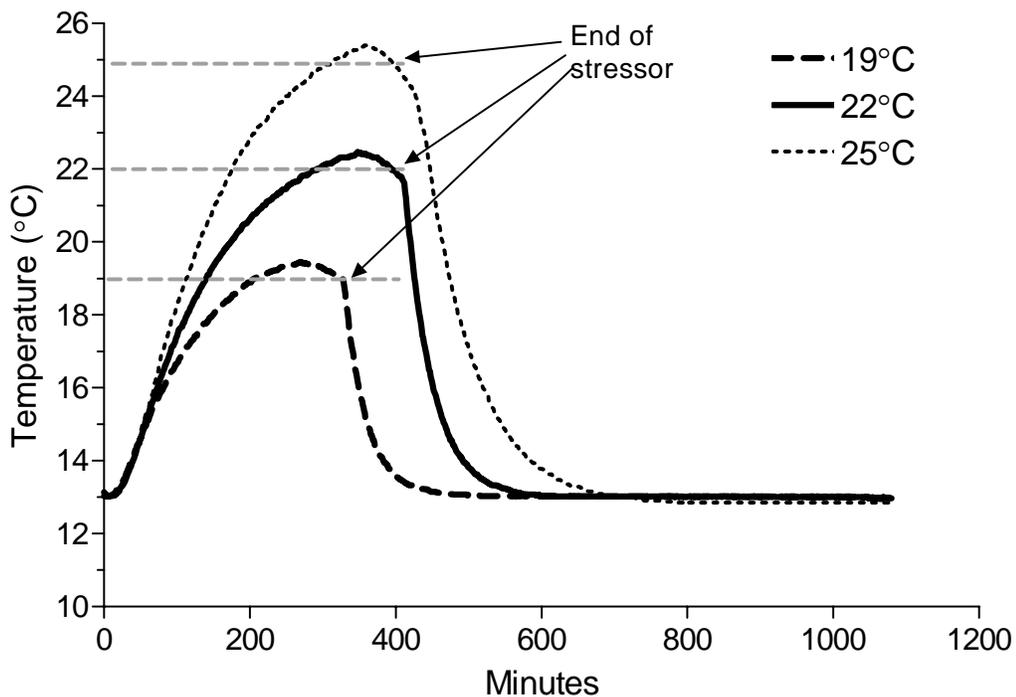


Figure 6- Thermal profile of the 19, 22, and 25°C temperature stressors. The ambient water temperature (13°C) was raised to target stressors of 19, 22, or 25°C and maintained above the target temperature for 90-120 minutes. After the water temperature in the tank dropped below the target stressor, ambient water was added at > 5 liters/minute. Fish were lethally sampled 12 hours after reintroduction of 13°C water.

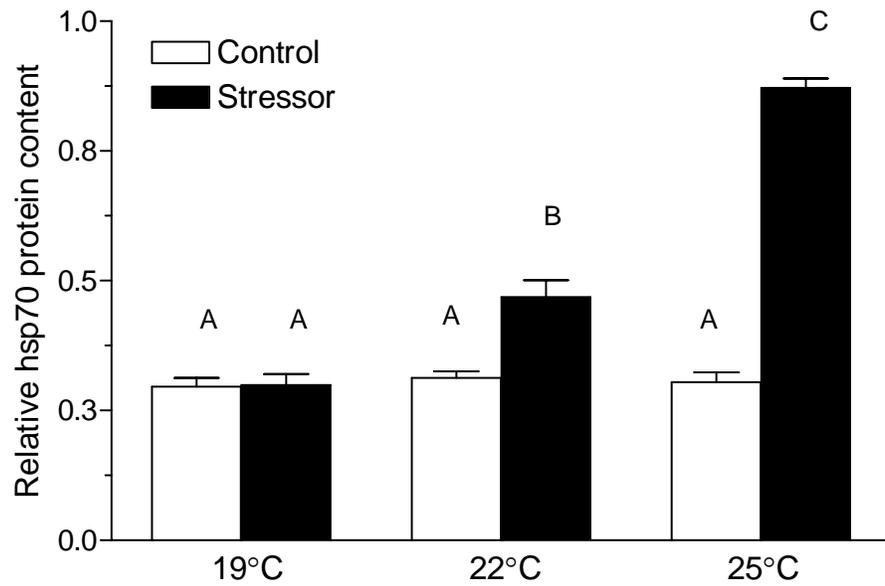


Figure 7- Relative heat shock protein 70 (hsp70) content in liver tissue from rainbow trout (*Oncorhynchus mykiss*) acclimated to 13°C and exposed to an acute temperature stressor of 19, 22, or 25°C followed by a 12-hour recovery at 13°C before sampling. Open bars represent fish sampled before the temperature stressor (control) and dark bars represent fish exposed to the stressor. Values represent mean densitometry values of protein bands ( $\pm 1$  standard error of the mean) detected by Western blotting;  $n = 18$  for each bar. Shared letters indicate a non-significant difference ( $P > 0.05$ ) in hsp70 values.

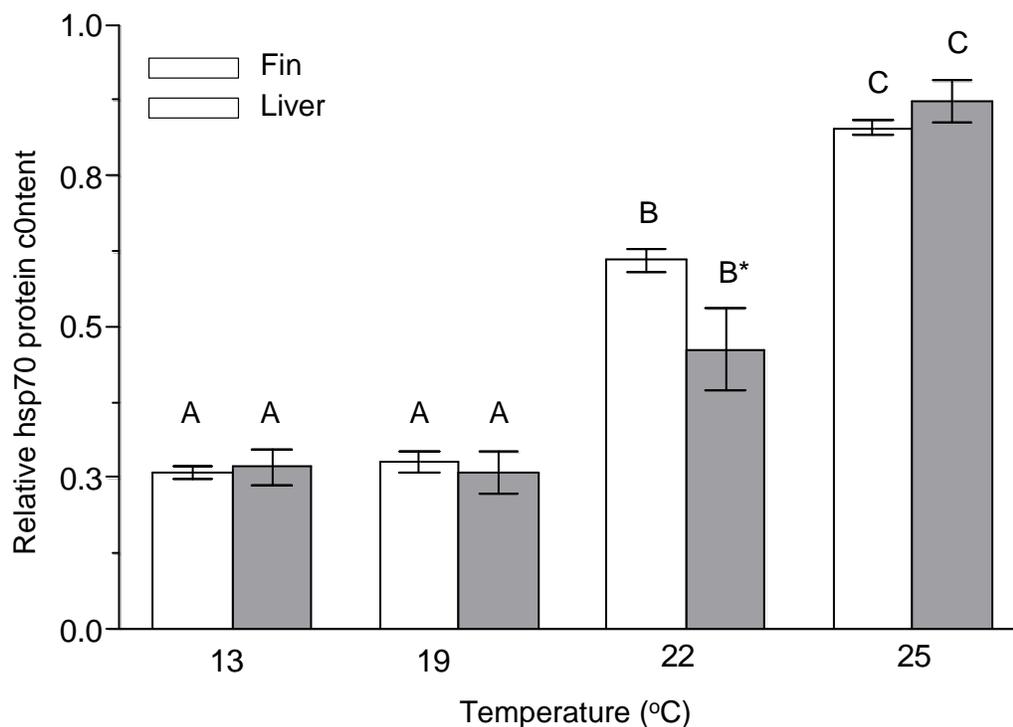


Figure 8- Relative heat shock protein 70 (hsp70) content in caudal fin and liver tissue from rainbow trout (*Oncorhynchus mykiss*) acclimated to ambient water temperature (13°C) and exposed to an acute temperature stress of 19, 22, or 25°C followed by a 12-hour recovery at 13°C. Values represent mean densitometry values of protein bands ( $\pm$  1 standard error of the mean) detected by Western blotting;  $n = 8$  for each bar. Shared letters indicate a non-significant difference ( $P > 0.05$ ) in hsp70 values within a tissue type. The asterisk indicates a significant difference between fin and liver tissue at 22°C (paired t-test;  $P = 0.043$ ).

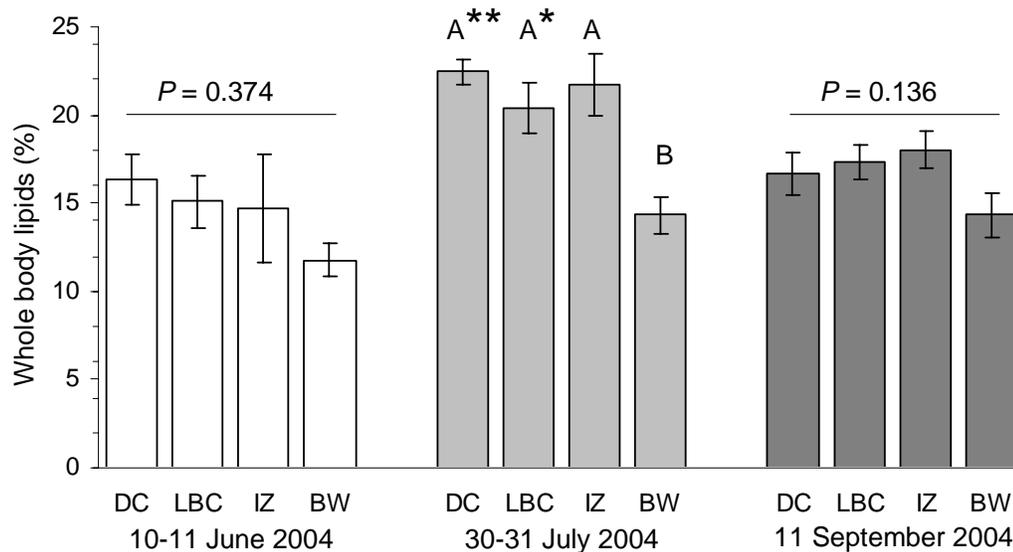


Figure 9- Whole body lipids (mean %;  $\pm 1$  standard error of the mean) in juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) collected from the South Fork John Day River. Fish were collected from 10-11 June, 30-31 July, and 11 September 2004 at Deer Creek (DC), lower Black Canyon (LBC), Izee Falls (IZ), and below Wind Creek (BW).  $n = 7$  for each bar in June;  $n = 9$  in for each bar in July (except BW,  $n = 8$ , in July) and September. Shared letters indicate a non-significant difference ( $P > 0.05$ ). A single asterisk represents a significant difference ( $P < 0.05$ ) from June to July. A double asterisk represents a significant difference from June to July and a significant difference from July to September.

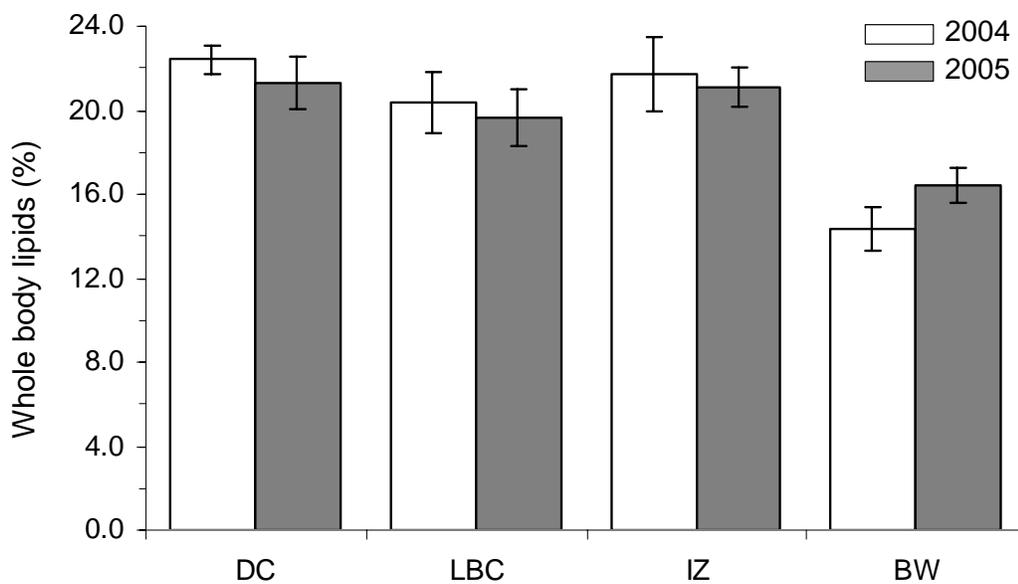


Figure 10- Comparison between years of whole body lipids (mean %;  $\pm 1$  standard error of the mean) in juvenile rainbow trout (*Oncorhynchus mykiss gairdneri*) collected from the South Fork John Day River. Fish were collected on 30-31 July 2004 (white bars) and 23-24 July 2005 (grey bars) at Deer Creek (DC), lower Black Canyon (LBC), Izee Falls (IZ), and below Wind Creek (BW). In 2004, each bar represents  $n = 9$ , except BW  $n = 8$ . In 2005, each bar represents  $n = 9$ .

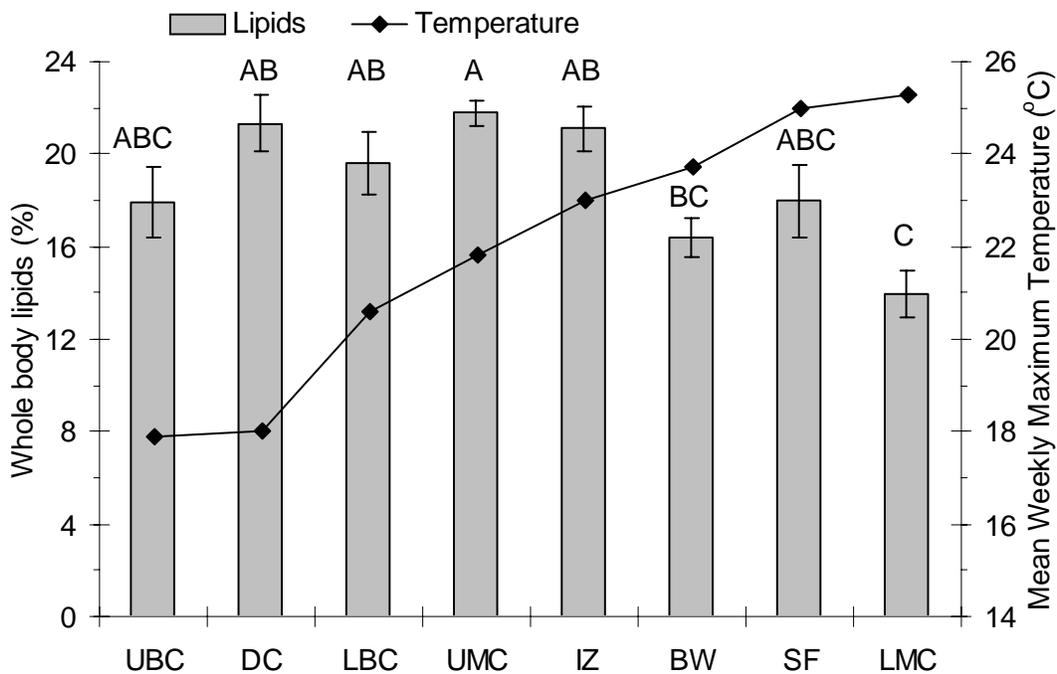


Figure 11- Mean weekly maximum temperature ( $^{\circ}\text{C}$ ) compared to whole body lipids (mean %;  $\pm 1$  standard error of the mean) in juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) collected from the South Fork John Day River on 24-25 July 2005. Fish were collected at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF). Shared letters indicate a non-significant difference at  $P > 0.05$ . Each bar represents  $n = 10$ .

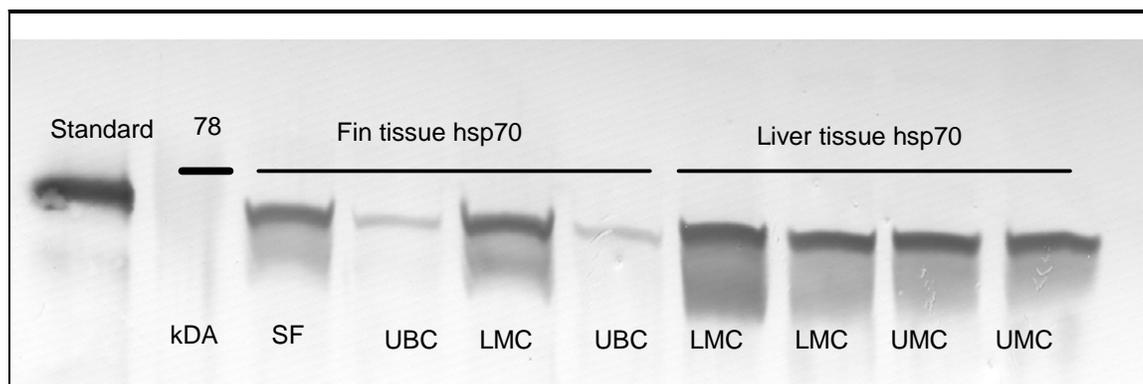


Figure 12- Representative Western blot of hsp70 protein detected in liver and fin tissue of juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) collected on 23-24 July 2005. Samples represented here were collected from fish sampled from the SFJD at river kilometer 24 (SF), upper Black Canyon (UBC), and lower and upper Murderers Creek (LMC, UMC). Proteins were visualized colorimetrically using an alkaline phosphatase conjugate substrate (Biorad). A recombinant chinook salmon hsp70 protein (Standard; StressGen Biotechnologies Corp) was applied to each gel to serve as an internal standard for molecular weight determination and blotting efficiency. Band density readings were adjusted for background.

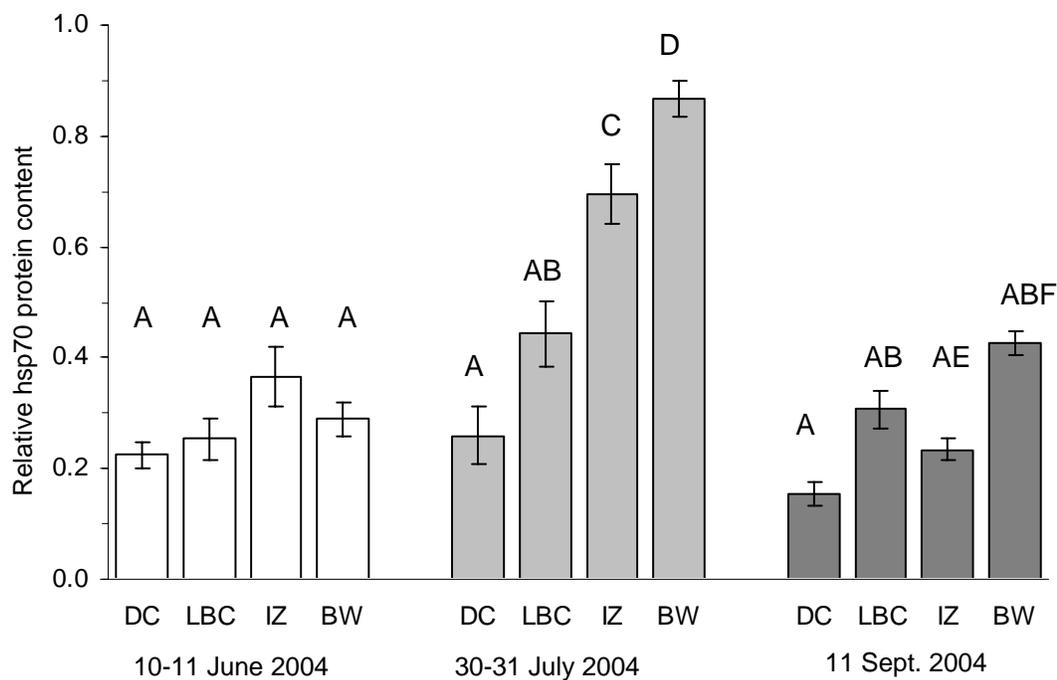


Figure 13- Relative heat shock protein 70 (hsp70) band density (mean densitometry value  $\pm$  1 standard error of the mean) in liver tissue from redband rainbow trout (*Oncorhynchus mykiss gairdneri*). Fish were collected from the South Fork John Day River on 10-11 June, 30-31 July, and 11 September 2004 at Deer Creek (DC), lower Black Canyon (LBC), Izee Falls (IZ), and below Wind Creek (BW).  $n = 10$  for each bar, except  $n = 9$  for LBC and IZ in July, and  $n = 9$  for IZ. Shared letters indicate a non-significant difference ( $P > 0.05$ ) in hsp70 values.

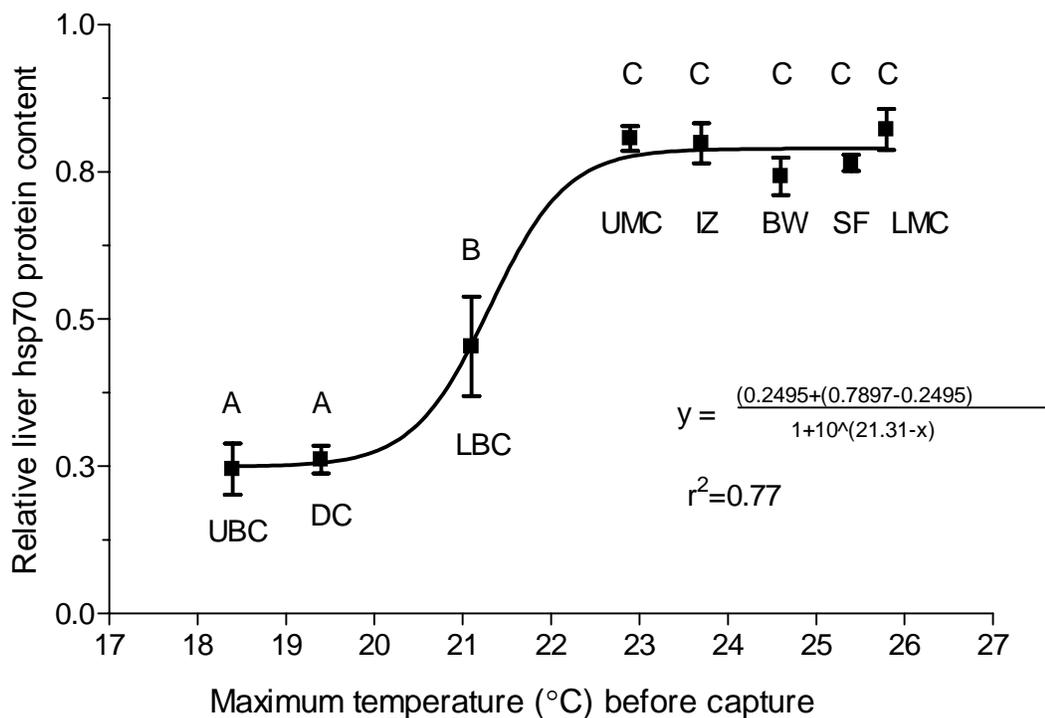


Figure 14- Relation between relative heat shock protein (hsp70) band density (mean densitometry value  $\pm$  1 standard error of the mean) in liver tissue of redband rainbow trout (*Oncorhynchus mykiss gairdneri*) and the maximum temperature within 5 days of capture. Fish were collected in the South Fork John Day River on 23-24 July 2005 at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF). Shared letters indicate a non-significant difference at  $P > 0.05$ . Each symbol represents  $n = 10$  except UBC and BW ( $n = 9$ ) and UMC ( $n = 8$ ).

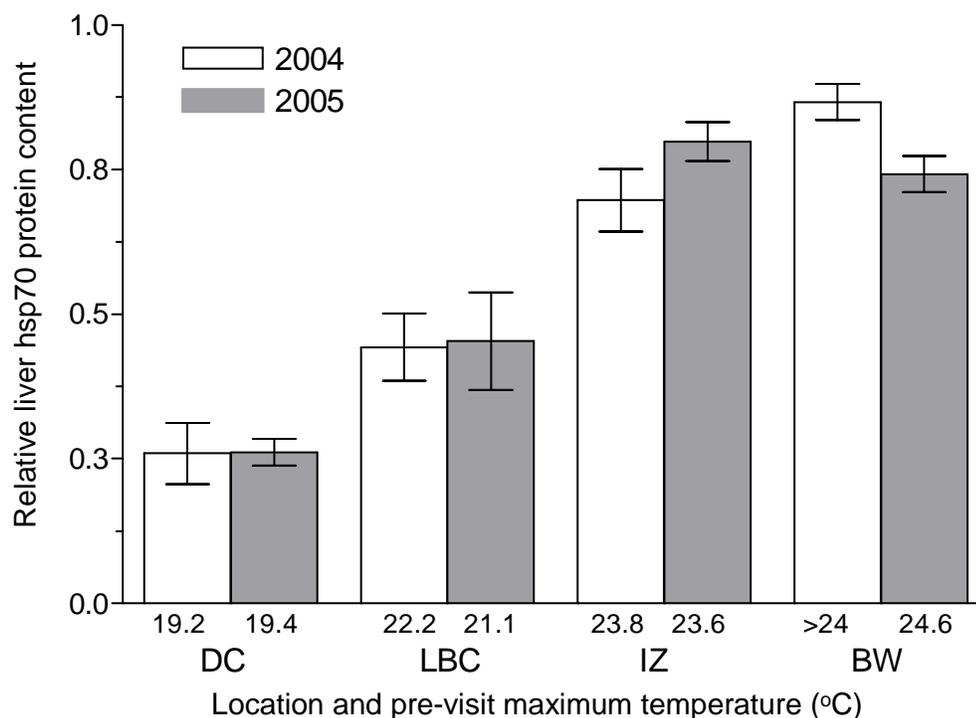


Figure 15- Relative heat shock protein 70 (hsp70) band density (mean densitometry value  $\pm$  1 standard error of the mean) in liver tissue from redband rainbow trout (*Oncorhynchus mykiss gairdneri*). Fish were collected from the South Fork John Day River on 30-31 July 2004 (white bars) and 24-25 July 2005 (grey bars) at Deer Creek (DC), lower Black Canyon (LBC), Izee Falls (IZ), and below Wind Creek (BW). Each bar represents  $n = 10$  except LBC and IZ in 2004 ( $n = 9$ ) and BW in 2005 ( $n = 9$ ).

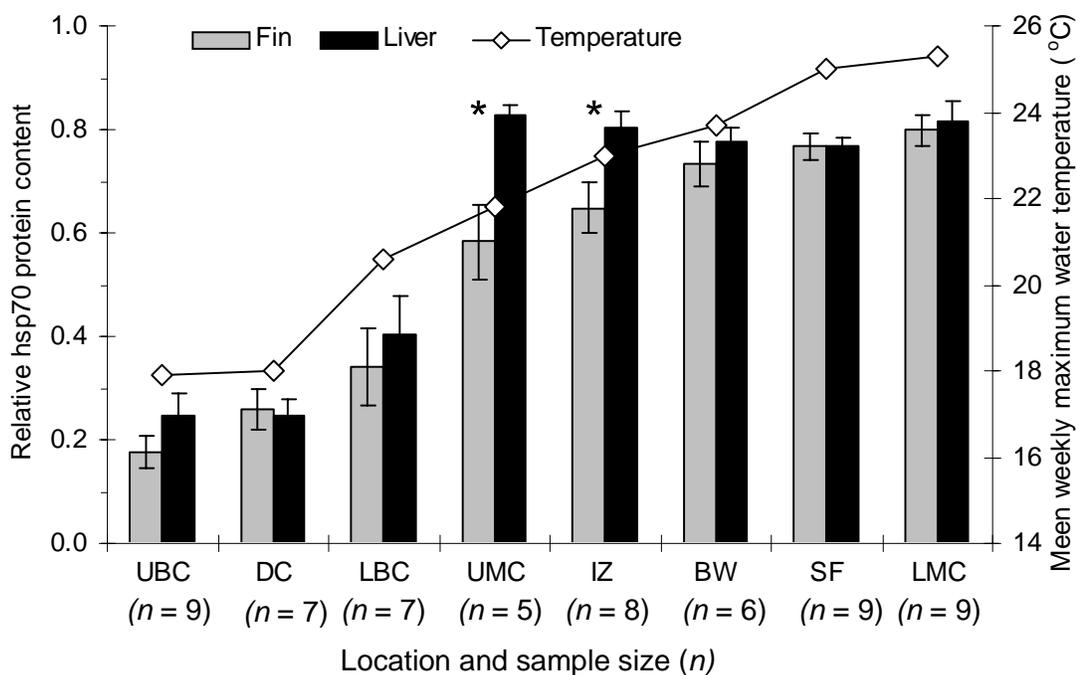


Figure 16- Relative heat shock protein 70 (hsp70) band density (mean densitometry value  $\pm$  1 standard error of the mean) in liver (black bars) and fin (grey bars) from redband rainbow trout (*Oncorhynchus mykiss gairdneri*). Fish were collected from the South Fork John Day River (SFJD) on 23-24 July 2005 at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF). Temperature values represent the maximum water temperature ( $^{\circ}$ C) within 5 days of fish collection. The asterisk represents a significant difference (paired t-test,  $P < 0.05$ ) in hsp70 band density between liver and fin tissue.

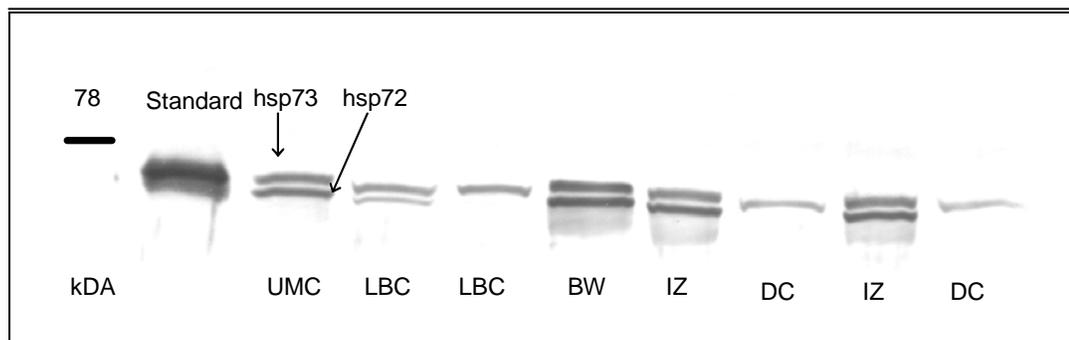


Figure 17- Representative Western blot of hsp72 and hsp73 protein detected in white muscle tissue of juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) collected on 23-24 July 2005. Samples represented here were collected from fish sampled at upper Murderers Creek (UMC), lower Black Canyon (LBC), below Wind Creek (BW), Izee falls (IZ), and Deer Creek (DC). Proteins were visualized colorimetrically using an alkaline phosphatase conjugate substrate (Biorad). A recombinant chinook salmon hsp70 protein (Standard; StressGen Biotechnologies Corp) was applied to each gel to serve as an internal standard for molecular weight determination and blotting efficiency. Band density readings were adjusted for background.

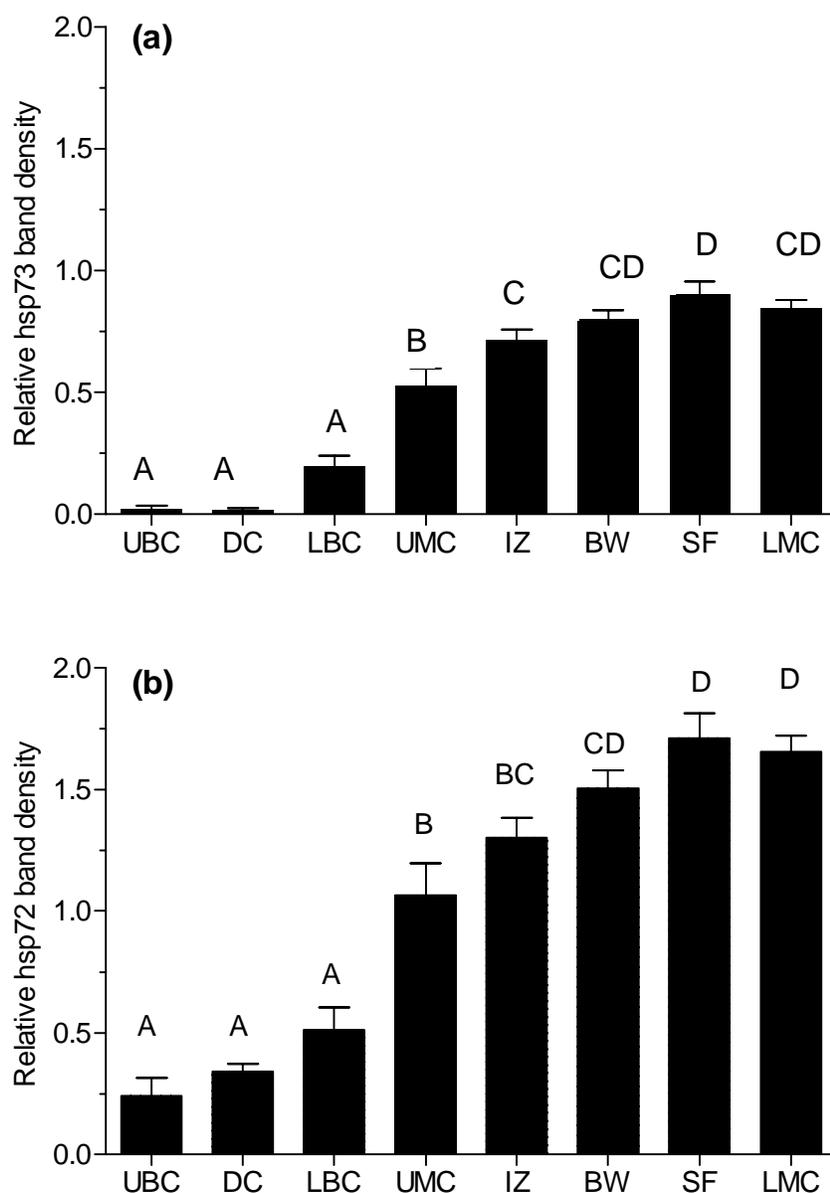


Figure 18- Relative expression of (a) hsp72 (hsp70) and (b) hsp73 (hsc70) in white muscle tissue from redband rainbow trout (*Oncorhynchus mykiss gairdneri*). Fish were collected on 23-24 July 2005 from upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the South Fork John Day River at river kilometer 24 (SF). Hsp70 values represent mean densitometry values of protein bands ( $\pm 1$  standard error of the mean). Shared letters indicate a non-significant difference ( $P > 0.05$ ). Each bar represents  $n = 10$ , except UBC ( $n = 9$ ).

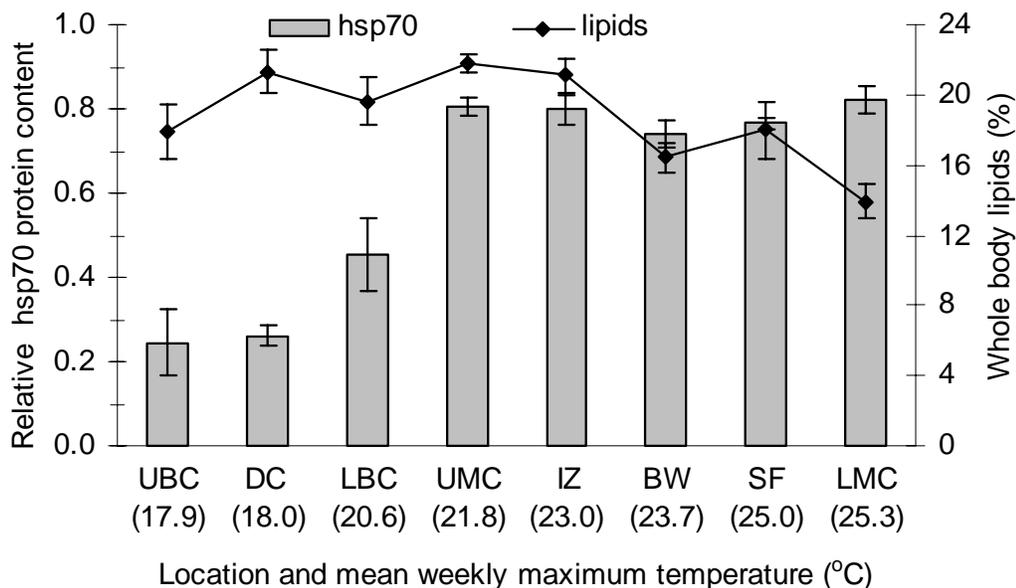


Figure 19- The mean weekly maximum temperature (MWMT) compared to whole body lipids (%) and relative hsp70 band density in liver tissue of juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*). Fish were collected from the South Fork John Day River on 23-24 July 2005 at upper and lower Black Canyon (UBC, LBC), upper and lower Murderers Creek (UMC, LMC), Deer Creek (DC), Izee Falls (IZ), and below Wind Creek (BW). Whole body lipid values represent the mean ( $\pm 1$  standard error of the mean;  $n = 10$  for each symbol) determined through proximate analysis. For hsp70 band density, values represent the mean ( $\pm 1$  standard error of the mean), and each bar represents  $n = 10$  except BW and UBC ( $n = 9$ ) and UMC ( $n = 8$ ).

### References

- Ackerman, P.A., R.B. Forsyth, C.F. Mazur & G.K. Iwama. 2000. Stress hormones and the cellular stress response in salmonids. *Fish Physiology and Biochemistry* 23: 327-336.
- Anthony, J.A., D.D. Roby & K.R. Turco. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. *Journal of Experimental Marine Biology and Ecology* 248: 53-78.
- Basu, N., C.J. Kennedy, P.V. Hodson & G.K. Iwama. 2001. Altered stress responses in rainbow trout following a dietary administration of cortisol and beta-naphthoflavone. *Fish Physiology and Biochemistry* 25: 131-140.
- Baltz, D.M., B. Vondracek, L.R. Brown & P.B. Moyle. 1987. Influence of Temperature on Microhabitat Choice by Fishes in a California Stream. *Transactions of the American Fisheries Society* 116: 12-20.
- Behnke, R.J. & American Fisheries Society. 1992. Native trout of western North America. American Fisheries Society, Bethesda, Md. 275 pp.
- Beitinger, T.L., W.A. Bennett & R.W. McCauley. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental biology of fishes* 58: 237-275.
- Benke, A.C. 1990. A perspective on America's vanishing streams. *Journal of the North American Benthological Society* 9: 77-88.
- Bierkens, J.G.E.A. 2000. Applications and pitfalls of stress-proteins in biomonitoring. *Toxicology* 153: 61-72.
- Brannon, E.L., M.S. Powell, T.P. Quinn & A. Talbot. 2004. Population structure of Columbia River Basin chinook salmon and steelhead trout. *Reviews in Fisheries Science* 12: 99-232.
- Ciechanover, A. & A.L. Schwartz. 1998. The ubiquitin-proteasome pathway: The complexity and myriad functions of proteins death. *Proceedings of the National Academy of Sciences of the United States of America* 95: 2727-2730.
- Clarkson, K., J.D. Kieffer & S. Currie. 2005. Exhaustive exercise and the cellular stress response in rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* 140: 225-232.

- Cunjak, R.A. & J.M. Green. 1986. Influence of water temperature on behavioural interactions between juvenile brook charr, *Salvelinus fontinalis*, and rainbow trout, *Salmo gairdneri*. *Canadian Journal of Zoology* 64: 1288-1291.
- Fader, S.C., Z. Yu & J.R. Spotila. 1994. Seasonal Variation in Heat Shock Proteins (hsp70) in Stream Fish Under Natural Condition. *Journal of Thermal Biology* 19: 335-341.
- Fausch, K.D., C.E. Torgersen, C.V. Baxter & H.W. Li. 2002. Landscapes to Riverscapes: Bridging the Gap between Research and Conservation of Stream Fishes. *Bioscience* 52: 483-498.
- Feder, M.E. & G.E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243-282.
- Feist, B.E., E.A. Steel, G.R. Pess & R.E. Bilby. 2003. The influence of scale on salmon habitat restoration priorities. *Animal Conservation* 6: 271-282.
- Forsyth, R.B., E.P.M. Candido, S.L. Babich & G.K. Iwama. 1997. Stress protein expression in Coho salmon with bacterial kidney disease. *Journal of Aquatic Animal Health* 9: 18-25.
- Gamperl, A.K., K.J. Rodnick, H.A. Faust, E.C. Venn, M.T. Bennett, L.I. Crawshaw, E.R. Keeley, M.S. Powell & H.W. Li. 2002. Metabolism, swimming performance, and tissue biochemistry of high desert redband trout (*Oncorhynchus mykiss ssp.*): Evidence for phenotypic differences in physiological function. *Physiological and Biochemical Zoology* 75: 413-431.
- Hargis, M.T. 1998. Heat shock proteins as biomarkers for thermal stress in chinook salmon. Ph.D. dissertation, University of Nevada Reno.
- Hassanein, H.M.A., M.A. Banhaway, F.M. Soliman, S.A. Abdel-Rehim, W.E.G. Muller & H.C. Schroder. 1999. Induction of hsp70 by the herbicide oxyfluorfen (Goal) in the Egyptian Nile fish, *Oreochromis niloticus*. *Archives of Environmental Contamination and Toxicology* 37: 78-84.
- Hochachka, P.W. & G.N. Somero. 2002. *Biochemical adaptation: mechanism and process in physiological evolution*. Oxford University Press, New York. xi, 466 pp.
- Hofmann, G.E., S.G. Lund, S.P. Place & A.C. Whitmer. 2005. Some like it hot, some like it cold: the heat shock response is found in New Zealand but not Antarctic notothenioid fishes. *Journal of Experimental Marine Biology and Ecology* 316: 79-89.

- Hofmann, G.E. & G.N. Somero. 1995. Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussel *Mytilus trossulus*. *The Journal of Experimental Biology* 198: 1509-1518.
- Hofmann, G.E. & G.N. Somero. 1996. Protein ubiquitination and stress protein synthesis in *Mytilus trossulus* occurs during recovery from tidal emersion. *Molecular Marine Biology and Biotechnology* 5: 175-184.
- Iwama, G.K., L.O.B. Afonso, A. Todgham, P. Ackerman & K. Nakano. 2004. Are hsps suitable for indicating stressed states in fish? *Journal of Experimental Biology* 207: 15-19.
- Iwama, G.K., P.T. Thomas, R.H.B. Forsyth & M.M. Vijayan. 1998. Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries* 8: 35-56.
- Iwama, G.K., M.M. Vijayan, R.B. Forsyth & P.A. Ackerman. 1999. Heat shock proteins and physiological stress in fish. *American Zoologist* 39: 901-909.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Leitzinger, Eric J. 1993. The Influence of Riparian Habitat and Salmonid Microhabitat Selection on Fish Assemblage Structure in the Upper John Day Basin, Oregon. M.S. Thesis. Oregon State University, Corvallis, OR.
- Li, H.W., G.A. Lamberti, T.N. Pearsons, C.K. Tait, J.L. Li & J.C. Buckhouse. 1994. Cumulative Effects of Riparian Disturbances along High Desert Trout Streams of the John Day Basin, Oregon. *Transactions of the American Fisheries Society* 123: 627-640.
- Linton, T.K. & I. Morgan. 1998. Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth and metabolism. *Canadian Journal of Fisheries and Aquatic Science* 55: 576-589.
- Lund, S.G., D. Caissie, R.A. Cunjak, M.M. Vijayan & B.L. Tufts. 2002. The effects of environmental heat stress on heat-shock mRNA and protein expression in Miramichi Atlantic salmon (*Salmo salar*) parr. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1553-1562.
- Lund, S.G., M.E.A. Lund & B.L. Tufts. 2003. Red blood cell Hsp 70 mRNA and protein as bioindicators of temperature stress in the brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* 60: 460-470.

- Mazur, C.F. 1996. The heat shock protein response and physiological stress in aquatic organisms. Ph.D., University of British Columbia, Vancouver.
- Mesa, M.G., L.K. Weiland & P. Wagner. 2002. Effects of acute thermal stress on the survival, predator avoidance, and physiology of juvenile fall chinook salmon. *Northwest Science* 76: 118-128.
- Milne, K.J. & E.G. Noble. 2002. Exercise-induced elevation of HSP70 is intensity dependent. *Journal of Applied Physiology* 93: 561-568.
- Myrick, C.A. & J.J. Cech. 2000. Temperature influences on California rainbow trout physiological performance. *Fish Physiology and Biochemistry* 22: 245-254.
- Nollen, E.A.A. & R.I. Morimoto. 2002. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J Cell Sci* 115: 2809-2816.
- Norris, C.E., P.J. diIorio, R.J. Schultz & L.E. Hightower. 1995. Variation in Heat Shock Proteins within Tropical and Desert Species of Poeciliid Fishes. *Mol. Biol. Evol.* 12: 1048-1062.
- Oregon Department of Environmental Quality. 811 SW Sixth Avenue, Portland, OR 97204-1390. Site accessed on 11 July, 2005.  
<<http://www.deq.state.or.us/wq/303dlist/303dpage.htm>>
- Parsell, D.A. & S. Lindquist. 1994. Heat Shock Proteins and Stress Tolerance. pp. 457-494. In: R.I. Morimoto, A. Tissières & C. Georgopoulos (ed.) *The Biology of heat shock proteins and molecular chaperones*, Cold Spring Harbor Laboratory Press, Plainview, N.Y.
- Place, S.P., M.L. Zippay & G.E. Hofmann. 2004. Constitutive roles for inducible genes: evidence for the alteration in expression of the inducible hsp70 gene in Antarctic notothenioid fishes. *Am J Physiol Regul Integr Comp Physiol* 287: 429-436.
- Railsback, S.F. & K.A. Rose. 1999. Bioenergetics modeling of stream trout growth: Temperature and food consumption effects. *Transactions of the American Fisheries Society* 128: 241-256.
- Rechsteiner, M. 1987. Ubiquitin-mediated pathways for intracellular proteolysis. *Annual Review of Cell Biology* 3: 1-30.
- Reynolds, S. & T. Kunz. 2001. Standard methods for destructive composition analysis. pp. 242. In: J.R. Speakman (ed.) *Body composition analysis of animals: a handbook of non-destructive methods*, Cambridge University Press, Cambridge ; New York.

- Richter, A. & S.A. Kolmes. 2005. Maximum temperature limits for chinook, coho, and chum salmon, and steelhead trout in the Pacific Northwest. *Reviews in Fisheries Science* 13: 23-49.
- Rodnick, K.J., K.A. Gamperl, K.R. Lizars, M.T. Bennett, R.N. Rausch & E.R. Keeley. 2004. Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. *Journal of Fish Biology* 64: 310-335.
- Sanders, B.M. 1993. Stress Proteins in Aquatic Organisms: An Environmental Perspective. *Critical Reviews in Toxicology* 23: 49-75.
- SAS Institute. 2003. SAS OnlineDoc, version 9. 1. SAS Institute, Cary, North Carolina.
- Sathiyaa, R., T. Campbell & M.M. Vijayan. 2001. Cortisol modulates HSP90 mRNA expression in primary cultures of trout hepatocytes. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 129: 679-685.
- Schreck, C.B. 2000. Accumulation and long-term effects of stress in fish. pp. 147-158. *In: G.P. Moberg & J.A. Mench (ed.) The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*, CABI Publishing, Walingford, UK.
- Schreck, C.B., B.L. Olla & M.W. Davis. 1997. Behavioural responses to stress. pp. 145-180. *In: G.W. Iwama, J. Sumpter, A.D. Pickering & C.B. Schreck (ed.) Fish Stress and Health in Aquaculture*, Cambridge University Press, Cambridge.
- S-Plus version 6.2 for windows, Insightful Corporation, Seattle, Washington USA.
- Tattam, Ian. 2006. M.S. thesis. Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon.
- Towbin, H., T. Staehelin, J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America*, Vol 16 (9): 4340-4354.
- The Upper John Day River Local Advisory Committee. 2002. Upper Mainstem and South Fork John Day River Agricultural Water Quality Management Area Plan. Guidance Document Developed with assistance from Oregon Department of Agriculture and Grant Soil and Water Conservation District.
- Torgersen, C.E., D.M. Price, H.W. Li & B.A. McIntosh. 1999. Multiscale thermal refugia and stream habitat associations of chinook salmon in northeastern Oregon. *Ecological Applications* 9: 301-319.

- Viant, M.R., I. Werner, E.S. Rosenblum, A.S. Gantner, R.S. Tjeerdema & M.L. Johnson. 2003. Correlation between heat-shock protein induction and reduced metabolic condition in juvenile steelhead trout (*Oncorhynchus mykiss*) chronically exposed to elevated temperature. *Fish Physiology and Biochemistry* 29: 159-171.
- Vijayan, M.M., C. Pereira, R.B. Forsyth, C.J. Kennedy & G.K. Iwama. 1997. Handling stress does not affect the expression of hepatic heat shock protein 70 and conjugation enzymes in rainbow trout treated with beta-naphthoflavone. *Life Sciences* 61: 117-127.
- Vijayan, M.M., C. Pereira, G. Kruzynski & G.K. Iwama. 1998. Sublethal concentrations of contaminant induce the expression of hepatic heat shock protein 70 in two salmonids. *Aquatic Toxicology* 40: 101-108.
- Werner, I., T.B. Smith, J. Feliciano & M.L. Johnson. 2005. Heat shock proteins in juvenile steelhead reflect thermal conditions in the Navarro River watershed, California. *Transactions of the American Fisheries Society* 134: 399-410.
- Zarate, J. & T.M. Bradley. 2003. Heat shock proteins are not sensitive indicators of hatchery stress in salmon. *Aquaculture* 223: 175-187.
- Zoellick, B.W. 1999. Stream temperatures and the elevational distribution of redband trout in southwestern Idaho. *Great Basin Naturalist* 59: 136-143.
- Zoellick, B.W. 2004. Density and biomass of redband trout relative to stream shading and temperature in southwestern Idaho. *Western North American Naturalist* 64: 18-26.
- Zugel, U. & S.H.E. Kaufmann. 1999. Role of Heat Shock Proteins in Protection from and Pathogenesis of Infectious Diseases. *Clin. Microbiol. Rev.* 12: 19-39.

## Chapter 4: General Discussion

In this thesis, I examined the utility of heat shock proteins (hsp70 family) and whole body lipids as indices of fish condition as a method for determining physiologically stressful water temperatures in juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*). Understanding physiological temperature limits is important for defining and classifying summer thermal habitat requirements. I also documented the effect of handling and temperature stressors on hsp70 levels in laboratory studies. Results from the laboratory temperature manipulation study helped explain the patterns of hsp70 expression observed in fish sampled from the South Fork John Day (SFJD) River (chapter 3). Results from the PIT tag experiment (Chapter 2) also provided information about short term effects of PIT tagging on plasma cortisol and hepatic hsp70 levels. This research also examined ecological and management implications for using heat shock proteins as an indicator of thermal stress.

Results of the PIT tag experiment (Chapter 2) show that hsp70 levels are not affected by PIT tag handling stress. Furthermore, there were no long term (> 24 h) increases in plasma cortisol levels in PIT tagged fish. There was an increase in plasma cortisol levels following the initial tagging procedure at Time 2h, but PIT tagging was no more stressful than handling. There was some evidence that PIT tagged fish had higher plasma cortisol levels at 6 hours post-tagging than control fish, but the variability in plasma cortisol and the small sample size leave this conclusion open to debate. Moreover, changes in plasma cortisol levels did not measurably alter hsp70 expression. This finding is important because PIT tags are used throughout the Columbia River Basin to track movement and habitat use of endangered salmonids. Tagging methods that have negative impacts on performance of endangered salmonids should be discouraged. Since PIT tagging does not alter hepatic hsp70 levels, and the effects of PIT tagging on plasma cortisol levels are minimal, it should be possible to use PIT tags in combination with lab or field manipulations to examine the effect of specific or multiple stressors on hepatic hsp70 levels.

A major finding of this research was that juvenile redband steelhead trout in the SFJD experience summertime water temperatures that increase cellular hsp70

levels in liver, white muscle, and fin tissue. The finding that hsp70 can be measured in fin tissue is important because it provides a non-lethal procedure for measuring hsp70 levels. To my knowledge, this is the first documented use of fin clips as a non-lethal technique for quantifying cellular hsp70 levels in wild populations of rainbow trout.

The general pattern of hsp70 expression in relation to temperature was similar in fin, liver and white muscle tissue. However, there was some evidence of a difference in cellular hsp70 levels between these three tissues. For example, in the laboratory study, fin tissue had greater hsp70 expression following the 22°C temperature stress than liver tissue. A similar finding was found for fish collected from the SFJD River at the UMC and IZ study location when MWMT were between 21 and 23°C. However, at UMC and IZ, hsp70 levels were higher in liver tissue than in fin tissue. It is also important to recognize that estimated threshold temperatures (i.e. temperature that induce hsp70 synthesis) were higher in white muscle tissue than liver tissue. Differential hsp70 expression in tissues is an important consideration because it provides an example of the danger in comparing the hsp70 response among studies using different tissues. The variability in estimated induction temperature using different temperature summaries, and different tissues, is important because it demonstrates how different temperature summaries lead to different conclusions about sublethal temperatures causing protein damage.

There was also a general relation between increased levels of hsp70 and decreased body condition (whole body lipids). It is not clear which physiological mechanisms best explains the general decrease in body lipids when MWMT exceed 23°C. One possibility is that metabolic costs associated with maintaining an elevated hsp70 response for a prolonged period of time (e.g., > 1 week) contribute to a net energy loss. Other explanations for decreased lipid levels include differences in food availability among study locations (e.g., community level impacts of water temperature), increased metabolic rates (e.g.,  $Q_{10}$ ), behavioral modification (e.g., appetite suppression), or a combination of effects.

There might be a seasonal component that helps explains hsp70 levels. However, the relation between water temperature and hsp70 expression in this study

make it difficult to determine if the effect of season acts independently of temperature effects. For example, for fish collected from SFJD River, there is greater variation in hsp70 levels among locations (Figure 13) and temperatures (Table 2) on 10-11 June 2004, than there is in Deer Creek from June to September. These findings suggest future research needs to carefully consider variation within a season and among different locations, and the effects of past thermal history on hsp70 expression.

The combination of hsp70 measurements and whole body lipid content is an example of how physiology can be used to help define habitat criteria for fish. However, indices used to define physiologically suitable habitat should be carefully considered. It is important to develop physiological indicators of fish performance that can be used to rapidly evaluate a fish's condition in a given habitat. Large quantities of money are being spent on habitat restoration projects. In general, the goal of habitat restoration efforts is to see an increase in the fish population that can be attributed to the restoration activity. Unfortunately, waiting for a population level response to evaluate the success of a given restoration activity might take years. If there is a change in the fish population, can it be proven that the change was a function of the restoration activity? Other explanations for the population change might include changes in ocean conditions that modify ocean survival of juvenile fish, or some other downstream modification (e.g., removal or building a dam) that effected survival. By using an index of population health, such as measurements of hsp70 to determine if fish are experiencing thermal stress, the impact of recovery and restoration efforts can be evaluated on a much shorter time scale. Another benefit of physiological indicators is that the effects of the restoration activity can be evaluated within the restoration area. For example, in Murderers Creek in the South Fork John Day River, the riparian zone has been protected by fencing that keeps cows out of the riparian zone. In the fenced areas, there is more vegetation. One way to evaluate the effect of this restoration activity on the fish community in Murderers creek would be to compare the physiological condition of fish in fenced areas with the condition of fish in unfenced areas.

Although this research suggests juvenile redband rainbow trout in the SFJD River can tolerate summer daily maximum temperatures in excess of 22°C, there are

physiological costs. The rapid increase in cellular hsp70 levels is an example of a physiological mechanism that allows cells to withstand temperature increases, but this also means cellular processes are being threatened. There is also a difference between cellular stress and stress behaviors associated with distress. It is premature to presume that cellular stress is a superior or inferior indicator of physiological status if distress—which can have behavioral consequences (e.g., increased predation risk)—acts through mechanisms that do not involve cellular alterations in hsp70 levels. Compared to streams and rivers flowing through cities, the most prominent stressor in the SFJD River is temperature. Would juvenile trout still be able to cope with temperatures in excess of 22°C if trout were exposed to multiple or sequential stressors (e.g., heat stress + pesticides + disease)?

The positive or negative impacts of altering hsp levels and the subsequent changes in the physiological status of fish are poorly understood. For example, how does thermal stress influence growth and development, long term survival, or life history strategies? At what point do hsp70 levels become detrimental to cellular processes? Although the ability to use hsps as a biomarker of “stress” is still questionable, carefully directed research measuring hsps in combination with other physiological indicators of cellular stress (e.g., ubiquitin) show promise for advancing the understanding of how temperature affects physiological processes in fish.

In conclusion, juvenile redband rainbow trout in the SFJD River are experiencing thermal stress during the summer. Although these fish are surviving summertime water temperatures that approach 26°C, this research does not prove or disprove the hypothesis that redband rainbow trout in the SFJD River are better suited to warm water than any other rainbow trout subpopulation. The definition of suitable thermal habitat for juvenile trout requires an understanding of physiological temperature limits, and a differences of 1 or 2°C is an important distinction when defining “suitable” and “unsuitable” habitat.

## BIBLIOGRAPHY

- Ackerman, P.A., R.B. Forsyth, C.F. Mazur & G.K. Iwama. 2000. Stress hormones and the cellular stress response in salmonids. *Fish Physiology and Biochemistry* 23: 327-336.
- Anthony, J.A., D.D. Roby & K.R. Turco. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. *Journal of Experimental Marine Biology and Ecology* 248: 53-78.
- Basu, N., C.J. Kennedy, P.V. Hodson & G.K. Iwama. 2001. Altered stress responses in rainbow trout following a dietary administration of cortisol and beta-naphthoflavone. *Fish Physiology and Biochemistry* 25: 131-140.
- Baltz, D.M., B. Vondracek, L.R. Brown & P.B. Moyle. 1987. Influence of Temperature on Microhabitat Choice by Fishes in a California Stream. *Transactions of the American Fisheries Society* 116: 12-20.
- Behnke, R.J. & American Fisheries Society. 1992. Native trout of western North America. American Fisheries Society, Bethesda, Md. 275 pp.
- Beitinger, T.L., W.A. Bennett & R.W. McCauley. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental biology of fishes* 58: 237-275.
- Benke, A.C. 1990. A perspective on America's vanishing streams. *Journal of the North American Benthological Society* 9: 77-88.
- Bierkens, J.G.E.A. 2000. Applications and pitfalls of stress-proteins in biomonitoring. *Toxicology* 153: 61-72.
- Bouwes, N. ed. 2005. Integrated status and effectiveness monitoring John Day pilot program, 2005 draft annual report. Compiled and edited by Eco Logical Research, Inc. Providence, UT for NOAA Fisheries Service and the Bonneville Power Administration.
- Brannon, E.L., M.S. Powell, T.P. Quinn & A. Talbot. 2004. Population structure of Columbia River Basin chinook salmon and steelhead trout. *Reviews in Fisheries Science* 12: 99-232.
- Ciechanover, A. & A.L. Schwartz. 1998. The ubiquitin-proteasome pathway: The complexity and myriad functions of proteins death. *Proceedings of the National Academy of Sciences of the United States of America* 95: 2727-2730.

- Clarkson, K., J.D. Kieffer & S. Currie. 2005. Exhaustive exercise and the cellular stress response in rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* 140: 225-232.
- Cunjak, R.A. & J.M. Green. 1986. Influence of water temperature on behavioural interactions between juvenile brook charr, *Salvelinus fontinalis*, and rainbow trout, *Salmo gairdneri*. *Canadian Journal of Zoology* 64: 1288-1291.
- Cucherousset, J., J.M. Roussel, R. Keeler, R.A. Cunjak & R. Stump. 2005. The use of two new portable 12-mm PIT tag detectors to track small fish in shallow streams. *North American Journal of Fisheries Management* 25: 270-274.
- Dare, M.R. 2003. Mortality and Long-Term Retention of Passive Integrated Transponder Tags by Spring Chinook Salmon. *North American Journal of Fisheries Management* 23: 1015-1019.
- Fader, S.C., Z. Yu & J.R. Spotila. 1994. Seasonal Variation in Heat Shock Proteins (hsp70) in Stream Fish Under Natural Condition. *Journal of Thermal Biology* 19: 335-341.
- Fausch, K.D., C.E. Torgersen, C.V. Baxter & H.W. Li. 2002. Landscapes to Riverscapes: Bridging the Gap between Research and Conservation of Stream Fishes. *Bioscience* 52: 483-498.
- Feder, M.E. & G.E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243-282.
- Feist, B.E., E.A. Steel, G.R. Pess & R.E. Bilby. 2003. The influence of scale on salmon habitat restoration priorities. *Animal Conservation* 6: 271-282.
- Forsyth, R.B., E.P.M. Candido, S.L. Babich & G.K. Iwama. 1997. Stress protein expression in Coho salmon with bacterial kidney disease. *Journal of Aquatic Animal Health* 9: 18-25.
- Foster, L.B., and R.T. Dunn. 1974. Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clinical Chemistry* 20: 365-368.
- Fretwell, S.D. and H.L. Lucas. 1970. On territorial behavior and other factors influencing habitat distribution in birds. I. Theoretical development. *Acta Biotheor.* 19:16-36.

- Gamperl, A.K., K.J. Rodnick, H.A. Faust, E.C. Venn, M.T. Bennett, L.I. Crawshaw, E.R. Keeley, M.S. Powell & H.W. Li. 2002. Metabolism, swimming performance, and tissue biochemistry of high desert redband trout (*Oncorhynchus mykiss ssp.*): Evidence for phenotypic differences in physiological function. *Physiological and Biochemical Zoology* 75: 413-431.
- Gries, G. & B.H. Letcher. 2002. Tag Retention and Survival of Age-0 Atlantic Salmon following Surgical Implantation with Passive Integrated Transponder Tags. *North American Journal of Fisheries Management* 22: 219-222.
- Hargis, M.T. 1998. Heat shock proteins as biomarkers for thermal stress in chinook salmon. Ph.D. dissertation, University of Nevada Reno.
- Hassanein, H.M.A., M.A. Banhaway, F.M. Soliman, S.A. Abdel-Rehim, W.E.G. Muller & H.C. Schroder. 1999. Induction of hsp70 by the herbicide oxyfluorfen (Goal) in the Egyptian Nile fish, *Oreochromis niloticus*. *Archives of Environmental Contamination and Toxicology* 37: 78-84.
- Hazon, N. & R. Balment. 1998. Endocrinology. pp. 519. *In*: D.H. Evans (ed.) *The physiology of fishes*, CRC Press, Boca Raton
- Hochachka, P.W. & G.N. Somero. 2002. *Biochemical adaptation: mechanism and process in physiological evolution*. Oxford University Press, New York. xi, 466 pp.
- Hockersmith, E.E., W.D. Muir, S.G. Smith, B.P. Sandford, R.W. Perry, N.S. Adams & D.W. Rondorf. 2003. Comparison of migration rate and survival between radio-tagged and PIT-tagged migrant yearling chinook salmon in the Snake and Columbia rivers. *North American Journal of Fisheries Management* 23: 404-413.
- Hofmann, G.E., S.G. Lund, S.P. Place & A.C. Whitmer. 2005. Some like it hot, some like it cold: the heat shock response is found in New Zealand but not Antarctic notothenioid fishes. *Journal of Experimental Marine Biology and Ecology* 316: 79-89.
- Hofmann, G.E. & G.N. Somero. 1995. Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussel *Mytilus trossulus*. *The Journal of Experimental Biology* 198: 1509-1518.
- Hofmann, G.E. & G.N. Somero. 1996. Protein ubiquitination and stress protein synthesis in *Mytilus trossulus* occurs during recovery from tidal emersion. *Molecular Marine Biology and Biotechnology* 5: 175-184.

- Hughes, N.F. & T.C. Grand. 2000. Physiological ecology meets the ideal-free distribution: Predicting the distribution of size-structured fish populations across temperature gradients. *Environmental Biology of Fishes* 59: 285-298.
- Iwama, G.K., P.T. Thomas, R.H.B. Forsyth & M.M. Vijayan. 1998. Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries* 8: 35-56.
- Iwama, G.K., L.O.B. Afonso, A. Todgham, P. Ackerman & K. Nakano. 2004. Are hsp90 suitable for indicating stressed states in fish? *Journal of Experimental Biology* 207: 15-19.
- Iwama, G.K., M.M. Vijayan, R.B. Forsyth & P.A. Ackerman. 1999. Heat shock proteins and physiological stress in fish. *American Zoologist* 39: 901-909.
- Jepsen, N., L.E. Davis, C.B. Schreck & B. Siddens. 2001. The Physiological Response of Chinook Salmon Smolts to Two Methods of Radio-Tagging. *Transactions of the American Fisheries Society* 130: 495-500.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Leitzinger, Eric J. 1993. The Influence of Riparian Habitat and Salmonid Microhabitat Selection on Fish Assemblage Structure in the Upper John Day Basin, Oregon. M.S. Thesis. Oregon State University, Corvallis, OR.
- Li, H. W., and M. D. Bowen. 2003. Indexing carrying capacity of salmonids on the basis of longitudinal stream temperatures. United States Bureau of Reclamation, Denver, Colorado.
- Li, H.W., G.A. Lamberti, T.N. Pearsons, C.K. Tait, J.L. Li & J.C. Buckhouse. 1994. Cumulative Effects of Riparian Disturbances along High Desert Trout Streams of the John Day Basin, Oregon. *Transactions of the American Fisheries Society* 123: 627-640.
- Linton, T.K. & I. Morgan. 1998. Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth and metabolism. *Canadian Journal of Fisheries and Aquatic Science* 55: 576-589.
- Lund, S.G., D. Caissie, R.A. Cunjak, M.M. Vijayan & B.L. Tufts. 2002. The effects of environmental heat stress on heat-shock mRNA and protein expression in Miramichi Atlantic salmon (*Salmo salar*) parr. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1553-1562.

- Lund, S.G., M.E.A. Lund & B.L. Tufts. 2003. Red blood cell Hsp 70 mRNA and protein as bioindicators of temperature stress in the brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 60: 460-470.
- Mazur, C.F. 1996. The heat shock protein response and physiological stress in aquatic organisms. Ph.D., University of British Columbia, Vancouver.
- Mesa, M.G., L.K. Weiland & P. Wagner. 2002. Effects of acute thermal stress on the survival, predator avoidance, and physiology of juvenile fall chinook salmon. Northwest Science 76: 118-128.
- Milne, K.J. & E.G. Noble. 2002. Exercise-induced elevation of HSP70 is intensity dependent. Journal of Applied Physiology 93: 561-568.
- Muir, W.D., S.G. Smith, J.G. Williams, E.E. Hockersmith & J.R. Skalski. 2001. Survival Estimates for Migrant Yearling Chinook Salmon and Steelhead Tagged with Passive Integrated Transponders in the Lower Snake and Lower Columbia Rivers, 1993-1998. North American Journal of Fisheries Management 21: 269-282.
- Myrick, C.A. & J.J. Cech. 2000. Temperature influences on California rainbow trout physiological performance. Fish Physiology and Biochemistry 22: 245-254.
- Nollen, E.A.A. & R.I. Morimoto. 2002. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. J Cell Sci 115: 2809-2816.
- Norris, C.E., P.J. diIorio, R.J. Schultz & L.E. Hightower. 1995. Variation in Heat Shock Proteins within Tropical and Desert Species of Poeciliid Fishes. Mol. Biol. Evol. 12: 1048-1062.
- Ombredane, D., J.L. Bagliniere & F. Marchand. 1998. The effects of Passive Integrated Transponder tags on survival and growth of juvenile brown trout (*Salmo trutta L.*) and their use for studying movement in a small river. Hydrobiologia 372: 99-106.
- Oregon Department of Environmental Quality. 811 SW Sixth Avenue, Portland, OR 97204-1390. Site accessed on 11 July, 2005.  
<<http://www.deq.state.or.us/wq/303dlist/303dpage.htm>>
- Parsell, D.A. & S. Lindquist. 1994. Heat Shock Proteins and Stress Tolerance. pp. 457-494. In: R.I. Morimoto, A. Tissiaeres & C. Georgopoulos (ed.) The Biology of heat shock proteins and molecular chaperones, Cold Spring Harbor Laboratory Press, Plainview, N.Y.

- Peterson, N.P., E.F. Prentice & T.P. Quinn. 1994. Comparison of Sequential Coded Wire and Passive Integrated Transponder Tags for Assessing Overwinter Growth and Survival of Juvenile Coho Salmon. *North American Journal of Fisheries Management* 14: 879-873.
- Place, S.P., M.L. Zippay & G.E. Hofmann. 2004. Constitutive roles for inducible genes: evidence for the alteration in expression of the inducible hsp70 gene in Antarctic notothenioid fishes. *Am J Physiol Regul Integr Comp Physiol* 287: 429-436.
- Prentice, E.F., T.A. Flagg, C.S. McCutcheon & D.F. Brastow. 1990a. PIT-tag Monitoring Systems for Hydroelectric Dams and Fish Hatcheries. *American Fisheries Society Symposium* 7: 323-334.
- Prentice, E.F., T.A. Flagg & C.S. McCutcheon. 1990b. Feasibility of Using Implantable Passive Integrated Transponder (PIT) Tags in Salmonids. *American Fisheries Society Symposium* 7: 317-322
- Railsback, S.F. & K.A. Rose. 1999. Bioenergetics modeling of stream trout growth: Temperature and food consumption effects. *Transactions of the American Fisheries Society* 128: 241-256.
- Redding, J.M., Schreck, C.B., Birks, E.K., and Ewing, R.D. 1984. Cortisol and Its Effects on Plasma Thyroid Hormone and Electrolyte Concentrations in Fresh Water and during seawater Acclimation in Yearling Coho Salmon, *Oncorhynchus kisutch*. *General and Comparative Endocrinology* 56: 146-155.
- Rechsteiner, M. 1987. Ubiquitin-mediated pathways for intracellular proteolysis. *Annual Review of Cell Biology* 3: 1-30.
- Reynolds, S. & T. Kunz. 2001. Standard methods for destructive composition analysis. pp. 242. *In: J.R. Speakman (ed.) Body composition analysis of animals: a handbook of non-destructive methods*, Cambridge University Press, Cambridge ; New York.
- Richter, A. & S.A. Kolmes. 2005. Maximum temperature limits for chinook, coho, and chum salmon, and steelhead trout in the Pacific Northwest. *Reviews in Fisheries Science* 13: 23-49.
- Rodnick, K.J., K.A. Gamperl, K.R. Lizars, M.T. Bennett, R.N. Rausch & E.R. Keeley. 2004. Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. *Journal of Fish Biology* 64: 310-335.
- Roussel, J.M., R.A. Cunjak, R. Newbury, D. Caissie & A. Haro. 2004. Movements and habitat use by PIT-tagged Atlantic salmon parr in early winter: the influence of anchor ice. *Freshwater Biology* 49: 1026-1035.

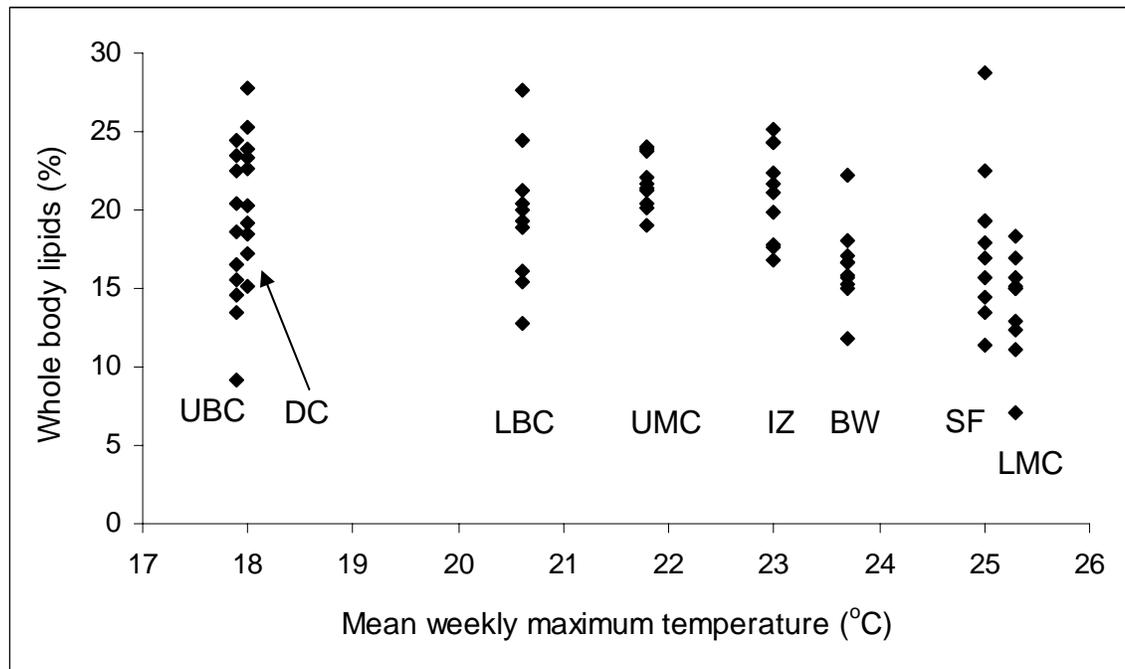
- Roussel, J.M., A. Haro & R.A. Cunjak. 2000. Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology. *Canadian Journal of Fisheries and Aquatic Sciences* 57: 1326-1329.
- Sanders, B.M. 1993. Stress Proteins in Aquatic Organisms: An Environmental Perspective. *Critical Reviews in Toxicology* 23: 49-75.
- Sathiyaa, R., T. Campbell & M.M. Vijayan. 2001. Cortisol modulates HSP90 mRNA expression in primary cultures of trout hepatocytes. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 129: 679-685.
- Schreck, C.B. 2000. Accumulation and Long-term Effects of Stress in Fish. *In: The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare* (Eds. G.P. Moberg and J.A. Mench), pp. 147-158. Walingford, UK: CABI Publishing.
- Schreck, C.B., B.L. Olla & M.W. Davis. 1997. Behavioural responses to stress. pp. 145-180. *In: G.W. Iwama, J. Sumpter, A.D. Pickering & C.B. Schreck (ed.) Fish Stress and Health in Aquaculture*, Cambridge University Press, Cambridge.
- Schreck, C.B., W. Contreras-Sanchez & M.S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* 197: 3-24.
- SAS Institute. 2003. SAS OnlineDoc, version 9. 1. SAS Institute, Cary, North Carolina.
- S-Plus version 6.2 for windows, Insightful Corporation, Seattle, Washington USA.
- Tattam, Ian. 2006. M.S. thesis. Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon.
- The Upper John Day River Local Advisory Committee. 2002. Upper Mainstem and South Fork John Day River Agricultural Water Quality Management Area Plan. Guidance Document Developed with assistance from Oregon Department of Agriculture and Grant Soil and Water Conservation District.
- Torgersen, C.E., D.M. Price, H.W. Li & B.A. McIntosh. 1999. Multiscale thermal refugia and stream habitat associations of chinook salmon in northeastern Oregon. *Ecological Applications* 9: 301-319.

- Towbin, H., T. Staehelin, J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America*, Vol 16 (9): 4340-4354.
- Viant, M.R., I. Werner, E.S. Rosenblum, A.S. Gantner, R.S. Tjeerdema & M.L. Johnson. 2003. Correlation between heat-shock protein induction and reduced metabolic condition in juvenile steelhead trout (*Oncorhynchus mykiss*) chronically exposed to elevated temperature. *Fish Physiology and Biochemistry* 29: 159-171.
- Vijayan, M.M., C. Pereira, R.B. Forsyth, C.J. Kennedy & G.K. Iwama. 1997. Handling stress does not affect the expression of hepatic heat shock protein 70 and conjugation enzymes in rainbow trout treated with beta-naphthoflavone. *Life Sciences* 61: 117-127.
- Vijayan, M.M., C. Pereira, G. Kruzynski & G.K. Iwama. 1998. Sublethal concentrations of contaminant induce the expression of hepatic heat shock protein 70 in two salmonids. *Aquatic Toxicology* 40: 101-108.
- Washburn, B.S., J.J. Moreland, A.M. Slaughter, I. Werner, D.E. Hinton & B.M. Sanders. 2002. Effects of handling on heat shock protein expression in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* 21: 557-560.
- Werner, I., T.B. Smith, J. Feliciano & M.L. Johnson. 2005. Heat shock proteins in juvenile steelhead reflect thermal conditions in the Navarro River watershed, California. *Transactions of the American Fisheries Society* 134: 399-410.
- Zarate, J. & T.M. Bradley. 2003. Heat shock proteins are not sensitive indicators of hatchery stress in salmon. *Aquaculture* 223: 175-187.
- Zugel, U. & S.H.E. Kaufmann. 1999. Role of Heat Shock Proteins in Protection from and Pathogenesis of Infectious Diseases. *Clin. Microbiol. Rev.* 12: 19-39.
- Zoellick, B.W. 1999. Stream temperatures and the elevational distribution of redband trout in southwestern Idaho. *Great Basin Naturalist* 59: 136-143.
- Zoellick, B.W. 2004. Density and biomass of redband trout relative to stream shading and temperature in southwestern Idaho. *Western North American Naturalist* 64: 18-26.

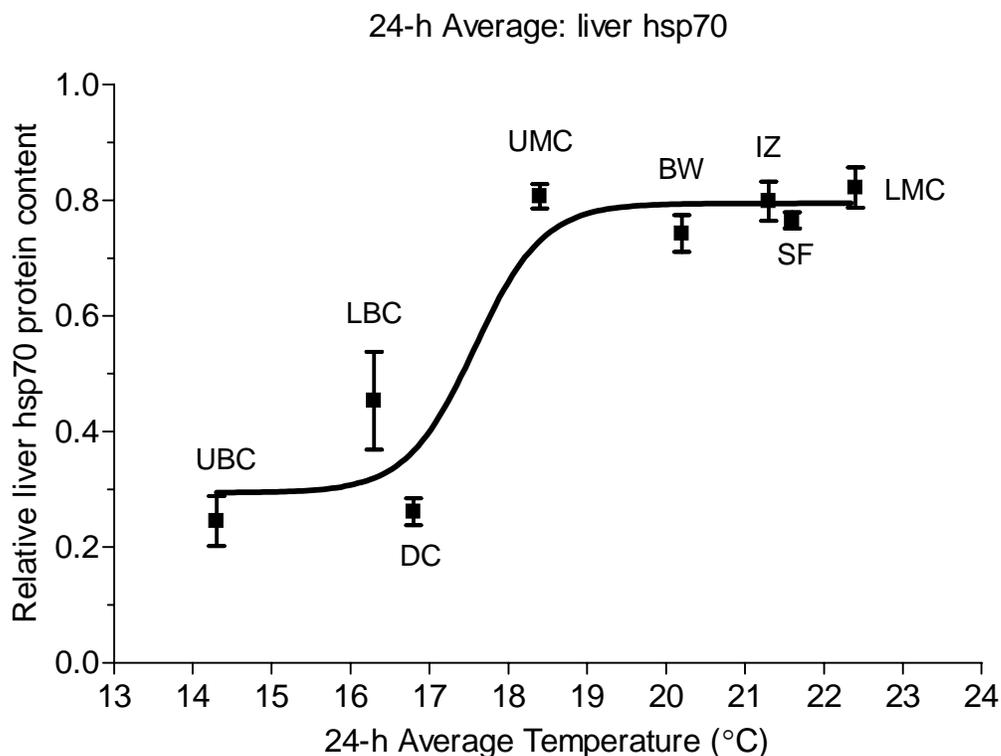
## APPENDICES

Appendix A-Summary of mean whole body lipids (%) and water content (%) of juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) collected from the South Fork John Day River. Fish were collected from 10-11 June, 30-31 July, and 11 September 2004, and 23-24 July 2005 at lower and upper Black canyon (LBC, UBC), the South Fork John Day at river kilometer 24 (SF), lower and upper Murderers creek (LMC, UMC), below Wind creek (BW), Deer Creek (DC), and Izee falls (IZ).

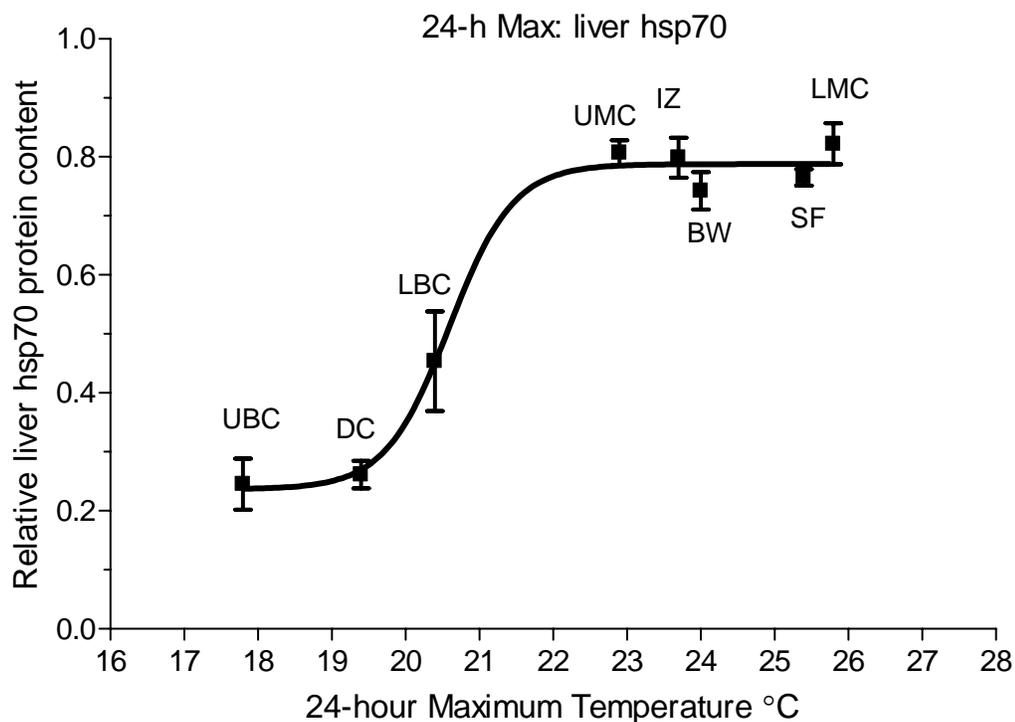
Sampling Location	Sampling date	n	Whole Body Lipids (%)		Water content (%)	
			Mean	SE	Mean	SE
Lower Black Canyon	10 June 04	7	15.10	1.50	75.92	0.59
Lower Black Canyon	31 July 04	9	20.39	1.45	73.76	0.47
Lower Black Canyon	11 Sept. 04	9	17.28	0.99	73.76	0.47
Below Wind	11 June 04	7	11.78	0.89	76.87	0.46
Below Wind	31 July 04	8	14.35	1.04	75.79	0.33
Below Wind	11 Sept. 04	9	14.31	1.27	75.79	0.33
Izee Falls	11 June 04	7	14.73	3.06	75.91	0.87
Izee Falls	30 July 04	9	21.72	1.78	72.16	0.64
Izee Falls	11 Sept. 04	9	18.04	1.08	72.87	0.27
Deer Creek	10 June 04	7	16.35	1.43	75.02	0.54
Deer Creek	30 July 04	9	22.44	0.68	73.85	0.37
Deer Creek	11 Sept. 04	9	16.7	1.22	73.54	0.37
Upper Black Canyon	25 July 05	10	17.98	1.58	75.21	0.5
Lower Black Canyon	25 July 05	10	19.62	1.37	75.17	0.6
South Fork	25 July -05	10	17.98	1.58	75.21	0.5
Upper Murderers Creek	24 July 05	10	21.76	0.54	73.52	0.23
Lower Murderers Creek	24 July 05	10	13.94	1.02	75.62	0.32
Below Wind	25 July 05	10	16.42	0.84	76.03	0.58
Izee Falls	24 July 05	10	21.1	0.1	73.84	0.37
Deer Creek	24 July 05	10	21.32	1.24	74.16	0.35
Total		N = 172				



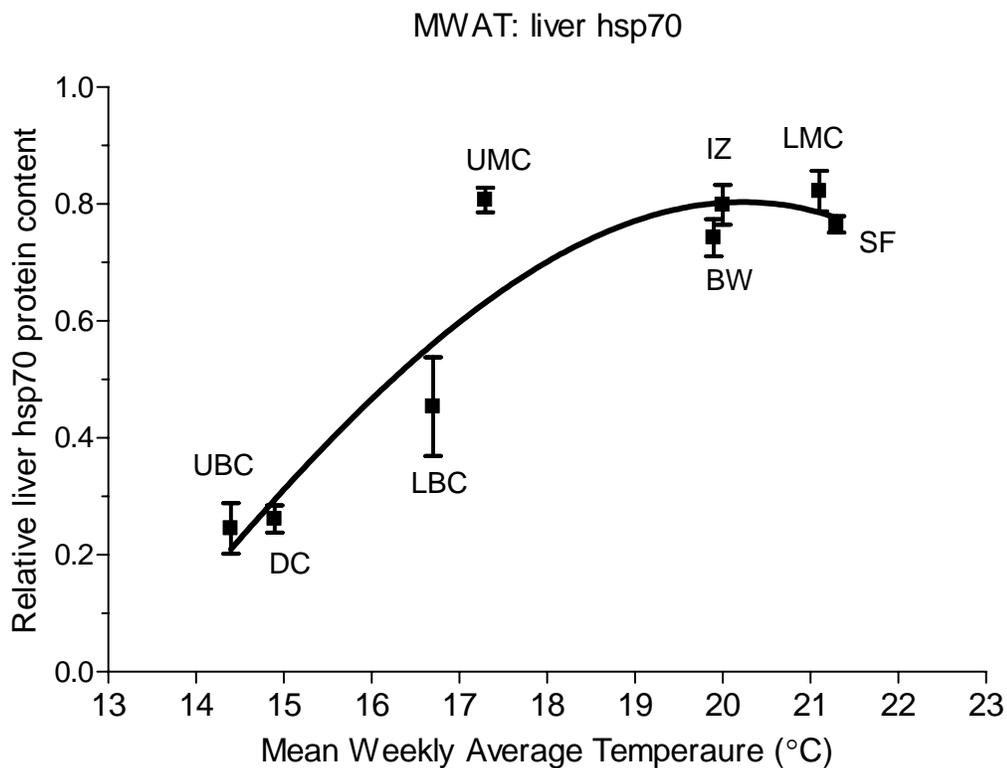
Appendix B- Scatter plot of whole body lipids (%) compared to the mean weekly maximum temperature (°C) before capture for juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) collected on 23-24 July 2005. Fish were collected at lower and upper Black canyon (LBC, UBC), the South Fork John Day at river kilometer 24 (SF), lower and upper Murderers creek (LMC, UMC), below Wind creek (BW), Deer Creek (DC), and Izee falls (IZ).



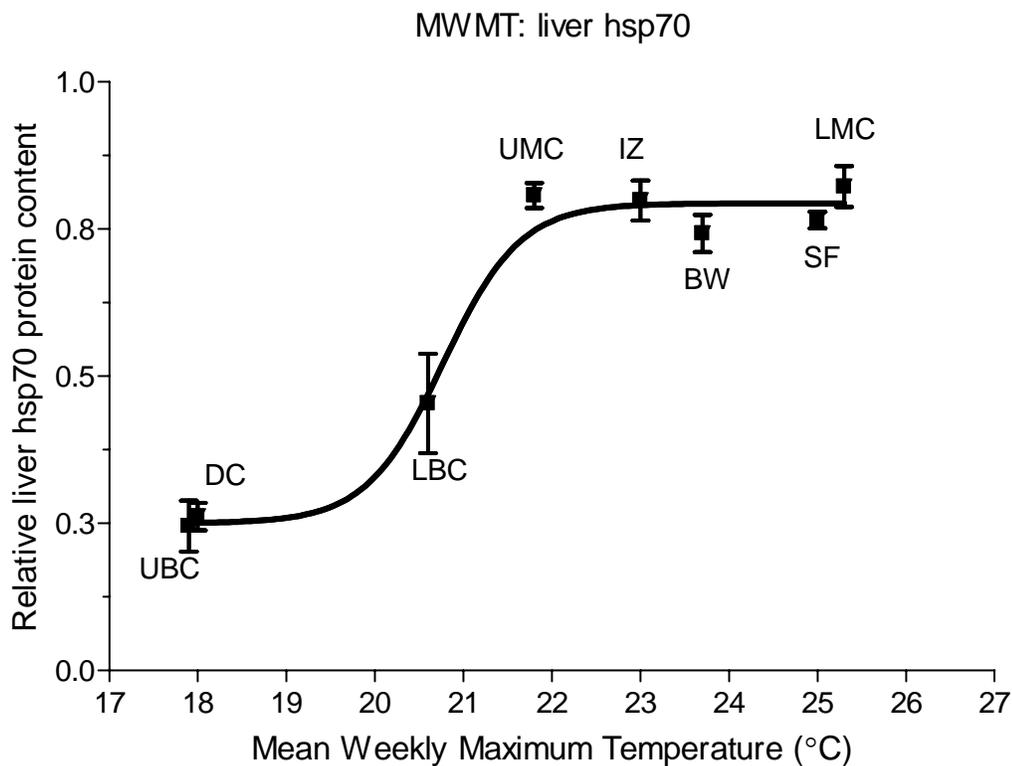
Appendix C- Relation between liver tissue hsp70 protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the 24-hour average daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



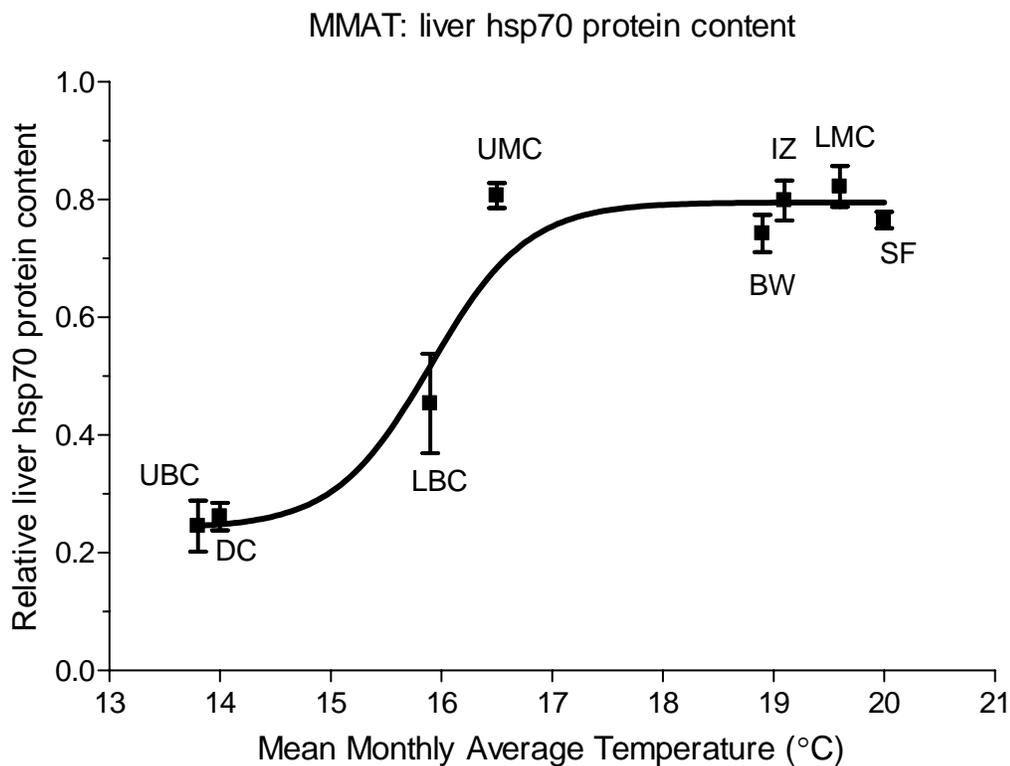
Appendix D- Relation between liver tissue hsp70 protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the 24-hour maximum daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



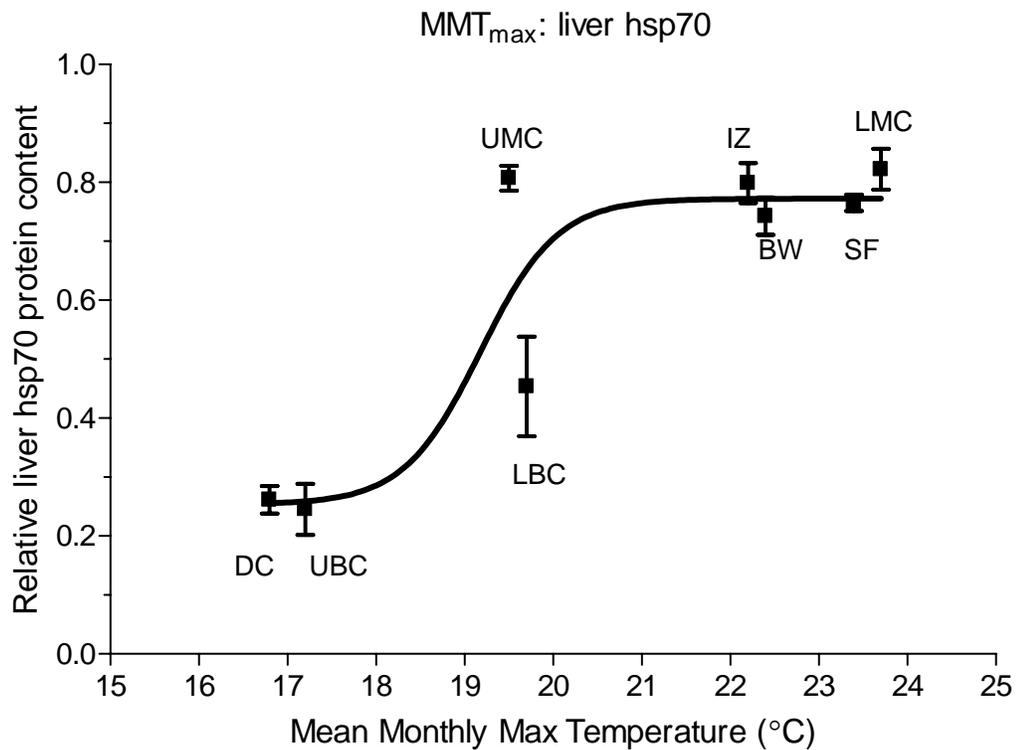
Appendix E- Relation between liver tissue hsp70 protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean weekly average daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



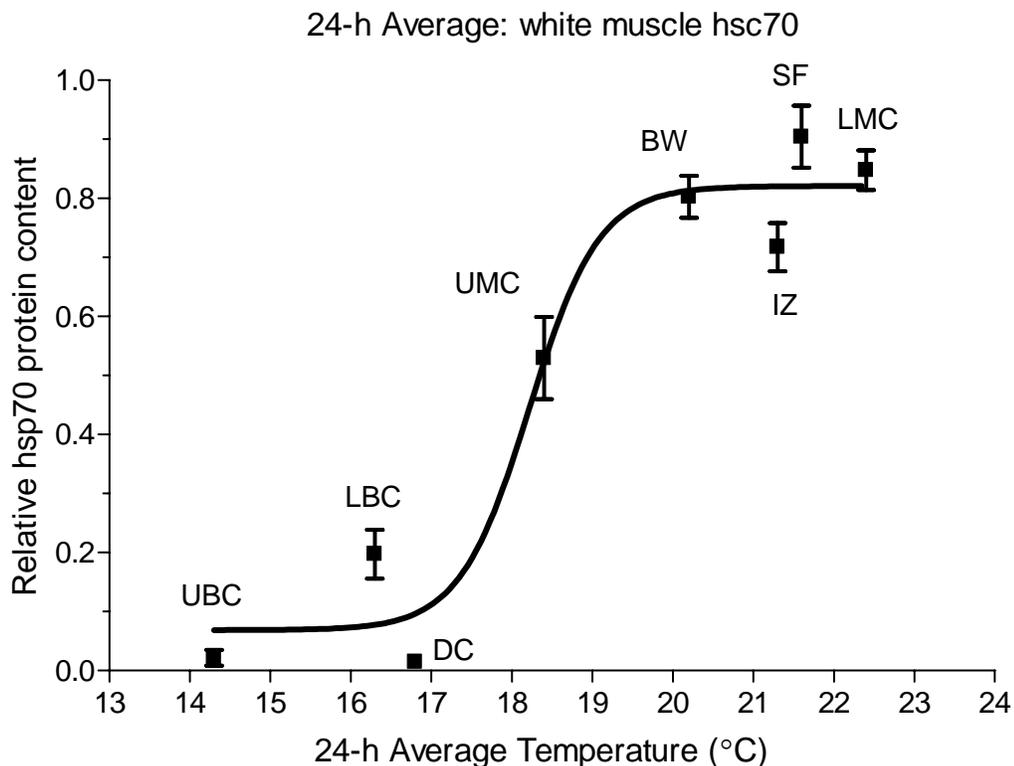
Appendix F- Relation between liver tissue hsp70 protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean weekly maximum daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



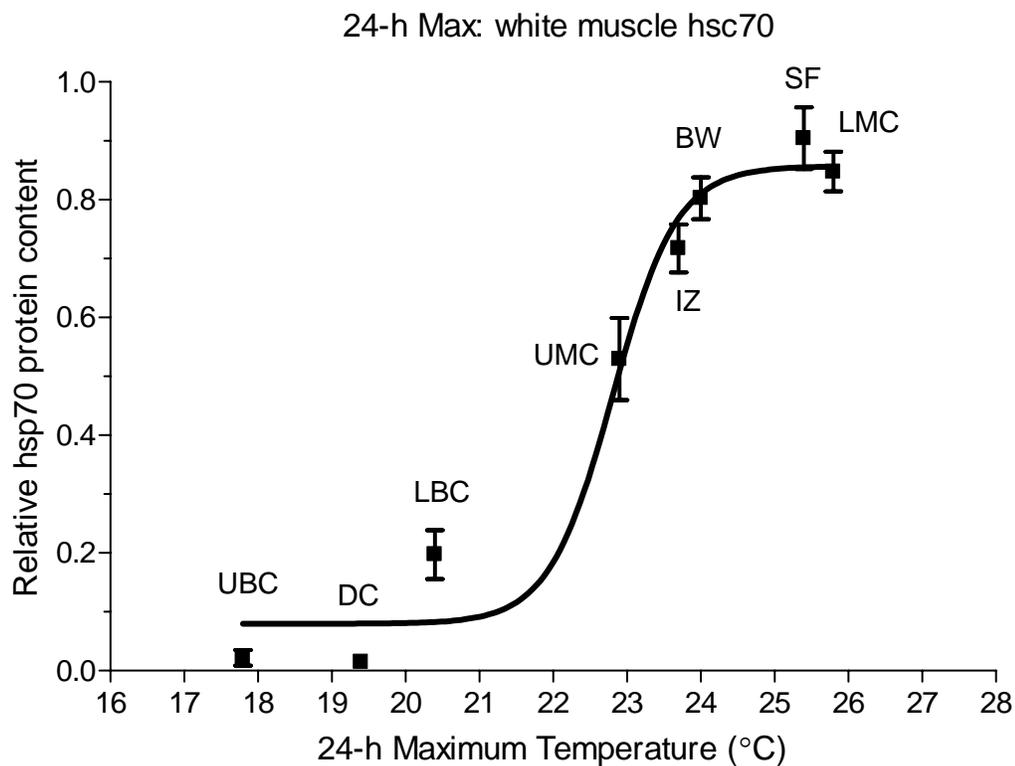
Appendix G- Relation between liver tissue hsp70 protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean monthly average daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



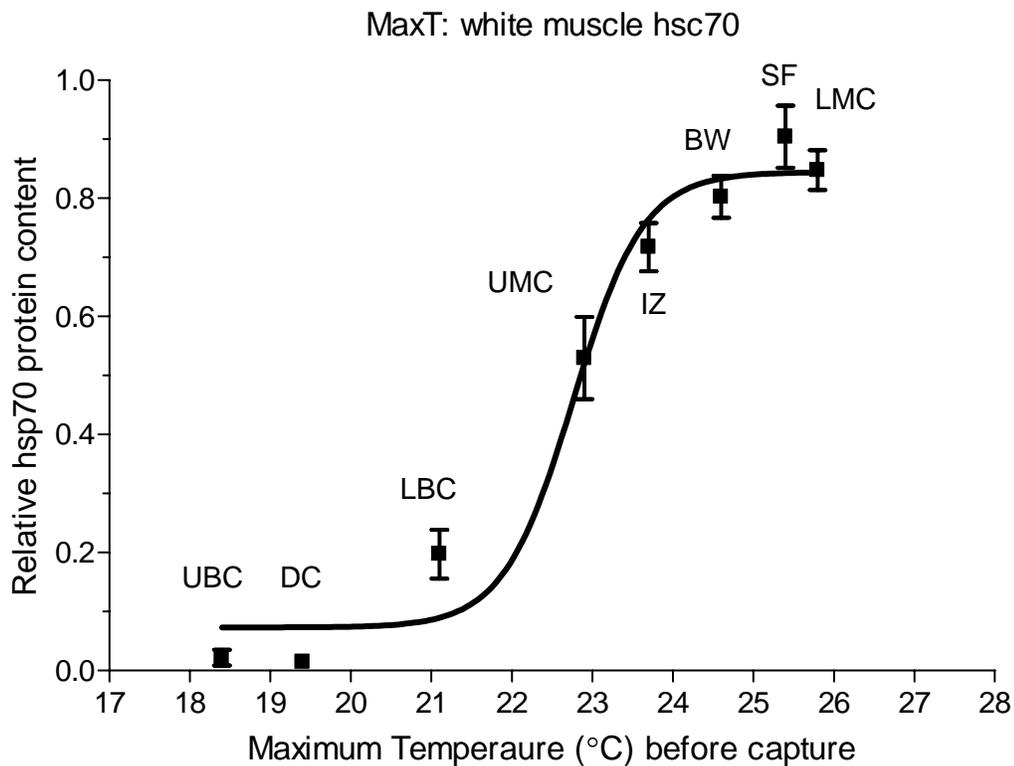
Appendix H- Relation between liver tissue hsp70 protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean monthly maximum daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



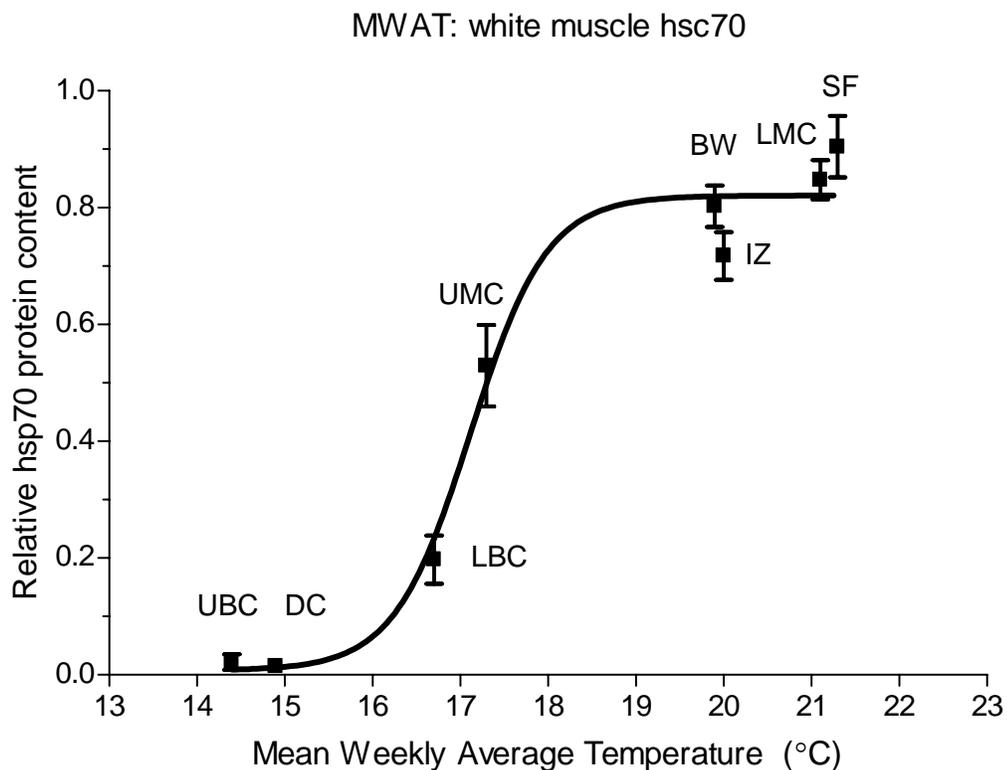
Appendix I- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the 24-hour average daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



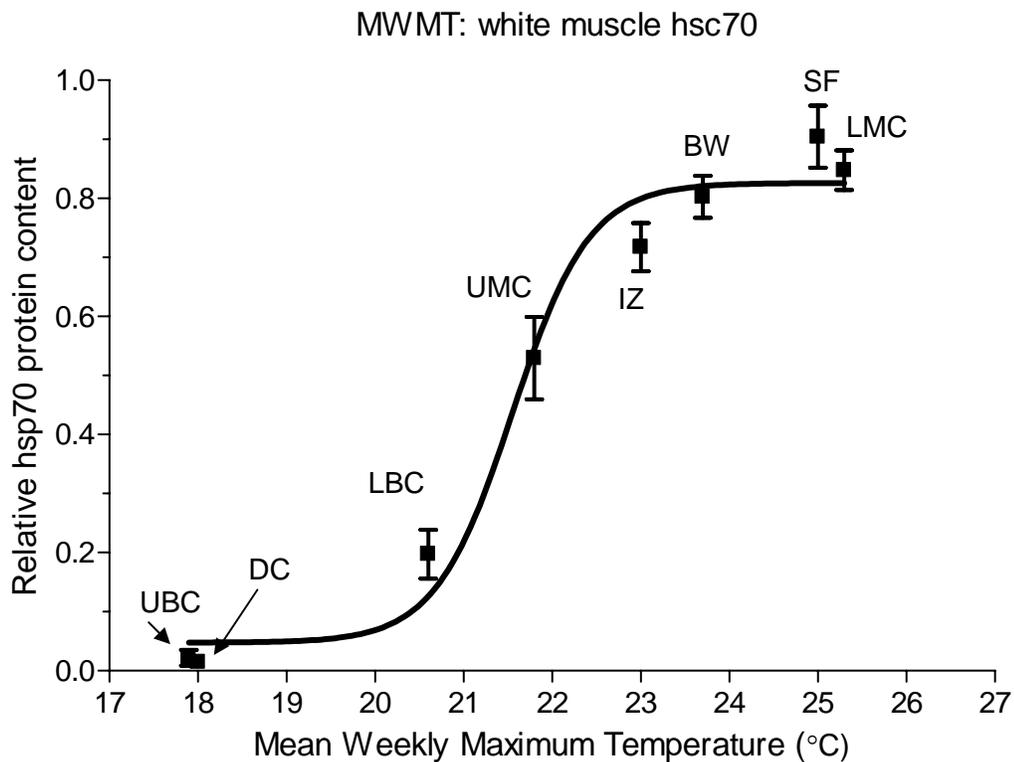
Appendix J- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the 24-hour maximum daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



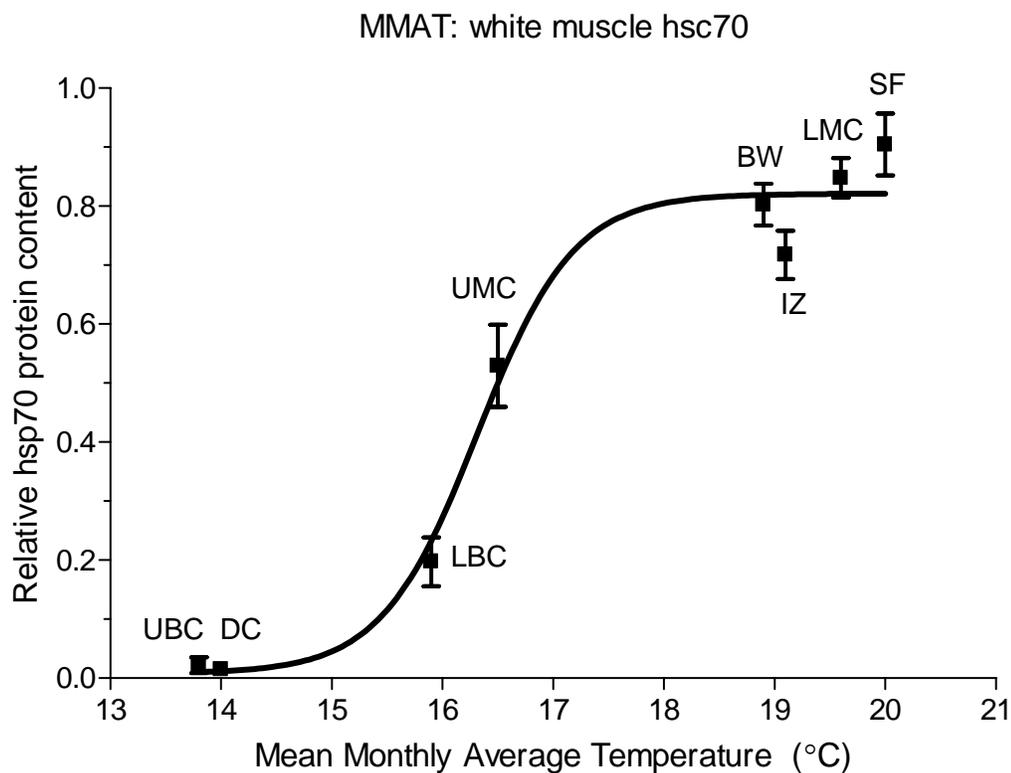
Appendix K- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the maximum water temperature ( $^{\circ}$ C) within five days of capture. Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



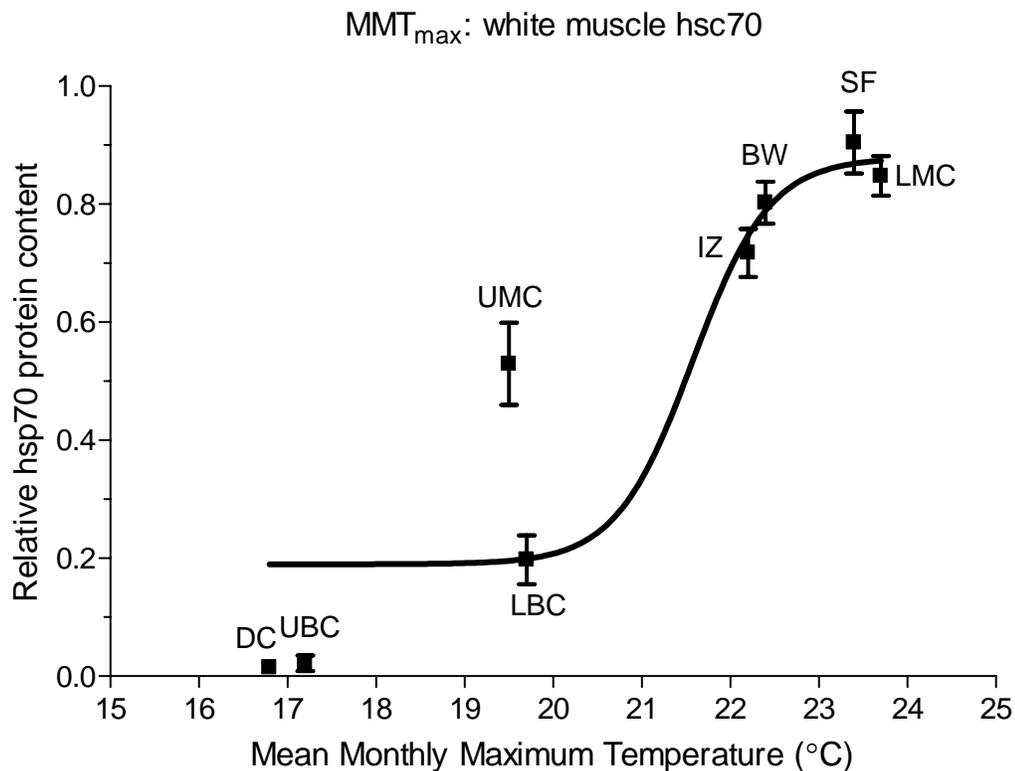
Appendix L- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean weekly daily average water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



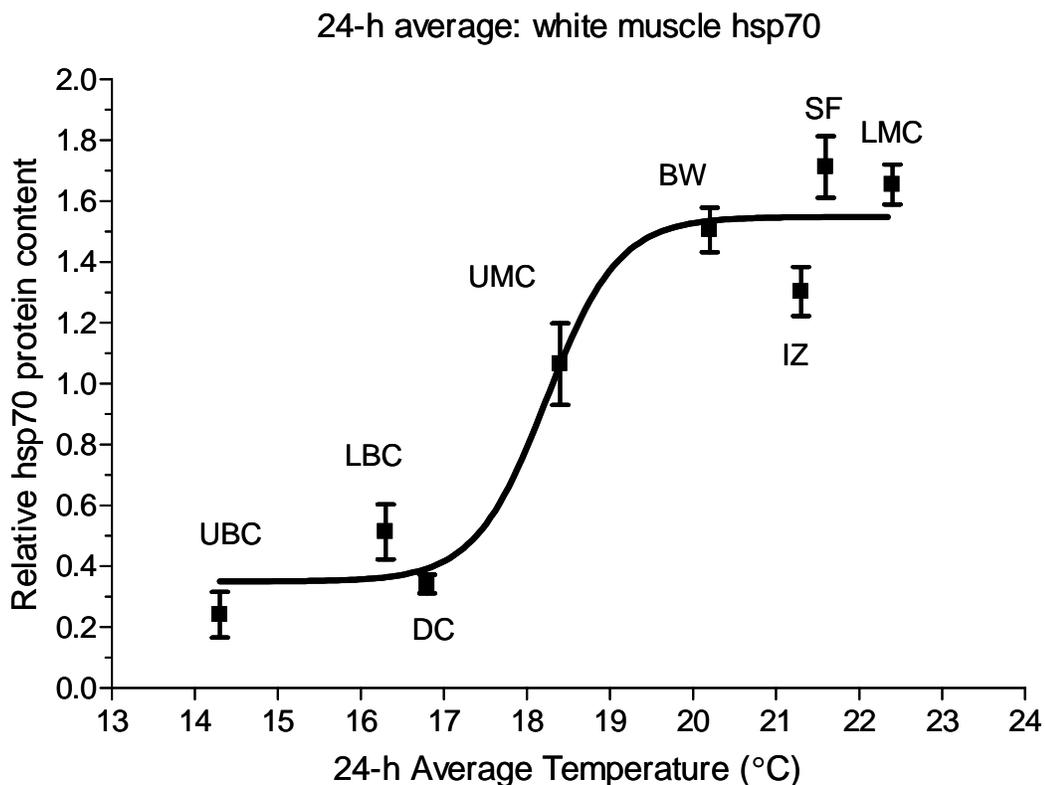
Appendix M- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean weekly maximum daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



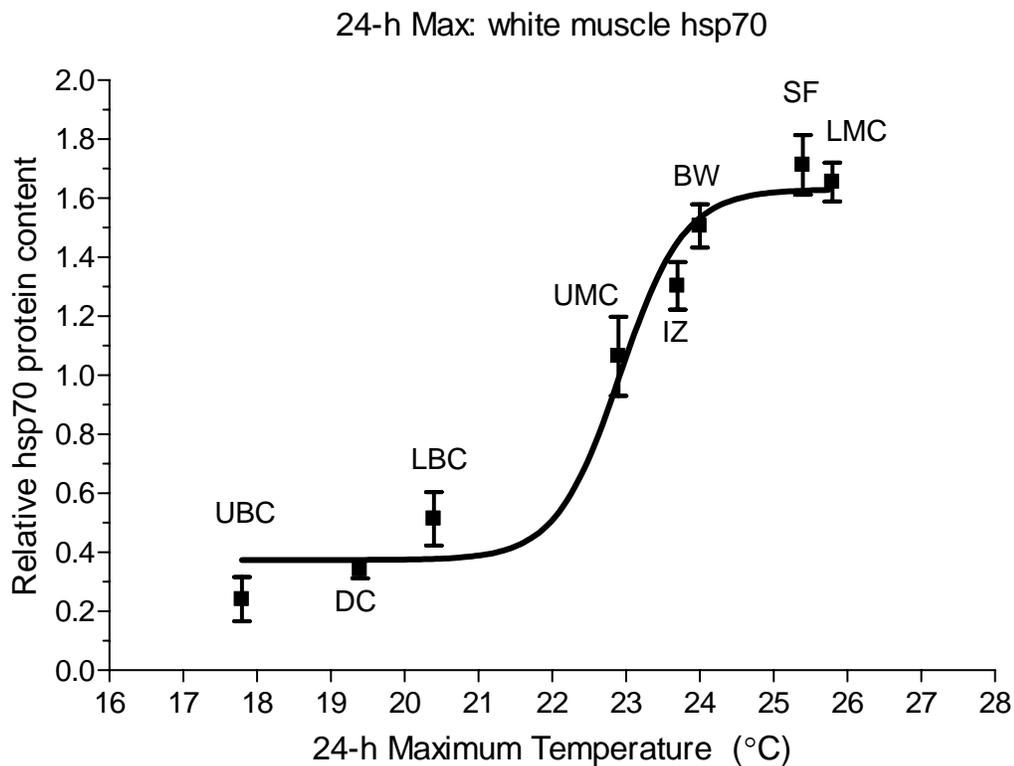
Appendix N- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean monthly average daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



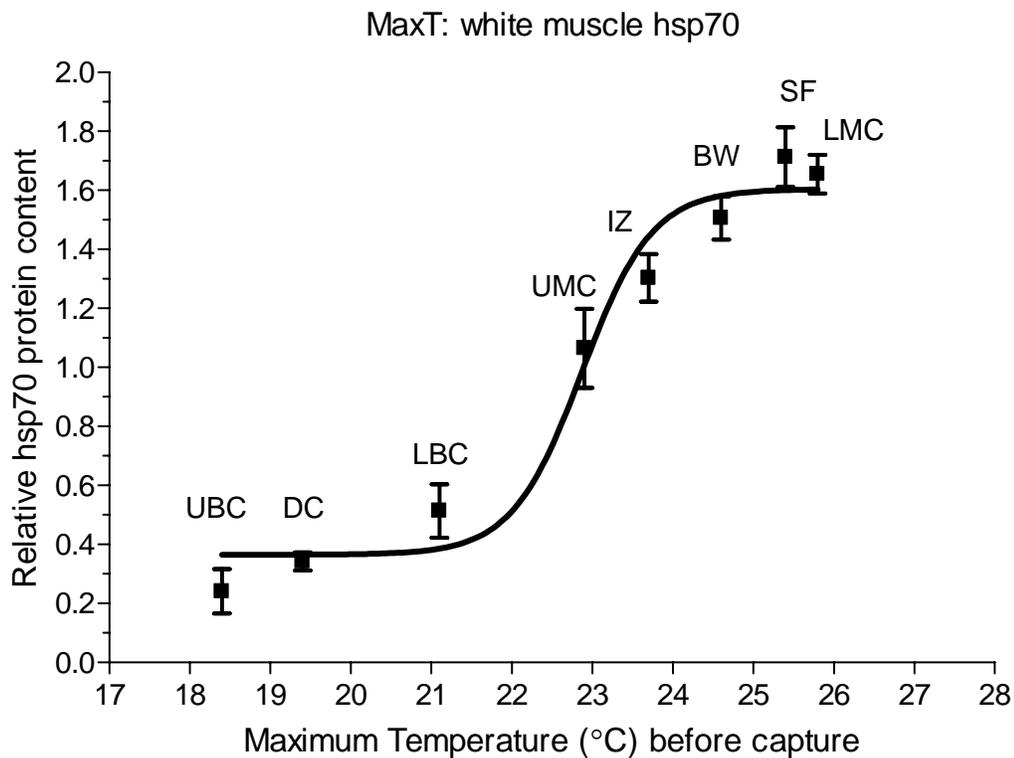
Appendix O- Relation between white muscle tissue inducible hsp73 (hsc70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean monthly maximum daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



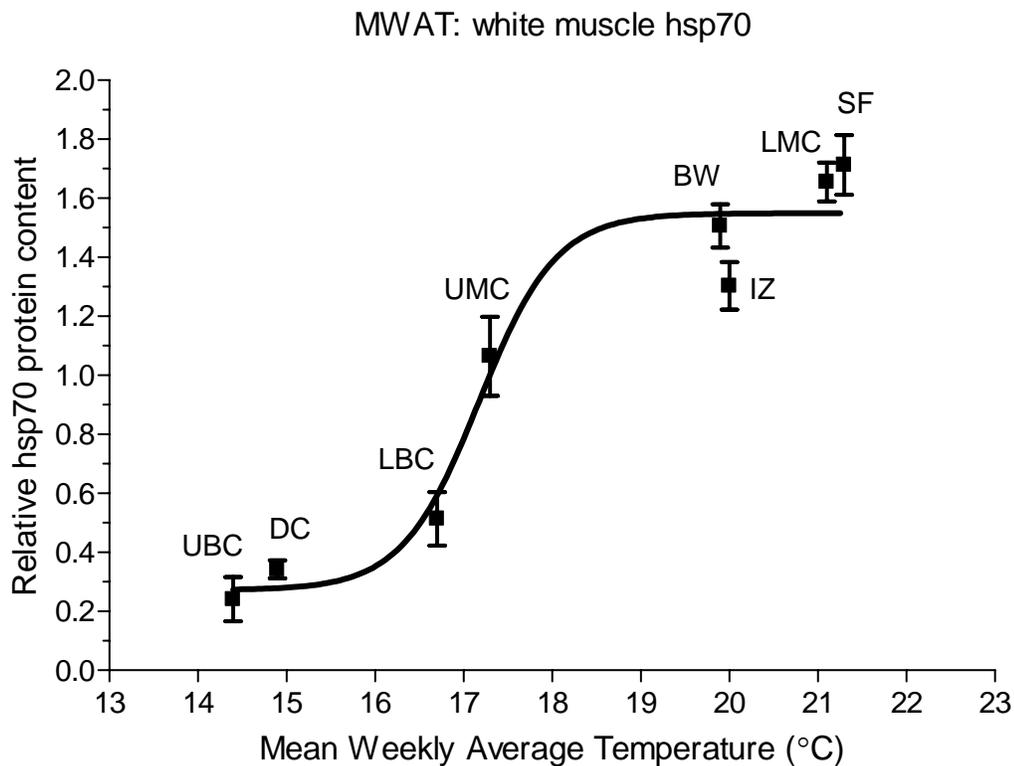
Appendix P- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the 24-hour average daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



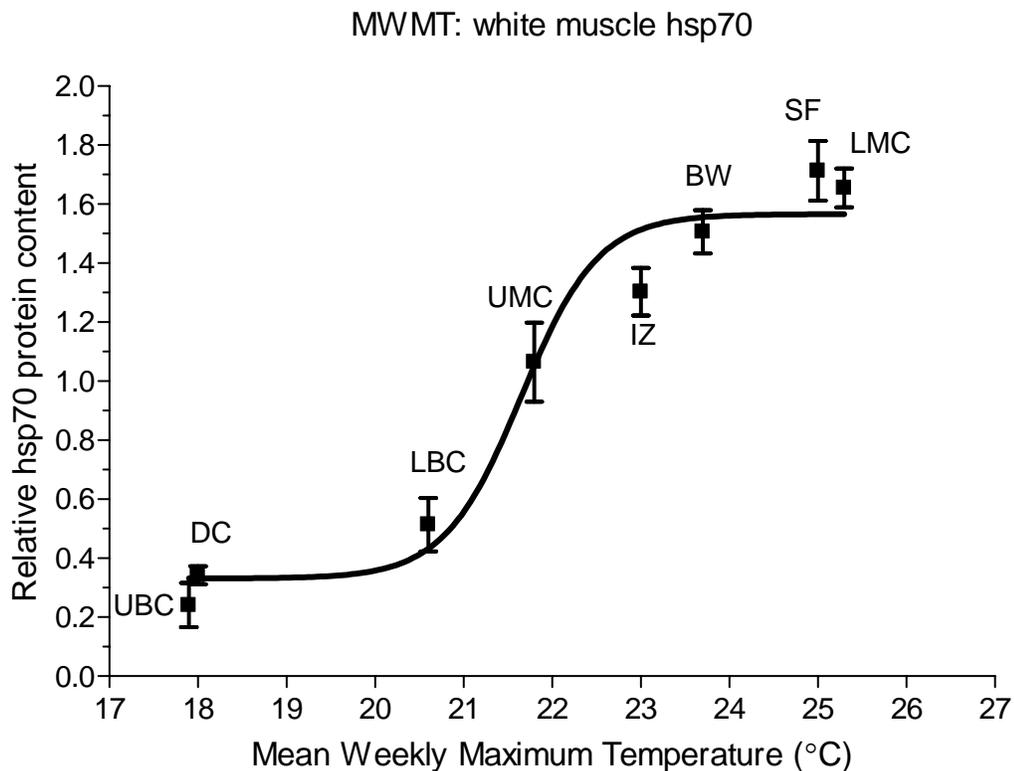
Appendix Q- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the 24-hour maximum daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



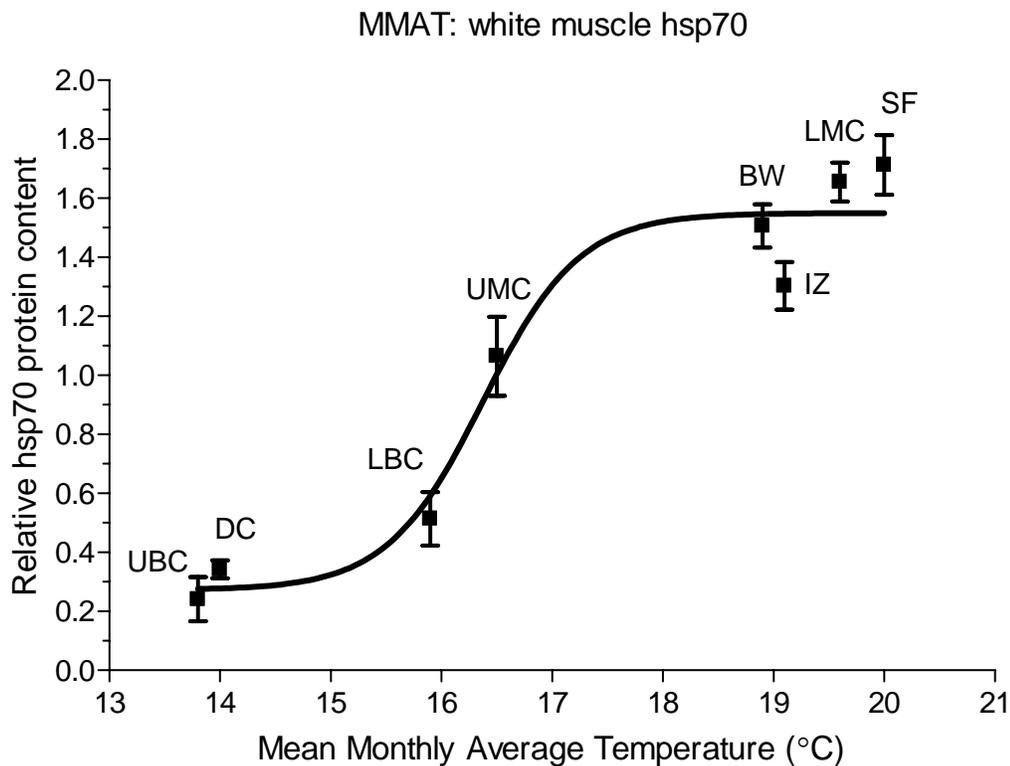
Appendix R- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the maximum water temperature ( $^{\circ}$ C) within five days of capture. Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



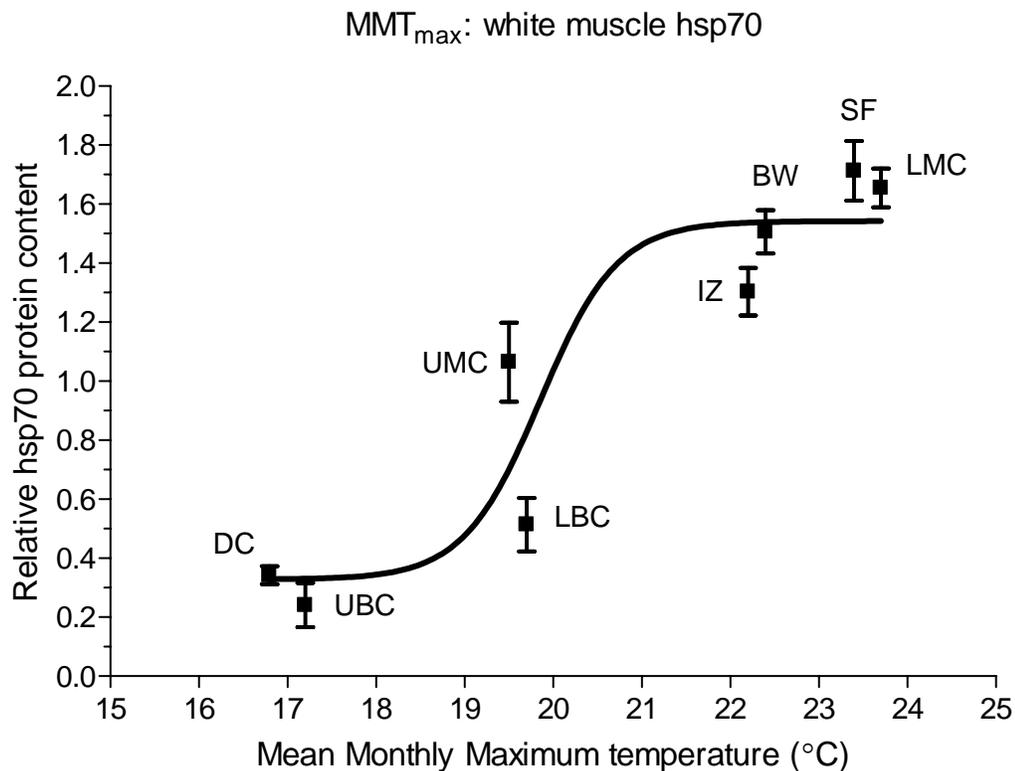
Appendix S- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean weekly daily average water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



Appendix T- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean weekly daily maximum water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



Appendix U- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and the mean monthly average daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).



Appendix V- Relation between white muscle tissue inducible hsp72 (hsp70) protein band density (mean densitometry value  $\pm$  1 standard error of the mean) and mean monthly maximum daily water temperature ( $^{\circ}$ C). Juvenile redband rainbow trout (*Oncorhynchus mykiss gairdneri*) were collected from the South Fork John Day River on 23-24 July 2005 in the South Fork John Day River at upper and lower Black Canyon (UBC, LBC), Deer Creek (DC), upper and lower Murderers Creek (UMC, LMC), Izee falls (IZ), below Wind Creek (BW), and the SFJD at river kilometer 24 (SF).

5. **THESIS: Movement Patterns Of Redband Trout In Relation To Push-Up Dams And Site Productivity Over Different Seasons**  
**Ian Tattam**

AN ABSTRACT OF THE THESIS OF

Ian A. Tattam for the degree of Master of Science in Fisheries Science presented on December 22, 2006.

Title: Seasonal Life History of *Oncorhynchus mykiss* in the South Fork John Day River Basin, Oregon

Abstract approved:

---

Hiram W. Li

Understanding seasonal changes in growth, survival, and movement rates is crucial to salmonid management. These life history characteristics provide a context for evaluation of management actions. We evaluated the life history of individually marked *Oncorhynchus mykiss gairdneri* in the South Fork John Day River basin in Northeastern Oregon. This thesis focused on Murderers and Black Canyon creeks, two tributaries to the South Fork John Day. These are semi-arid, mid elevation basins with naturally reproducing populations of summer steelhead and redband trout (both *O. mykiss*). Summer steelhead populations in this basin have declined from historic abundances, and are protected under the Endangered Species Act.

In Chapter 2, we evaluated life history variation in Murderers Creek during four consecutive seasons. Growth rate varied significantly with season ( $F_{3,88} = 62.56$ ,  $P < 0.0001$ ), with most growth occurring during spring. Location and season interacted ( $F_{6,88} = 4.45$ ,  $P < 0.001$ ) to influence individual growth rates. As a result, areas with high growth potential shifted up and down-stream between seasons. However, we found low rates of summer movement ( $< 3\%$ ) in Murderers Creek, suggesting that individuals did not track resource availability at a large scale. Apparent survival rate

varied among reaches, but was consistently higher in the upstream most reach than in the two lowermost reaches. Survival rates were similar between summer and fall, indicating that there was not an increase in mortality with the onset of winter.

A shift in population distribution occurs during fall (September through December), when some *O. mykiss* emigrate from tributaries into the South Fork John Day. In Chapter 3, we investigated differences in fall life history between and within tributaries. A significantly greater proportion of *O. mykiss* emigrated from Murderers Creek than from Black Canyon Creek during two consecutive years ( $P < 0.001$  for both years). There were no significant differences in proportion of emigrants between years within either stream ( $P > 0.10$  for both streams). In Murderers Creek, the odds of emigration were related to location of tagging. Odds of fall emigration were also significantly and positively related to body length in fall and growth rate experienced during summer. This suggests that “winners” volitionally emigrated from Murderers Creek during fall. After emigration, *O. mykiss* dispersed primarily downstream into the Mainstem John Day River. Radio-telemetry indicated that the majority of fall emigrants occupied a  $< 6$  km section of the Mainstem John Day. Fall emigrants shifted into an alternative niche and had growth rates during winter that were significantly ( $P < 0.001$ ) higher than those of individuals remaining in tributaries.

This study underscores the need to monitor *O. mykiss* growth, movement, and survival during all seasons to accurately characterize habitat quality. Monitoring life history patterns is important for measuring population response to environmental change. This thesis provides an ecological context for monitoring recovery of *O. mykiss* populations in the South Fork John Day River basin.

©Copyright by Ian A. Tattam  
December 22, 2006  
All Rights Reserved

Seasonal Life History of *Oncorhynchus mykiss* in the South Fork John Day River Basin,  
Oregon

by

Ian A. Tattam

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

Presented December 22, 2006  
Commencement June 2007

Master of Science thesis of Ian A. Tattam presented on December 22, 2006.

APPROVED:

---

Major Professor, representing Fisheries Science

---

Head of the Department of Fisheries and Wildlife

---

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

---

Ian A. Tattam, Author

## ACKNOWLEDGMENTS

First of all, I thank my major professor, Hiram Li, for always giving me the freedom to be overly ambitious in this research. I have learned a lot about field research design and implementation from trying many different ideas and seeing some things not pan out. Guillermo Giannico, Jim Ruzycki, and Stephen Schoenholtz have offered exceptional guidance and suggestions as my committee members. They have always asked the right questions to help refine the work presented herein.

I have enjoyed working with the whole desert basins research crew; Seth White, Joseph Feldhaus, Francisco Madrinan, George Boxall, Jeremiah Osborne-Gowey, Peter Bayley, and Scott Heppell. I have surely learned more from them than they have from me. Additionally, I have been fortunate to learn a tremendous amount from officemates Aaron Johnston, Darren Clark, and Anthony Olegario.

Over the course of three years of field research, I have learned some things you can't garner from a textbook. In no particular order: 1) You really can live for months on only energy bars and water; 2) You should take a shower monthly, whether you need it or not; 3) If an old growth tree falls in the woods, and you happen to be ten feet from the point of impact, it makes a lot of noise; and 4) A  $\frac{3}{4}$  ton four wheel drive can make it through flowing water that is over the headlights. Learning these lessons firsthand made sitting in statistics class bearable. Finally, I have to acknowledge the reason we do this; the wild steelhead of the John Day Basin. I hope that decades into the future, one can still live along a stream full of wild juvenile steelhead as I have during the past three years.

## CONTRIBUTION OF AUTHORS

Hiram W. Li and Guillermo R. Giannico assisted in study design, data collection, and data analysis for Chapter 2. James R. Ruzycki and Wayne H. Wilson assisted with study design and data collection for Chapter 3. Hiram W. Li and Guillermo R. Giannico assisted with data analysis for Chapter 3. James R. Ruzycki and Wayne H. Wilson assisted with study design and data collection for Appendix B. Peter B. Bayley assisted with data analysis for Appendix B.

## TABLE OF CONTENTS

	<u>Page</u>
CHAPTER 1. GENERAL INTRODUCTION.....	1
CHAPTER 2. SPATIAL AND TEMPORAL INFLUENCES ON LIFE HISTORY PHENOTYPES OF <i>ONCORHYNCHUS MYKISS</i> IN MURDERERS CREEK, OREGON.....	5
Abstract.....	6
Introduction.....	7
Methods.....	9
Study Location Description.....	9
Seasonal Changes in Relative Growth Rate.....	12
Seasonal Patterns of Individual Movement and Emigration.....	14
Seasonal Survival Rate.....	16
Environmental Correlates of Survival Rate.....	17
Seasonal Density and Biomass of <i>O. mykiss</i> .....	17
Environmental Characteristics.....	18
Results.....	19
Seasonal Changes in Relative Growth Rate.....	19
Seasonal Patterns of Individual Movement and Emigration.....	20
Seasonal Survival Rate.....	21
Environmental Correlates of Survival Rate.....	22
Seasonal Density and Biomass of <i>O. mykiss</i> .....	23
Discussion.....	23
Seasonal Changes in Relative Growth Rate.....	23
Seasonal Patterns of Individual Movement and Emigration.....	26
Seasonal Survival Rate.....	27
Interrelation of Growth, Movement, Survival and Density.....	29
References.....	33

TABLE OF CONTENTS (Continued)

	<u>Page</u>
CHAPTER 3. INFLUENCES ON AND EFFECTS OF FALL MOVEMENT BY <i>ONCORHYNCHUS MYKISS</i> IN THE SOUTH FORK JOHN DAY RIVER.....	52
Abstract.....	53
Introduction.....	54
Methods.....	57
Study Location Description.....	57
Prevalence of Fall Migration and subsequent Winter Distribution...	58
Individual Based Monitoring of Fall Life History.....	62
Influence of Fall Life History.....	65
Results.....	66
Prevalence of Fall Migration and subsequent Winter Distribution...	66
Individual Based Monitoring of Fall Life History.....	68
Influence of Fall Life History.....	69
Discussion.....	71
Prevalence of Fall Migration and subsequent Winter Distribution...	71
Individual Based Monitoring of Fall Life History.....	74
Influence of Fall Life History.....	75
References.....	78
CHAPTER 4. CONCLUSIONS AND IMPLICATIONS.....	99
BIBLIOGRAPHY.....	103
APPENDICES.....	113
APPENDIX A. POPULATION ABUNDANCE ESTIMATION.....	113
APPENDIX B. 2005 SOUTH FORK JOHN DAY RIVER SCREW TRAP CALIBRATION.....	120

## LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
2.1 Location of the South Fork John Day River basin (SFJD), and Murderers Creek, the principal study area location.....	40
2.2 Space-time schematic of monitoring in Murderers Creek during 2004-2006.....	41
2.3 Seasonal pattern in relative growth rates of PIT tagged <i>O. mykiss</i> in Murderers Creek from December 2004 through January 2006.....	42
2.4 Seasonal averages of daily mean stream temperatures at Murderers Creek sentinel sites .....	43
2.5 Detections of upstream migrant <i>O. mykiss</i> at fixed PIT arrays in Murderers Creek during 2005.....	44
2.6 Detections of downstream migrant <i>O. mykiss</i> at fixed PIT arrays in Murderers Creek during 2005.....	45
2.7 Seasonal pattern in apparent survival rate of PIT tagged <i>O. mykiss</i> in Murderers Creek .....	46
2.8 Seasonal survival rate of PIT tagged <i>O. mykiss</i> in Murderers Creek .....	47
2.9 Estimated mean density (individuals per lineal stream meter) of <i>O. mykiss</i> (> 75mm fork length) at Murderers Creek sentinel sites .....	48
3.1 Location of the South Fork John Day River basin, including Black Canyon and Murderers creeks .....	85
3.2 Locations of recapture and redetection of PIT tagged <i>O. mykiss</i> during fall-winter 2004.....	86
3.3 Location of recapture and redetection of PIT tagged <i>O. mykiss</i> in the South Fork John Day River (SFJD) and tributaries during Fall-Winter 2005.....	87
3.4 Recapture location of PIT tagged <i>O. mykiss</i> in the South Fork John Day river (SFJD).....	88

## LIST OF FIGURES (Continued)

<u>Figure</u>	<u>Page</u>
3.5 Winter distribution of radio-tagged <i>O. mykiss</i> downstream from the South Fork John Day River screw trap .....	89
3.6 Comparison of individual growth rates experienced during fall by sedentary <i>O. mykiss</i> in Black Canyon and Murderers creeks .....	90
3.7 Chronology of skeletal growth of individually marked <i>O. mykiss</i> in the South Fork John Day River.....	91
3.8 Comparison of average individual growth rates during winter 2005.....	92
3.9 Correlation between julian day of detection at John Day Dam (JDA) and julian day of detection in the Columbia River estuary (TWX).....	93
3.10 Influence diagram of factors affecting the fall life history of an individual <i>O. mykiss</i> .....	94

## LIST OF TABLES

<u>Table</u>		<u>Page</u>
2.1	Stream temperatures by reach and season in Murderers Creek during 2005.....	49
2.2	Model selection results for Cormack-Jolly-Seber apparent survival rate models fit to PIT tag mark-recapture sampling .....	50
2.3	Frequency of body scars or cysts observed on <i>O. mykiss</i> .....	51
3.1	Estimated fall emigration of PIT tagged <i>O. mykiss</i> from Black Canyon and Murderers creeks .....	95
3.2	Radio-telemetry monitoring of fall migrating <i>O. mykiss</i> in the John Day River basin.....	96
3.3	Model selection results for AICc analysis of fall emigration from Murderers Creek.....	97
3.4	Parameter estimates for a logistic regression model of the probability of fall emigration from Murderers Creek.....	98

## LIST OF APPENDIX FIGURES

<u>Figure</u>	<u>Page</u>
B.1 South Fork John Day River screw trap during typical streamflow levels observed during the study period.....	129
B.2 Schematic representation of release site locations and times used in the South Fork John Day River during fall 2005.....	130
B.3 Comparison of the relative odds of recapture among release strategies used during fall 2005.....	131
B.4 Comparisons of the rotary screw trap recapture probability among size classes during fall 2005.....	132

## LIST OF APPENDIX TABLES

<u>Table</u>		<u>Page</u>
A.1	Selected simulations conducted in Program Capture to determine an appropriate estimator for mark-recapture population estimates.....	118
A.2	Summary of <i>O. mykiss</i> captures during mark-release-recapture sampling events in Murderers Creek .....	119

SEASONAL LIFE HISTORY OF ONCORHYNCHUS MYKISS IN THE SOUTH  
FORK JOHN DAY RIVER BASIN, OREGON

## CHAPTER 1 GENERAL INTRODUCTION

Native anadromous salmonids in the Columbia River basin have declined in abundance during the past century (Nehlsen et al. 1991; Lichatowich 1999). Suspected causes of this decline are widespread, ranging from localized sources of migration mortality (e.g., hydropower dams on the Columbia River), to dispersed, subtle changes in stream habitat characteristics (McIntosh 1996). Returns of adult steelhead to middle Columbia River tributaries in north-central and northeastern Oregon have declined sufficiently from historic levels to spur a “threatened” listing under the federal Endangered Species Act (Federal Register, Vol. 64, No. 57, March 25, 1999, p. 14517). The Oregon tributaries of the middle Columbia River are the Deschutes, John Day and Umatilla basins. Steelhead returning to these rivers are sometimes referred to as “redband steelhead” (*Oncorhynchus mykiss gairdneri*; see Behnke 2002). These are “summer-run” populations, denoted by their strategy of returning to the Columbia during July-September, then overwintering in large rivers prior to migrating upstream into spawning tributaries the following spring. These *O. mykiss* populations are iteroparous, and also display partial migration (Jonsson and Jonsson 1993). *O. mykiss* populations found in tributary streams are hence composed of individuals which will become steelhead, and individuals which will mature as resident trout (these forms are visually indistinguishable as juveniles).

One strategy for regaining the historic abundance of summer steelhead focuses on restoring habitat in tributary streams which are primarily used for spawning and

subsequent juvenile rearing (NMFS 2000). A range of habitat management actions are being undertaken, ranging from passively allowing stream channel recovery, to replacing physical impediments to fish passage with alternative “fish-friendly” structures. A critical need for assessment of this recovery strategy is evaluation of habitat management actions at the population scale. That is, do these habitat changes increase the abundance of *O. mykiss* at a tributary or basin scale?

*O. mykiss* frequently have generation times in excess of 4 years (Peven et al. 1994). Monitoring generation to generation changes in population abundance is impractical for short-term evaluation of recovery actions. An alternate strategy is to evaluate and monitor life history mechanisms which influence population abundance. The “life history” of an individual encompasses where and how it behaves, migrates, and reproduces (Lichatowich 1999). All of these variables are influenced by human activities and changes to a stream basin. The freshwater life history of each individual is not disjointed from migratory and marine phases. Rather, freshwater life history exerts substantial influence on when individuals migrate to the ocean, at what size and age they migrate, and their odds of survival (e.g., Ward et al. 1989; Lundqvist et al. 1994; Peven et al. 1994; Cucherousset et al. 2005). Therefore, the life history parameters of *O. mykiss* in tributary streams influence lifetime survival and production, and have a direct bearing on how the population responds to recovery actions.

Freshwater life history of salmonids is composed of several key factors, which essentially involve biological trade-offs. The factors which have been most intensively studied are: movement, growth, and survival. These factors are interdependent. For

instance, “movers” and “stayers” can experience different growth (e.g., Roni and Quinn 2001; Steingrimsson and Grant 2003) and survival (Martel and Dill 1995) rates as a result of movement (or lack thereof). Rates of feeding activity can influence survival (e.g., Metcalfe et al. 1999), as well as growth rate. Growth rate in turn influences the likelihood of migration (e.g., Cucherousset et al. 2005). Hence, these factors interact to create freshwater life history patterns, and need to be evaluated simultaneously.

The John Day River is the largest of the Oregon tributaries to the middle portion of the Columbia River. A large number of habitat improvement actions have occurred, or are occurring, in the John Day basin. The effectiveness of these actions, however, remains unclear. The objective of this thesis is to provide the life-history and ecological template for understanding how *O. mykiss* populations in the South Fork John Day River (as well as other similar locations) might respond to changes in habitat conditions. To this end, we conducted year-round study of growth, survival and movement patterns of *O. mykiss* in the South Fork of the John Day River from a “riverscape” perspective (Fausch et al. 2002). Potential population regulatory mechanisms need to be understood at this riverscape scale, across all seasons, as this is the spatio-temporal scale at which management occurs, and hence at which population responses should be monitored.

Understanding life history strategy provides the framework to monitor and detect changes through time. Changes may be more subtle than simply an increase or decrease in juvenile abundance. For instance, habitat improvement may result in a change in individual growth rate during a specific season, while not altering the abundance of juvenile *O. mykiss* in the project area. Even a short-term change in

growth rate, however, can influence later survival and life history tactics (Metcalf 1998). Therefore, we aimed to describe the current life history tactics, growth and survival rates of portions of the South Fork John Day river population, which could then serve as a baseline for long-term monitoring of population response.

In Chapter 2, we describe the spatial and temporal variation of several key biological parameters over the course of a year in Murderers Creek (a tributary of the South Fork John Day). Our objectives for Chapter 2 were to: i) determine seasonal patterns of individual growth rate throughout Murderers Creek; ii) describe seasonal patterns of movement within and between Murderers Creek and the South Fork John Day; and iii) estimate seasonal survival rate in Murderers Creek. In Chapter 3, we compare the prevalence and correlates of a fall migrant life history tactic between and within Black Canyon and Murderers creeks (neighboring tributaries of the South Fork John Day). Our objectives for chapter 3 were to: i) determine if there were differences in the proportion of fall emigrants between Murderers and Black Canyon creeks; ii) determine the winter distribution of these emigrants; iii) compare the prior physical characteristics and growth histories of individuals in Murderers Creek with their subsequent fall life history strategy; and iv) evaluate the long-term influence that fall life history has on individuals.

**CHAPTER 2****SPATIAL AND TEMPORAL INFLUENCES ON LIFE HISTORY PHENOTYPES OF  
*ONCORHYNCHUS MYKISS* IN MURDERERS CREEK, OREGON**

### Abstract

Growth, movement, and survival are driving variables on the expression of life history traits. These variables influence life history and hence production at the riverscape scale. We jointly studied these variables by marking and recapturing individually identifiable *Oncorhynchus mykiss gairdneri*. Capture and recapture occurred over the course of a year in locations throughout Murderers Creek, a semi-arid tributary to the South Fork John Day River in Northeastern Oregon. Individual growth rates differed significantly ( $P < 0.0001$ ) among reaches and seasons. There was a significant interaction ( $P < 0.001$ ) between stream reach and season. Areas with high growth potential therefore shifted across the riverscape between seasons. Stream reaches with high growth potential during the winter had low growth potential during summer, and vice-versa. Rates of movement during summer were low ( $< 3\%$ ), suggesting that individuals did not track these changes in growth potential at the reach scale. Apparent survival of *O. mykiss* differed among reaches, with differences among reaches remaining constant through time. Apparent survival was consistently higher in the upstream most reach than in the two lowermost reaches. Survival rates were similar between summer (range: 0.75 to 0.88) and fall (range: 0.69 to 0.83), indicating that there was not an increase in mortality with the onset of winter. This study demonstrates the need to monitor during all seasons to accurately characterize the relative habitat quality of different reaches within a stream.

## Introduction

Growth rate is an important indicator of fish development (Van den Avyle and Hayward 1999). In juvenile anadromous fish, freshwater growth rate influences age and size at smoltification. Size at smoltification is positively related to survival in the marine environment (Ward et al. 1989; Lundqvist et al. 1994; Tipping 1997). Increased growth rate decreases the time needed to attain minimum size thresholds (e.g., Peven et al. 1994; Cucherousset et al. 2005) for migration, potentially decreasing the risk of freshwater mortality. Many biotic and abiotic factors influence freshwater growth rate, such as food availability (Filbert and Hawkins 1995; Metcalfe et al. 1999; Imre et al. 2004), physical cover (Thorpe 1987), stream temperature (Myrick and Cech 2000; Marine and Cech 2004; Murphy et al. 2006), feeding intensity (Metcalfe et al. 1988), behavioral patterns (Metcalfe et al. 1999), and intraspecific (Keeley 2001) or interspecific (Reeves et al. 1987) competition. Growth rate, therefore, integrates the effects of the surrounding environment as it is actually experienced by an individual fish.

With the development of individual fish marking techniques such as Passive Integrated Transponder (PIT) tags, knowledge of growth patterns of individual wild, stream-dwelling salmonids has increased dramatically in recent years (Juanes et al. 2000; Bacon et al. 2005). Individual identification has facilitated evaluation of the influence of freshwater growth rates on life history (Juanes et al. 2000; Cucherousset et al. 2005) and subsequent survival (Juanes et al. 2000; Letcher et al. 2002). The relationship between movement and growth rate has also become better understood

(Kahler et al. 2001). Stream salmonids may be more mobile than previously thought (Gowan and Fausch 1996; Armstrong et al. 1997), and mobile individuals often fare better in terms of growth than sedentary individuals (Roni and Quinn 2001; Gowan and Fausch 2002; Steingrimsson and Grant 2003).

For phenotypically plastic species such as salmonids, a life history trade-off exists between anadromy and residency (Thorpe 1987; Zimmerman and Reeves 2000). Small changes in growth rate can influence an individuals' subsequent life history (Metcalf 1998; Morinville and Rasmussen 2003), and changes in environmental conditions can influence life history at the population or cohort level (Duston and Saunders 1997; Cucherousset et al. 2005). Interactions between growth, movement, and survival may drive the life history strategy of the population. Inter-annual differences in environmental conditions can create inter-cohort variation in life history expression (Cucherousset et al. 2005). It is equally important to understand how intra-annual environmental differences, expressed spatially, influence the distribution of life history traits among juvenile salmonid phenotypes within a single stream. Environmental conditions change longitudinally (Wright 2000), creating differential responses by juvenile salmonids in terms of growth, movement, and survival which may in turn trigger different life history patterns within the same population.

Many individual based studies have focused on the growth or movement patterns of individuals in short stream segments (Juanes et al. 2000; Letcher et al. 2002; Bacon et al. 2005). While valuable, we believe that examining multiple segments distributed at the stream scale allows detection of longitudinal environmental changes

and corresponds with the typical scale of management (Fausch et al. 2002). Management and theory require that the population(s) and its various life history phenotypes be understood at the stream scale. We assume that growth rate of individuals, along with the survival rate of cohorts (using tagged individuals as indicators), are key population regulators. Hence, it is important to know what environmental factors influence these metrics and at what spatial or temporal scales they act. Therefore, it is necessary to 'scale-up' our sampling of individual and cohort properties using a hierarchical design (Frissell et al. 1986). To address these issues, we studied both individual growth and cohort survival of *Oncorhynchus mykiss gairdneri* (anadromous redband trout) in Murderers Creek, a tributary of the South Fork John Day River, Oregon.

The three primary goals of this study were to: i) determine seasonal patterns of individual growth rate over a large spatial scale; ii) describe seasonal patterns of movement within and between Murderers Creek and the South Fork John Day; and iii) estimate seasonal survival of *Oncorhynchus mykiss gairdneri* in Murderers Creek. Addressing these three goals will clarify the spatial and temporal scales that these environmentally influenced population regulators operate at, such that we can monitor at those scales.

## **Methods**

### *Study Location Description*

The South Fork John Day River basin supports native, naturally reproducing steelhead, and sympatric resident redband trout. At this time it is not certain whether

these represent reproductively isolated populations or whether they are two phenotypes of the same breeding population of *Oncorhynchus mykiss gairdneri*. *O. mykiss gairdneri* are broadly distributed throughout the South Fork John Day River basin and four principal tributaries downstream of Izee Falls, which blocks upstream fish migration (Figure 2.1). We selected the largest of these four tributaries, Murderers Creek, as a location of intensive study over the course of an entire year (Figure 2.1). Most of the *O. mykiss gairdneri* in this stream are < 200 mm fork length, however no individuals are positively identifiable as adult resident redband trout or juvenile steelhead. Thus we use *O. mykiss* to encompass all individuals captured and tagged. Adult steelhead and resident redband trout spawn March-June, with juveniles reaching 60-80 mm fork length by the end of their first year. *O. mykiss* emigrate from the basin between October and June, typically at a size > 115 mm, and at ages commonly ranging from 1 to 4.

Murderers Creek is a fourth-order stream which encompasses 14,632 hectares in Northeast Oregon (Figure 2.1), and ranges in elevation from 710 meters to 2,133 meters. Uplands are characterized by open grasslands interspersed with western juniper (*Juniperus occidentalis*), transitioning into ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*) forests at higher elevations. Climate is semi-arid high desert, with most precipitation occurring during the winter and spring months, and has wide annual and diel temperature fluctuations. Stream temperatures vary from 0 °C (with ice cover during portions of the winter) to 27 °C. During summer there is a strong longitudinal increase in temperature of up to 10 °C from the upper to lower portions of

the study area. Much of the creek exceeds optimal growth temperatures of 15-19 °C during summer, with daily maximum temperatures near the mouth exceeding reported lethal limits (Richter and Kolmes 2005). Discharge varied by more than two orders of magnitude. A minimum of 0.07 m<sup>3</sup>/s occurred on August 28, 2005 and a maximum of 9.14 m<sup>3</sup>/s occurred on December 31, 2005.

Although the mainstem of Murderers Creek is approximately 40 kilometers in length, we studied only the lower 18 kilometers. A confined canyon segment immediately upstream of this area contained woody debris jams believed to prevent upstream passage of anadromous fish. This was inferred from the lack of steelhead spawning activity upstream of this canyon area in recent years (T. Unterwegner, ODFW John Day, personal communication). Therefore, our sample area included the majority of mainstem Murderers Creek available to use by adult steelhead during the study period.

The lower 18 km of Murderers Creek were partitioned into three reaches (Frissel et al. 1986), based on stream power and valley topography (Figure 2.2). Reach 1, the most downstream portion of the study area, is a confined canyon with limited forested canopy cover, and the highest summer temperatures. Reach 2 is an unconstrained alluvial valley section where the channel meanders across a broad valley floor. Forest cover is absent in Reach 2, with stream cover being entirely provided by riparian obligate species such as willows (*Salix* spp.), red osier dogwood (*Cornus sericea*), and limited black cottonwood (*Populus trichocarpa*) stands. Reach 3 is the most upstream portion of the study area in a higher gradient confined canyon segment with the coolest

summer temperatures. This reach has considerable forest canopy cover, primarily from ponderosa pine, in addition to riparian species such as red osier dogwood. One “sentinel site” was selected in each reach either randomly, or as representative of the physical characteristics of that reach (Figure 2.2). Each sentinel site consisted of 5 contiguous pool-riffle units. These sites ranged in thalweg length from 94 to 391 meters, and on average, were 61 times the summer wetted width.

#### *Seasonal Changes in Relative Growth Rate*

We captured and tagged *O. mykiss* with 12 mm full-duplex PIT tags in Murderers Creek and the South Fork John Day River from December 2004 through January 2006. Sampling occurred at sentinel sites in Murderers Creek during December 2004, March-April 2005, June 2005, September 2005 and January 2006. When stream temperatures were  $> 8$  °C, we captured *O. mykiss* primarily by using either a snorkeler or a backpack electrofisher (Smith-Root<sup>1</sup> 12-B POW electrofisher, Vancouver, WA) at mild settings (voltage, frequency, pulse length) to herd fish from pools into a bag seine (3.7 m wide and 1.2 m deep, with 6.4 mm mesh) held in the thalweg of the pool. Flyfishing with small barbless hooks was also used to collect fish from pool habitats. In riffles less than 40 cm deep we used either conventional pulsed-DC electrofishing techniques wherein we dipnetted fish from on or near the surface, or used the electrofisher to herd *O. mykiss* into the bag seine located in the thalweg of the riffle. In deeper riffles ( $>40$  cm), we again used either snorkeling or electrofishing to drive fish into the bag seine. Sampling proceeded in an upstream direction in both pools and riffles, that is, the location of the net progressively moved upstream.

When stream temperatures were  $< 8$  °C, we captured *O. mykiss* by night snorkeling and dipnetting. One snorkeler worked upstream with a dive light (C-8 UK Sunlight<sup>1</sup>, with Light Emitting Diode white bulb), and captured *O. mykiss* by backing an individual into a handheld dipnet positioned vertically, but near the substrate, immediately downstream of the fish. When the fish swam into the dipnet, it was lifted to entrap the fish. The diver made two passes through each pool (separated by 15 or more minutes) and one pass through all non-pool portions of the sentinel site which were deep enough to snorkel ( $> 40$  cm).

Captured *O. mykiss* were anesthetized (MS-222) and PIT tagged in the peritoneal cavity (Prentice et al. 1990, PTSC 1999), or recorded as a recapture if previously tagged. Date, fork length (nearest millimeter), location of capture (to the channel unit scale), and conditional status were recorded on each capture occasion. Fish were allowed sufficient recovery time in a dark, well oxygenated container before being returned to the channel unit of capture.

We compared relative growth rate (Van den Avyle and Hayward 1999), or change in length per starting length per unit time, at the reach scale in Murderers Creek for four consecutive seasons (winter 2005-fall 2005). We used two-way Analysis of Variance (ANOVA) with multiple comparisons (Bonferroni adjustment) between reaches and seasons. Eleven percent of the individuals (identifiable by their unique PIT code) used for growth rate measurement were measured during repeated seasons. We conducted the two-way ANOVA both with and without the repeatedly measured

individuals and found no differences in our results. Thus, we reported the analysis with all observations (including repeatedly measured individuals).

### *Seasonal Patterns of Individual Movement and Emigration*

Pit-tagging strategy, including placement of PIT tag arrays, could not address all potential movement patterns. We addressed questions of spring-summer upstream migration into and within Murderers Creek, and downstream emigration from Murderers Creek during fall. We hypothesized that upstream migration may occur during June, July and August, as streamflow decreased and temperature increased. Therefore, we conducted extensive PIT tagging in Reach 1 during June 2005, to assess the magnitude of this presumed upstream migrant life history pattern. Additionally, we PIT tagged *O. mykiss* from January-March 2005 in the South Fork John Day River up to 18 km downstream of the confluence of Murderers Creek. We PIT tagged *O. mykiss* in these two spatially discrete locations to define migration timing, and determine if there was a long-distance migrant population utilizing both Murderers Creek and the South Fork John Day (e.g. Armstrong et al. 1994).

Stationary PIT tag detection antennas were deployed to detect movements of *O. mykiss* PIT tagged in 2005 and prior years (2003-4). We used detection weirs composed of a submersible rectangular (30.48 cm by 80.01 cm inner diameter) antenna (Biomark Inc.<sup>1</sup>, Boise, ID) anchored upright in the thalweg with v-shaped weirs of 1.27 cm plastic mesh screening anchored upright and onto the stream bottom to direct fish through or over the antenna (depending on water depth). PIT tag antennas were

operated by a Destron Fearing<sup>1</sup> 2001F transceiver which recorded the date and time of all tag detections.

We placed a pair of weirs (separated by 237 m thalweg length to allow determination of movement directionality) in the central portion of the study location, 7.4 stream kilometers upstream from the confluence with the South Fork John Day (Figure 2.2). The detection weirs were operated in this location from April 14 through September 27, 2005; and hence detected summer movement between warmer downstream reaches of the creek, and cooler upstream reaches of the creek.

Prior observation suggested that high rates of movement, primarily emigration from tributaries into the South Fork John Day, occurred during fall (late September through winter freeze-up). In order to monitor movement of PIT tagged *O. mykiss* from all reaches of the stream, we shifted the array downstream to Reach 1 during fall. Antennas (separated by > 100 m to determine directionality) were placed downstream of the sentinel site in Reach 1, 0.9 kilometers upstream of the confluence with the South Fork John Day River (Figure 2.2). The array was operated from September 27, 2005 until disabled by ice flows on December 27, 2005.

We estimated detection efficiency of both array emplacements by two similar methods (following Zydlewski et al. 2006). During summer, we estimated the efficiency of the lower antenna at detecting upstream migrants through a ratio of fish detected at both antennas to those detected only at the upstream antenna. During fall, we estimated the efficiency of both antennas in combination through a ratio of fish detected at either antenna to those detected at a trap and array combination in the South

Fork John Day (at river km 10, Figure 2.1) downstream of the confluence with Murderers Creek.

### *Seasonal Survival Rate*

We PIT tagged *O. mykiss* throughout each sentinel site in December 2004, March-April 2005, June 2005, September 2005, and January 2006. During December and March-April, we conducted 1 sampling event at each site. In June and September, we conducted 2 sampling events at each site, and in January we conducted 3 sampling events at each site. We used Program MARK (White and Burnham 1999) to fit a Cormack-Jolly-Seber live recapture model to this series of individual mark-recapture histories for age 1+ (> 80 mm FL when tagged) and older *O. mykiss*. We used AICc selection within Program MARK to compare models with different variance structures for capture probability and survival rate. In this framework, we were able to compare models wherein capture probability and survival rate could each be either variable or fixed over time and space. Additionally, each of these parameters could covary across space or time, or interact across space and time. We tested for goodness of fit with the median  $\hat{c}$  procedure in Program MARK. The full model was only slightly overdispersed (estimate of  $c = 1.25$ ), thus we did not adjust for overdispersion in the final model. We used 95% Confidence Intervals generated by Program MARK to compare survival among reaches and seasons.

As we were unable to distinguish between mortality in-situ and emigration for most seasons, most estimates represent “apparent survival”. Apparent survival is the probability of a marked individual remaining both alive and in the sampling site.

However, we were able to quantify upstream emigration from Reach 1 during summer, and fall emigration from all reaches of Murderers Creek. Thus, for these reaches, we were able to estimate true survival via the equation: (true survival = apparent survival · (1-emigration rate)<sup>-1</sup>).

#### *Environmental Correlates of Survival Rate*

We quantified three variables which could potentially influence survival rate of *O. mykiss*. These metrics were; percent overstory canopy coverage, incidence of external scarring on *O. mykiss*, and incidence of external cysts on *O. mykiss*. Percent canopy coverage was measured with a densiometer while standing in the head of each sentinel site pool (total n = 15). Readings at all four cardinal directions were averaged for each pool. Percent canopy coverage was not continuous, and hence we compared coverage among sentinel sites with a Kruskal-Wallis non-parametric one-way ANOVA.

Each *O. mykiss* captured (both within and external to the sentinel sites), was visually inspected for body scars (typically beak marks which were present on both sides of a fish) and extruding cysts (normally ~10 mm diameter). We assumed that body scars and cysts were indicative of reaches with higher mortality rates. We used chi-square tests to determine if the frequency of occurrence of these conditions were uniformly distributed across reaches.

#### *Seasonal Density and Biomass of O. mykiss*

We used program Capture (White et al. 1982) to estimate abundance of *O. mykiss* at each of the sentinel sites in June 2005, September 2005 and January 2006 (detailed methods in Appendix A). We subsequently estimated the density of *O. mykiss*

> 75 mm per lineal stream meter, and the total combined fork length of *O. mykiss* (as a surrogate for biomass) per lineal stream meter. We used these metrics to examine density-dependent influences on growth and survival. Density and biomass (all seasons pooled) were compared between reaches with one-way ANOVA. Sample size was insufficient to use ANOVA analysis for comparison of reaches within a single season. Therefore, 95% Confidence Intervals were used to visually compare density estimates between reaches within seasons.

#### *Environmental Characteristics*

Stream temperatures were monitored continuously over the course of the study with i-Button<sup>1</sup> thermochron temperature recorders and Onset Optic Stowaway<sup>1</sup> temperature loggers. Temperature was monitored at locations near the sentinel sites during most seasons. We used one-way ANOVA and multiple comparisons (Bonferroni adjustment) to compare mean daily stream temperatures among reaches during summer and fall.

Stream height was continuously recorded by an Oregon Water Resources Department pressure gauge in Reach 1 of Murderers Creek, near the confluence with the South Fork John Day River. Stream flows were determined with a rating curve and retrieved from an Internet data storage site (<http://www.usbr.gov/pn/hydromet>). We used daily mean discharge ( $\text{m}^3/\text{s}^{-1}$ ) to represent trends in streamflow. Stream ice formation changed the channel profile and invalidated the rating curve during portions of December 2005, thus we omitted these periods.

## Results

### *Seasonal Changes in Relative Growth Rate*

Growth of *O. mykiss* in Murderers Creek showed substantial spatial and temporal variation (Figure 2.3). Season had a significant influence on relative growth rate ( $F_{3,88} = 62.56$ ,  $P < 0.0001$ ), and there was a significant first-order interaction in the model between season and reach ( $F_{6,88} = 4.45$ ,  $P < 0.001$ ). There was evidence of a difference ( $F_{2,88} = 3.02$ ,  $P = 0.05$ , Figure 2.3) in relative growth rates among reaches during winter (December 2004-March/April 2005). During winter, there was no significant difference between growth rate in Reaches 1 and 2 ( $t = -0.45$ ,  $df = 88$ ,  $P = 0.66$ ). There was evidence of a difference between Reaches 1 and 3 ( $t = 1.87$ ,  $df = 88$ ,  $P = 0.06$ ), and growth rate in Reach 3 was significantly lower than Reach 2 ( $t = 2.32$ ,  $df = 88$ ,  $P = 0.02$ ). Murderers Creek averaged 2.3 °C (Table 2.1) during winter.

Growth rates were highest during spring (March/April 2005-June 2005, Figure 2.3), and were homogenous across all reaches ( $F_{2,88} = 0.73$ ,  $P = 0.48$ ). Spring growth rates were significantly higher than growth rates for any other season or reach ( $P < 0.0001$  for all pairwise comparisons). Stream temperature averaged 9.0 °C (Table 2.1).

Growth rates differed significantly between reaches ( $F_{2,88} = 18.23$ ,  $P < 0.0001$ ) during summer (June 2005-September 2005) and displayed a spatial pattern opposite that observed in winter (Figure 2.3). Relative growth in Reach 1 was significantly lower than both Reach 2 ( $t = -4.71$ ,  $df = 88$ ,  $P < 0.0001$ ) and Reach 3 ( $t = -5.52$ ,  $df = 88$ ,  $P < 0.0001$ ). There was no significant difference between Reaches 2 and 3 ( $t = -0.94$ ,  $df = 88$ ,  $P = 0.35$ ). Mean daily stream temperatures during summer (Figure 2.4) varied

significantly among reaches ( $F_{2,225} = 51.33$ ,  $P < 0.0001$ ). The mean daily temperature in Reach 1 of 19.2 °C (95% Confidence Interval: 18.8 to 19.6) was significantly higher ( $P < 0.0001$ ) than the mean daily temperature of 17.5 °C (95% Confidence Interval: 17.2 to 17.9) in Reach 2. The mean daily temperature in Reach 3 of 16.3 °C (95% Confidence Interval: 15.9 to 16.6) was significantly lower ( $P < 0.0001$ ) than both Reaches 1 and 2 (Table 2.1, Figure 2.4).

Relative growth rates during fall (September 2005 through January 2006) were not significantly different between reaches ( $F_{2,88} = 1.48$ ,  $P = 0.23$ , Figure 2.3). Similarly, stream temperatures during fall were homogeneous ( $F_{2,294} = 0.13$ ,  $P = 0.88$ ) across different reaches (Table 2.1, Figure 2.4).

#### *Seasonal Patterns of Individual Movement and Emigration*

Rates of upstream migration during summer were low. Of 306 *O. mykiss* PIT tagged in Reach 1 of Murderers Creek during June 2005, we estimated that 2.6% migrated upstream through the antenna array in Reach 2 (Figure 2.2). Furthermore, of 458 *O. mykiss* PIT tagged in the South Fork John Day River, 0 were detected making long-distance migrations into Murderers Creek during 2005. *O. mykiss* PIT tagged in Murderers Creek during prior years were also detected migrating upstream through the array. Upstream migration timing, as inferred from the limited number of detections, was bimodal. One group of *O. mykiss* ( $n = 3$ ) passed the array during April, while the larger group ( $n = 6$ ) passed the array between June 17 and July 20 (statistical weeks 24 to 29; Figure 2.5).

The latter mode of upstream migration occurred on the descending limb of the hydrograph, but before summer baseflow (Figure 2.5), and was coincident with rising stream temperatures (Figure 2.5). This latter mode of upstream migration began concurrent with maximum temperatures at the antenna location increasing to 16 °C and ended concurrent with maximum temperatures of 23 °C. This migration period, however, concluded 11 days before peak temperatures in Reach 1 and 17 days before peak temperatures in Reach 2 (see Figure 2.5).

Downstream migration during fall was more prevalent than upstream migration during summer. Fall emigration from Murderers Creek began in late September and continued through December (statistical weeks 39 to 52). This migration also appeared to be bimodal in nature (Figure 2.6). The first mode of emigration in late October (statistical week 43) was coincident with the first decline in daily mean temperature below 8 °C. The second mode of emigration in late December (statistical week 51) was coincident with a high discharge event (Figure 2.6). Fall emigration differed between reaches in Murderers Creek. An estimated 35.2% of the *O. mykiss* in Reach 1 emigrated during fall. Similarly, an estimated 49.2% of *O. mykiss* in Reach 2 emigrated from the creek during fall. Conversely, only an estimated 8.5% of the *O. mykiss* in Reach 3 emigrated during fall.

#### *Seasonal Survival Rate*

Model selection indicated that constant probability of capture was most appropriate for the data (Table 2.2). Two models with constant capture probability were within 2 AICc units of each other, and hence were competing models. The first

model found that survival rate was significantly different among reaches. Differences among reaches remained constant through time (Table 2.2). The second of the competing models was structured such that survival rate was equal between reaches 1 and 2, but survival rate in these reaches was significantly different from Reach 3. In this model, differences in survival rate between these groups (reaches 1 and 2 vs. reach 3) again remained constant through time (Table 2.2).

Both models (Table 2.2) indicated that apparent survival rate was significantly higher in Reach 3 than either Reach 1 or Reach 2 during all seasons. Apparent survival rate was lowest during spring, and highest during summer (Figure 2.7), however, differences in apparent survival among seasons were not significant. Estimates of true survival during fall (Figure 2.8) were not significantly different among reaches. Fall survival rates were not significantly different from summer survival rates (Figure 2.8) for any reach.

#### *Environmental Correlates of Survival Rate*

Percent canopy coverage differed significantly between reaches ( $F_{2,12} = 27.2$ ,  $P < 0.0001$ ). Average canopy coverage in the reach 1 sentinel site was 7%. Average canopy coverage in the reach 2 sentinel site was 23%. Average canopy coverage in the reach 3 sentinel site was 74%. The occurrence of body scars and external cysts was low throughout Murderers Creek (Table 2.3). The proportion of *O. mykiss* with body scars was higher in reaches 1 and 2 than in reach 3, however, this difference was not statistically significant ( $\chi^2 = 3.77$ ,  $df = 2$ ,  $P = 0.15$ ). Similarly, a higher proportion of *O.*

*mykiss* in reaches 1 and 2 had external cysts than in reach 3. This difference was statistically significant ( $\chi^2 = 6.94$ ,  $df = 2$ ,  $P = 0.03$ ).

#### *Seasonal Density and Biomass of O. mykiss*

There was evidence of a difference in *O. mykiss* density among reaches ( $F_{2,6} = 4.98$ ,  $P = 0.05$ ). However, lower density in downstream reaches was somewhat offset by larger size of the individuals present. There was no significant difference in biomass of *O. mykiss* among reaches ( $F_{2,6} = 3.86$ ,  $P = 0.08$ ).

Comparison of 95% Confidence Intervals (Figure 2.9) suggests there were no significant differences among reaches in mean density of *O. mykiss* in June 2005. Conversely, during September 2005, Reach 1 had significantly lower density than Reach 2, and Reach 2 in turn had significantly lower density than Reach 3 (Figure 2.9). During January 2006 there was no significant difference in density between Reach 1 and Reach 2. Conversely, estimated density in Reach 3 was significantly higher than density in either Reach 1 or Reach 2 (Figure 2.9).

### **Discussion**

#### *Seasonal Changes in Relative Growth Rate*

Space and time interactively influenced individual growth rates of *O. mykiss*, and caused areas with high growth potential to shift across the landscape between seasons (Figure 2.3). Some areas with comparatively high growth potential during winter (e.g., Reach 1) had comparatively low growth potential during summer. This variability suggests that accurately characterizing the production potential of different stream segments requires year-round monitoring. Furthermore, relative growth rate

varied among individuals within each location and season. For instance, individual growth rates during summer in Reach 1 ranged from 0.00 (0% of the mean) to 0.17 (189% of the mean). Thus, to accurately measure production, individual monitoring should be nested within seasonal monitoring of different stream segments. The variable growth rates we observed are similar to those of Juanes et al. (2000), but differ with Bacon et al. (2005). Bacon et al. observed little variation in individual growth rates of Atlantic salmon parr. Different spatial scales of sampling likely contributed to these contrasting results. Bacon et al. (2005) sampled solely in a 140 m stream section, conversely, Juanes et al. (2000) sampled a 1 km section of stream. We sampled 848 m of stream distributed across 18 km. Given the environmental influences on individual growth rate, growth rate variation should increase with spatial extent, which can be observed in these three studies.

*O. mykiss* in Murderers Creek achieved the majority of their annual skeletal growth during spring (Figure 2.3). This growth occurred at mean temperatures (9.0 °C, Table 2.1) below those reported as optimal for growth of *O. mykiss* (15 to 19 °C; Richter and Kolmes 2005). Bacon et al. (2005) observed a similar discrepancy, with the fastest annual growth of Atlantic salmon parr occurring during spring in colder than “optimal” temperatures. Similarly, Juanes et al. (2000) also found growth to be greatest during spring. Bioenergetics studies defining “optimal growth temperatures” may overlook seasonal changes in fish response to temperature. For instance, Averett (1969) found that optimal temperatures for growth of coho salmon shifted between seasons, and were lower during winter and spring than summer. Optimal spring growth

temperatures for coho were 1/3 the optimal summer temperature (Averett 1969). Thus, although spring temperatures in Murderers Creek were below the optimum range summarized by Richter and Kolmes (2005), they likely met or exceeded seasonally optimal growth temperatures.

Growth rates were not driven solely by temperature, which is consistent with prior studies (Nicola and Almodovar 2004; Larsson et al. 2005). Winter growth rates differed among reaches (Figure 2.3) during a season when stream temperatures are typically homogenous. During summer, there was a significant difference in temperature between Reaches 2 and 3 (Figure 2.4), but no difference in growth rate (Figure 2.3). Differences in food availability or feeding rates must be present between Reach 2 and Reach 3, especially during summer in order to offset temperature-driven increases in metabolic demand in Reach 2 (Hughes and Grand 2000; Lovtang 2005). The annual pattern in growth rate throughout Murderers Creek (Figure 2.3) tracks changes in photoperiod more closely than temperature. For instance, temperatures during spring and fall (means and observed maxima) were comparable (Table 2.1, Figure 2.4), yet growth rates were higher in spring than fall (Figure 2.3). As summarized and observed by Higgins (1985), photoperiod appears to control the annual growth pattern of juvenile salmonids, with direction of change (i.e., increasing or decreasing) being more important than magnitude. The reach-scale differences in growth we observed during winter and summer were superimposed on this annual growth pattern, yet remained substantial enough to potentially alter survival or life history strategy of *O. mykiss*.

### *Seasonal Patterns of Individual Movement and Emigration*

Movement rates of PIT tagged individuals were low during spring and summer (Figure 2.5). This suggests that this population, at least in the year of study and at the reach scale, conformed to the “restricted movement paradigm” (Rodriguez 2002). The vast majority of the population did not move during the summer, in contrast to other more mobile salmonid populations (Gowan and Fausch 1996; Kahler et al. 2001; Gowan and Fausch 2002). Additionally, we found no evidence of “long-distance migrants” (Armstrong et al. 1994), as no PIT-tagged individuals immigrated from the South Fork John Day. The lack of movement is notable because individuals remained in stream segments where temperature reached 27 °C (Table 2.2), above reported lethal limits for *O. mykiss* (Richter and Kolmes 2005).

The paucity of movement from April through September suggests that individuals did not track resource availability, nor behaviorally thermoregulate at the reach scale. An upstream migration during summer of 6 kilometers would have resulted in a significant increase in growth (presuming density dependence did not reduce the observed growth rate, Figure 2.3) and decrease in daily mean temperatures (Figure 2.4). However, such migration occurred at a negligible rate. Individuals in the lower portion of the creek continued to add skeletal growth during summer even though temperatures exceeded levels where net growth of *O. mykiss* has been reported to cease (Richter and Kolmes 2005). *O. mykiss* in Murderers Creek appear to have adaptive tolerance of high water temperatures, similar to populations of resident redband trout in Southeast Oregon (Gamperl et al. 2002; Rodnick et al. 2004).

Movement rates were higher during fall than all other seasons. The majority of emigration occurred during a single week, coincident with the first decline in stream temperature to  $< 8^{\circ}\text{C}$  (Figure 2.6). Juanes et al. (2000) also observed the largest downstream migration of Atlantic salmon parr coincident with water temperature declining to near or below  $8^{\circ}\text{C}$ . Bjornn (1971) similarly observed the largest number of emigrant *O. mykiss* coincident with temperature declining to  $8^{\circ}\text{C}$ , although he could not establish a causal link between temperature and emigration in experimental channels. Eight degrees is the temperature at which juvenile salmonids shift from diurnal to nocturnal behavior, and in turn conceal in substrate during daylight hours (Riehle and Griffith 1993; Grunbaum 1996). It is likely, however, that both abiotic (availability of concealment habitat) and biotic (size and prior growth rate) factors influence this downstream migration (more thoroughly addressed in Chapter 3), which may occur in both anadromous and potamodromous life histories. If seasonal movement is an indicator of habitat quality (Belanger and Rodriguez 2002), winter may be the limiting season in Murderers Creek. Conversely, high rates of emigration may be a result of individual metabolic demands becoming limited by seasonal food availability (Cucherousset et al. 2005), rather than physical habitat.

#### *Seasonal Survival Rate*

Apparent survival rates were higher in reach 3 than either reaches 1 or 2. High rates of emigration (mainly in the form of smoltification) during winter and spring make comparison of survival during those seasons tenuous. However, low rates ( $<3\%$ ) of individual movement during summer suggest that, for summer, apparent survival

approximates true survival. Survival rates during summer were equal between reaches 1 and 2, but both of these reaches had lower survival than reach 3. Thus, survival rate differed at the reach scale, similar to individual growth rate.

We observed correlation between patterns of survival rate, and evidence of avian predation. While not statistically significant at an alpha of 0.05, the majority of all observed body scars occurred in reaches 1 and 2 (Table 2.3). This suggests that avian predation in all reaches of Murderers Creek was primarily concentrated in the lower, more open stream segments (reaches 1 and 2). Canopy coverage was significantly lower in reaches 1 and 2 than in reach 3. The dense canopy coverage in Reach 3 likely impeded common aerial predators such as Belted Kingfisher (*Ceryle alcyon*) and Great Blue Heron (*Ardea herodias*) and hence contributed to higher survival rates. Furthermore, warmer summer temperatures in reaches 1 and 2 may have interacted with reduced canopy coverage to decrease survival rate. Predator avoidance capability declines with warmer water temperatures (Marine and Cech 2004), and we also noted that occurrence of cysts was significantly higher in reaches 1 and 2 than 3 (Table 2.3). Thus, high stream temperatures may have indirectly contributed to lower survival rates in reaches 1 and 2.

We found no evidence of decreased survival rate during the fall-winter transition. True survival rate estimates during fall were comparable to summer survival rates (Figure 2.8) for all reaches. Increased mortality rates have been reported for salmonid parr during winter (Cunjak 1996; Letcher et al. 2002). For instance, Letcher et al. (2002) found two-fold lower survival of Atlantic salmon parr during winter as

summer. Much of this mortality has been attributed to the transition or “acclimation” phase of late fall and early winter (Cunjak et al. 1987; Berg and Bremset 1988; Smith and Griffith 1994; Carlson and Letcher 2003). We sampled in January, after the reported period of primary winter mortality, and after stream temperatures had remained at 0°C for an extended period (approximately 2 continuous weeks, Figure 2.6). Thus, the timing of our sampling was appropriate to detect winter mortality. Energy reserve levels (lipids) may influence overwinter survival (Finstad et al. 2004). Lipid reserve levels increase with size (Metcalf and Thorpe 1992; Biro et al. 2004). Since many of the age 1+ *O. mykiss* we tagged were larger than Atlantic salmon parr, they may have had inherently higher winter survival owing to their larger size. Nevertheless, we found that *O. mykiss* in Murderers Creek handled the transition to winter temperatures with no detectable decrease in survival rate from prior seasons. Moreover, this capacity was retained after exposure of some individuals (fish in reaches 1 and 2) to summer temperatures at physiologically stressful levels (Richter and Kolmes 2005; Feldhaus 2006).

#### *Interrelation of Growth, Movement, Survival and Density*

*O. mykiss* largely remained sedentary in Reach 1 of Murderers Creek during summer, despite experiencing slower growth and higher temperatures relative to upstream reaches. Conversely, *O. mykiss* in Reach 1 experience higher growth during winter relative to upstream areas (Reach 3). Higher growth in Reach 1 during winter may give individuals a “jump start” on necessary annual growth. At the beginning of summer, individuals in Reach 1 were, on average, 4.9 mm longer than individuals in

Reach 2, and 15.5 mm longer than individuals in Reach 3. Salmonids can monitor and allocate their lipid levels (Bull et al. 1996). Smolting in the upcoming year is affected by growth rate trajectories and seemingly affects energy and time budgets (i.e., upper and lower modal groups; Thorpe 1987). Atlantic salmon parr will adjust their activity levels, depending on whether they are on a smolt trajectory (Metcalf et al. 1988). Thus, individuals in Reach 1 may have “anticipated” that their size or energy levels (as a result of beginning summer at a larger size) were sufficient to stay on a desired growth trajectory. Density in Reach 1 during summer was lower than Reaches 2 and 3 (Figure 2.9). This lower density may have ensured suitable microhabitat locations for all individuals. Radiotagged individuals in this reach have been observed conducting 150-200 m diel movements (F. Madrinan, unpublished data), potentially facilitated by the low density of *O. mykiss*. Thus, lower density and biomass may have helped support growth rates despite high temperatures. Although *O. mykiss* in this region apparently have enhanced capability to function at high temperatures (Rodnick et al. 2004), we suggest that the interaction of growth rate during prior seasons and seasonal density may influence the response (or lack thereof) of individuals to high temperatures and help maintain survival rates.

In summary, we demonstrated that different life history patterns, and hence phenotypes can be present within a relatively small stream system. Whereas temperature is one of the major variables, other localized differences in the environment affect growth and survival and in turn, the expression and distribution of different phenotypes within a basin. These influences represent the riverscape (see Fausch et al.

2002), which we propose is the template that governs expression and distribution of life history phenotypes.

### **Acknowledgements**

We thank S. White, F. Madrinan, J. Feldhaus, P. Bayley, S. Heppell, J. Davis, B. Kingsley, V. Mueller, J. Togstad, J. Silbernagel, D. Myers, N. Weber, B. Tattam, and T. Tattam for assistance collecting data in Murderers Creek. We thank C. Moore of ODFW for providing us a field camp at the Murderers Creek Ranch. This project was funded by the U.S. Bureau of Reclamation, Pacific Northwest Region, through M. Newsom. Additional funding for I. Tattam was provided by the Oregon Chapter of the American Fisheries Society, Flyfishers Club of Oregon, Federation of Flyfishers Oregon Chapter, Washington County Flyfishers, and the Sunriver Anglers Club. C. Jordan of the NOAA Northwest Fisheries Science Center provided PIT tags. W. Wilson of ODFW helped with PIT tag data management. P. Bayley provided extensive comments which greatly improved the manuscript.

## References

- Armstrong, J. D., P. E. Shackley, and R. Gardiner. 1994. Redistribution of juvenile salmonid fishes after localized catastrophic depletion. *Journal of Fish Biology* 45:1027-1039.
- Armstrong, J. D., V. A. Braithwaite, and F. A. Huntingford. 1997. Spatial strategies of wild Atlantic salmon parr: exploration and settlement in unfamiliar areas. *Journal of Animal Ecology* 66:203-211.
- Averett, R. C. 1969. Influence of temperature on energy and material utilization by juvenile coho salmon. Doctoral dissertation, Oregon State University, Corvallis.
- Bacon, P. J., W. S. C. Gurney, W. Jones, I. S. McLaren, and A. F. Youngson. 2005. Seasonal growth patterns of wild juvenile fish: partitioning variation among explanatory variables, based on individual growth trajectories of Atlantic salmon (*Salmo salar*) parr. *Journal of Animal Ecology* 74:1-11.
- Belanger, G. and M. A. Rodriguez. 2002. Local movement as a measure of habitat quality in stream salmonids. *Environmental Biology of Fishes* 64:155-164.
- Berg, O. K., and G. Bremset. 1998. Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. *Journal of Fish Biology* 52:1272-1288.
- Biro, P. A., A. E. Morton, J. R. Post, and E. A. Parkinson. 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 61:1513-1519.
- Bjornn, T. C. 1971. Trout and salmon movement in two Idaho streams as related to temperature, food, stream flow, cover and population density. *Transactions of the American Fisheries Society* 100:423-438.
- Bull, C. D., N. B. Metcalfe, and M. Mangel. 1996. Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. *Proceedings of the Royal Society of London* 263:13-18.
- Carlson, S. M., and B. H. Letcher. 2003. Variation in brook and brown trout survival within and among seasons, species and age classes. *Journal of Fish Biology* 63:780-794.
- Cucherousset, J., D. Ombredane, K. Charles, F. Marchand, and J. Bagliniere. 2005. A continuum of life history tactics in a brown trout (*Salmo trutta*) population. *Canadian Journal of Fisheries and Aquatic Sciences* 62:1600-1610.

- Cunjak, R. A., R. A. Curry, and G. Power. 1987. Seasonal energy budget of brook trout in streams: implications of a possible deficit in early winter. *Transactions of the American Fisheries Society* 116: 817-828.
- Cunjak, R. A. 1996. Winter habitat of selected stream fishes and potential impacts from land-use activity. *Canadian Journal of Fisheries and Aquatic Sciences* 53(Suppl. 1):267-282.
- Duston, J., and R. L. Saunders. 1997. Life histories of Atlantic salmon altered by winter temperature and summer rearing in fresh- or sea-water. *Environmental Biology of Fishes* 50:149-166.
- Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. *BioScience* 52: 483-498.
- Feldhaus, J. W. 2006. Heat shock proteins and physiological stress in redband rainbow trout (*Oncorhynchus mykiss gairdneri*) in the South Fork John Day River, Oregon. Masters thesis, Oregon State University, Corvallis.
- Filbert, R. B., and C. P. Hawkins. 1995. Variation in condition of rainbow trout in relation to food, temperature, and individual length in the Green River, Utah. *Transactions of the American Fisheries Society* 124:824-835.
- Finstad, A. G., O. Ygedal, T. Forseth, and T. F. Naesje. 2004. Energy-related juvenile winter mortality in a northern population of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 61:2358-2368.
- Frissell, C. A., W. J. Liss, C. E. Warren, and M. D. Hurley. 1986. A hierarchical framework for stream habitat classification. *Environmental Management* 10:199-214.
- Gamperl, A. K., K. J. Rodnick, H. A. Faust, E. C. Venn, M. T. Bennett, L. I. Crawshaw, E. R. Keeley, M. S. Powell, and H. W. Li. 2002. Metabolism, swimming performance and tissue biochemistry of high desert redband trout (*Oncorhynchus mykiss* ssp.): evidence for phenotypic differences in physiological function. *Physiological and Biochemical Zoology* 75:413-431.
- Girard, I. L., J. W. A. Grant, and S. O. Steingrimsson. 2004. Foraging, growth, and loss rate of young-of-the-year Atlantic salmon (*Salmo salar*) in relation to habitat use in Catamaran Brook, New Brunswick. *Canadian Journal of Fisheries and Aquatic Sciences* 61:2339-2349.

- Gowan, C., and K. D. Fausch. 1996. Mobile brook trout in two high-elevation Colorado streams: re-evaluating the concept of restricted movement. *Canadian Journal of Fisheries and Aquatic Sciences* 53:1370-1381.
- Gowan, C., and K. D. Fausch. 2002. Why do foraging stream salmonids move during summer? *Environmental Biology of Fishes* 64:139-153.
- Grunbaum, J. B. 1996. Geographical and seasonal variation in diel habitat use by juvenile (age 1+) steelhead trout (*Oncorhynchus mykiss*) in coastal and inland Oregon streams. Master's thesis, Oregon State University, Corvallis.
- Higgins, P. J. 1985. Metabolic differences between Atlantic salmon (*Salmo salar*) parr and smolts. *Aquaculture* 45:33-53.
- Hughes, N. F., and T. C. Grand. 2000. Physiological ecology meets the ideal-free distribution: predicting the distribution of size-structured fish populations across temperature gradients. *Environmental Biology of Fishes* 59:285-298.
- Imre, I., J. W. A. Grant, and E. R. Keeley. 2004. The effect of food abundance on territory size and population density of *O. mykiss* trout (*Oncorhynchus mykiss*). *Oecologia* 138:371-378.
- Juanes, F., B. H. Letcher, and G. Gries. 2000. Ecology of stream fish: insights gained from an individual-based approach to juvenile Atlantic salmon. *Ecology of Freshwater Fish* 9:65-73.
- Kahler, T. H., P. Roni, and T. P. Quinn. 2001. Summer movement and growth of juvenile anadromous salmonids in small western Washington streams. *Canadian Journal of Fisheries and Aquatic Sciences* 58:1947-1956.
- Keeley, E. R. 2001. Demographic responses to food and space competition by juvenile steelhead trout. *Ecology* 82:1247-1259.
- Larsson, S., T. Forseth, I. Berglund, A. J. Jensen, I. Naslund, J. M. Elliott, and B. Jonsson. 2005. Thermal adaptation of Arctic charr: experimental studies of growth in eleven charr populations from Sweden, Norway and Britain. *Freshwater Biology* 50:353-368.
- Letcher, B. H., G. Gries, and F. Juanes. 2002. Survival of stream-dwelling Atlantic salmon: effects of life history variation, season and age. *Transactions of the American Fisheries Society* 131:838-854.
- Lovtang, J. C. 2005. Distribution, habitat use, and growth of juvenile Chinook salmon

- in the Metolius River Basin, Oregon. Masters thesis, Oregon State University, Corvallis.
- Lundqvist, H., S. McKinnell, H. Fangstam, and I. Berglund. 1994. The effect of time, size and sex on recapture rates and yield after river releases of *Salmo salar* smolts. *Aquaculture* 121:245-257.
- Marine, K. R., and J. J. Cech Jr. 2004. Effects of high water temperature on growth, smoltification, and predator avoidance in juvenile Sacramento River Chinook salmon. *North American Journal of Fisheries Management* 24:198-210.
- Metcalf, N. B., F. A. Huntingford, and J. E. Thorpe. 1988. Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon *Salmo salar*. *Journal of Animal Ecology* 57:463-474.
- Metcalf, N. B., and J. E. Thorpe. 1992. Anorexia and defended energy levels in over-wintering juvenile salmon. *Journal of Animal Ecology* 61:175-181.
- Metcalf, N. B. 1998. The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 55(Suppl. 1):93-103.
- Metcalf, N. B., N. H. C. Fraser, and M. D. Burns. 1999. Food availability and the nocturnal vs. diurnal foraging trade-off in juvenile salmon. *Journal of Animal Ecology* 68:371-381.
- Morinville, G. R., and J. B. Rasmussen. 2003. Early juvenile bioenergetic differences between anadromous and resident brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* 60:401-410.
- Murphy, M. H., M. J. Connerton, and D. J. Stewart. 2006. Evaluation of winter severity on young-of-the-year Atlantic salmon. *Transactions of the American Fisheries Society* 135:420-430.
- Myrick, C. A., and J. J. Cech Jr. 2000. Temperature influences on California rainbow trout physiological performance. *Fish Physiology and Biochemistry* 22:245-254.
- Nicola, G. G., and A. Almodovar. 2004. Growth pattern of stream-dwelling brown trout under contrasting thermal conditions. *Transactions of the American Fisheries Society* 133:66-78.
- Peven, C. M., R. R. Whitney, and K. R. Williams. 1994. Age and length of steelhead

smolts from the mid-Columbia River Basin, Washington. *North American Journal of Fisheries Management* 14:77-86.

- Prentice, E. F., T. A. Flagg, C. S. McCutcheon, D. F. Brastow, and D. C. Cross. 1990. Equipment, methods, and an automated data entry station for PIT tagging. Pages 335-340 in N. C. Parker, A. E. Giorgi, R. C. Hedinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. *Fish-marking techniques*. American Fisheries Society, Symposium 7, Bethesda, Maryland.
- PTSC (Pit Tag Steering Committee). 1999. *PIT Tag Marking Procedures Manual*. Pacific States Marine Fisheries Commission, Gladstone, Oregon.
- Reeves, G. H., F. H. Everest, and J. D. Hall. 1987. Interactions between the redband shiner (*Richardsonius balteatus*) and the steelhead trout (*Salmo gairdneri*) in western Oregon: the influence of water temperature. *Canadian Journal of Fisheries and Aquatic Sciences* 44:1603-1613.
- Richter, A., and S. A. Kolmes. 2005. Maximum temperature limits for Chinook, coho, and chum salmon, and steelhead trout in the Pacific Northwest. *Reviews in Fisheries Science* 13:23-49.
- Riehle, M. D., and J. S. Griffith. 1993. Changes in habitat use and feeding chronology of juvenile rainbow trout (*Oncorhynchus mykiss*) in fall and the onset of winter in Silver Creek, Idaho. *Canadian Journal of Fisheries and Aquatic Sciences* 50:2119-2128.
- Rodnick, K. J., A. K. Gamperl, K. R. Lizars, M. T. Bennett, R. N. Rausch, and E. R. Keeley. 2004. Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. *Journal of Fish Biology* 64:310-335.
- Rodriguez, M. A. 2002. Restricted movement in stream fish: the paradigm is incomplete, not lost. *Ecology* 83(1):1-13.
- Roni, P., and T. P. Quinn. 2001. Effects of wood placement on movements of trout and juvenile coho salmon in natural and artificial stream channels. *Transactions of the American Fisheries Society* 130:675-684.
- Smith, R. W., and J. S. Griffith. 1994. Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. *Transactions of the American Fisheries Society* 123:747-756.
- Steingrimsson, S. O., and J. W. A. Grant. 2003. Patterns and correlates of

- movement and site fidelity in individually tagged young-of-the-year Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 60:193-202.
- Tipping, J. M. 1997. Effect of smolt length at release on adult returns of hatchery-reared winter steelhead. *The Progressive Fish Culturist* 59:310-311.
- Thorpe, J. E. 1987. Environmental regulation of growth patterns in juvenile Atlantic salmon. Pages 463-474 *in*: R. C. Summerfeldt and G. E. Hall, editors. *Age and Growth of Fish*. Iowa State University Press, Ames, Iowa.
- Van den Avyle, M. J., and R. S. Hayward. 1999. Dynamics of exploited fish populations. Pages 127-166 *in*: C. C. Kohlyer and W. A. Hubert, editors. *Inland Fisheries Management in North America*. American Fisheries Society, Bethesda, Maryland.
- Ward, B. R., P. A. Slaney, A. R. Facchin, and R. W. Land. 1989. Size-biased survival in steelhead trout (*Oncorhynchus mykiss*): Back-calculated lengths from adult's scales compared to migrating smolts at the Keogh River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1853-1858.
- White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory LA-8787-NERP. 235 pp.
- White, G. C. and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. *Bird Study* 46 Supplement, 120-138.
- Wright, K. K. 2000. From continua to patches: Longitudinal patterns in the Middle Fork of the John Day River, Oregon. Doctoral dissertation, Oregon State University, Corvallis.
- Zimmerman, C. E., and G. H. Reeves. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. *Canadian Journal of Fisheries and Aquatic Sciences* 57:2152-2162.
- Zydlewski, G. B., G. Horton, T. Dubreuil, B. Letcher, S. Casey, and J. Zydlewski. 2006. Remote monitoring of fish in small stream: A unified approach using PIT tags. *Fisheries* 31:492-502.

### **Endnotes**

<sup>1</sup>Reference to trade names does not imply endorsement by the United States Geological Survey, Oregon Cooperative Fishery Research Unit, or Oregon State University.

## Tables and Figures

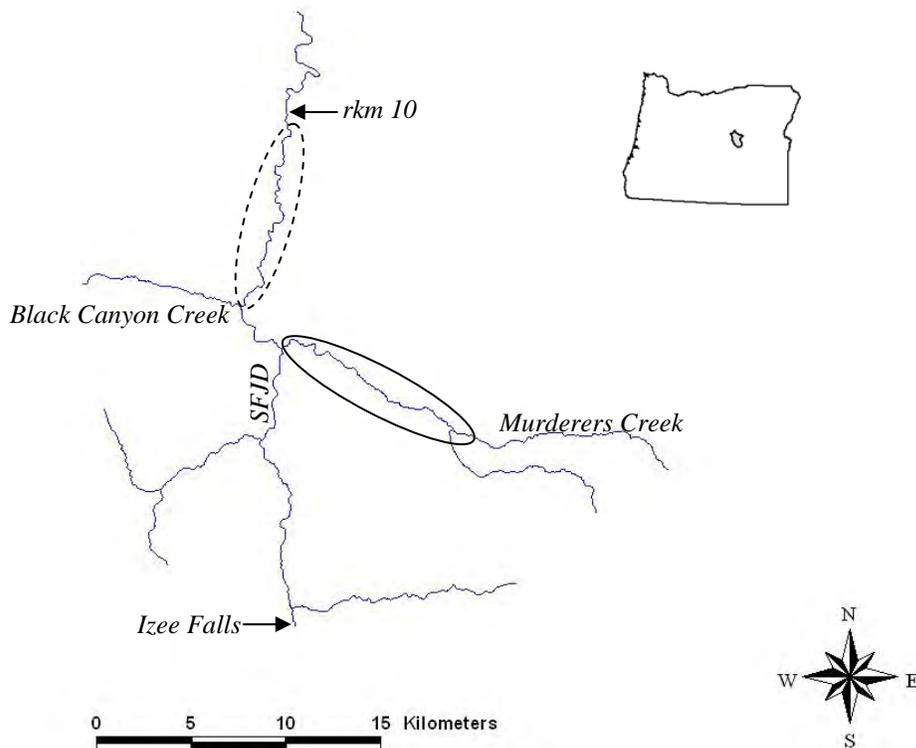


Figure 2.1. Location of the South Fork John Day River basin (SFJD), and Murderers Creek, the principal study area location. The South Fork John Day River flows from South to North, and is a fifth-order stream downstream (North) of the confluence with Murderers Creek. Inset shows the position of the South Fork basin within Oregon. Dashed oval denotes where PIT tagging occurred in the SFJD. Solid oval denotes the study section of Murderers Creek.

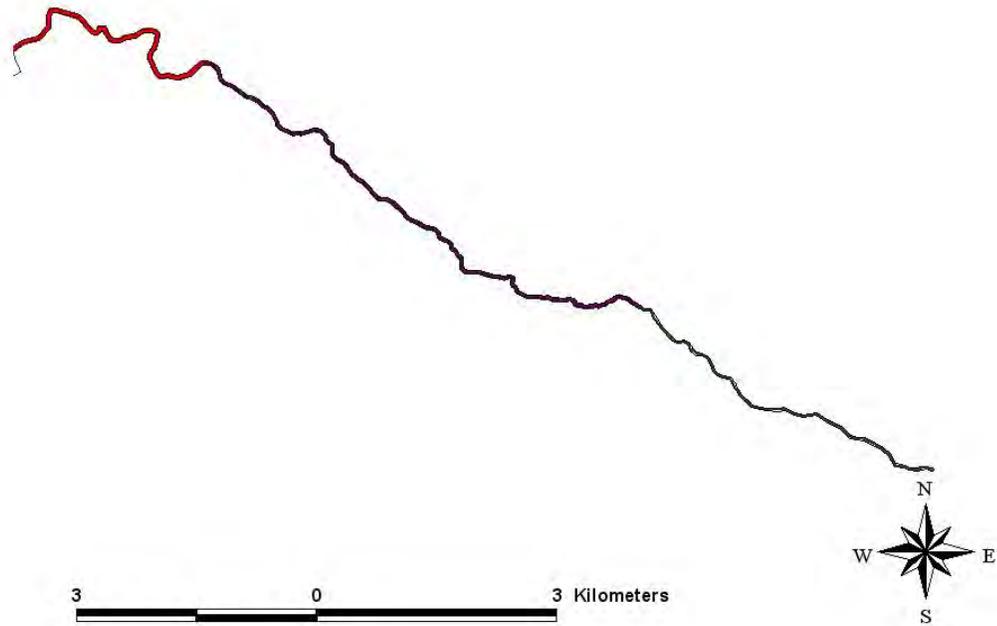


Figure 2.2. Space-time schematic of monitoring in Murderers Creek during 2004-2006. Red shading denotes Reach 1, purple shading denotes Reach 2, and green shading denotes Reach 3. The stream flows from east to west, and joins the South Fork John Day at the west edge of this figure. Circles are sentinel site locations within each reach. Capture and PIT tagging occurred at the circled locations in December 2004, March-April 2005, June 2005, September 2005, and January 2006. Two PIT tag antennas were located at the solid line from April 14, 2005 through September 27, 2005. The PIT tag antennas were moved to the location of the dashed line from September 27, 2005 through December 27, 2005.

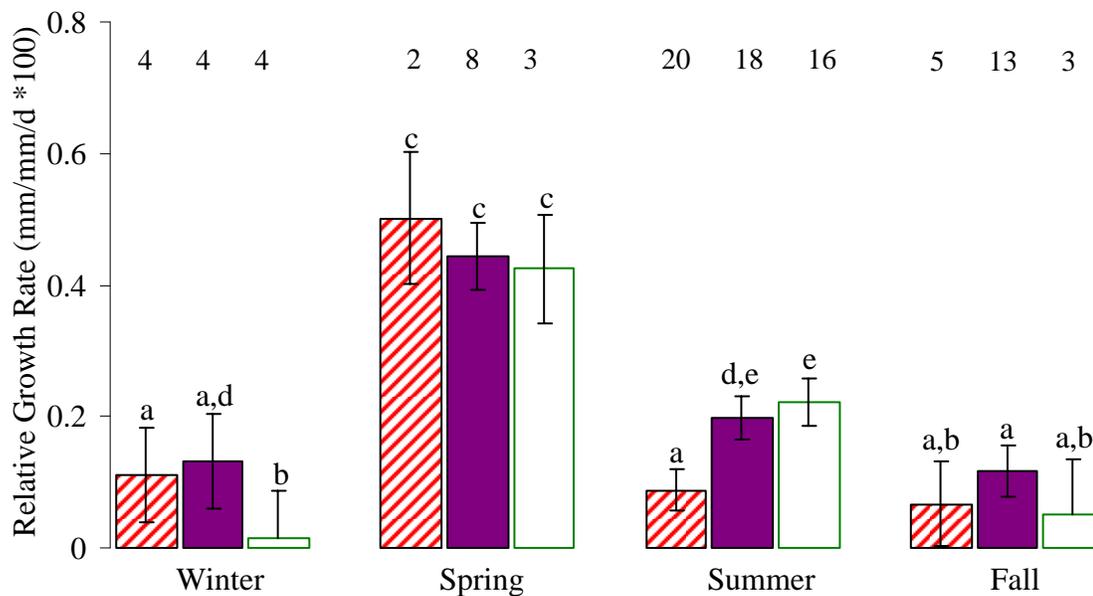


Figure 2.3. Seasonal pattern in relative growth rates of PIT tagged *O. mykiss* in Murderers Creek from December 2004 through January 2006. Striped bars denote Reach 1, the lowermost portion of the creek. Solid bars indicate Reach 2, the middle valley segment of the creek. Open bars represent Reach 3, the uppermost portion of the study area. Numerals at the top of the figure indicate the number of individuals measured for each reach and season. Error bars are 95% Confidence Intervals of the mean. Common letters indicate reaches that are not significantly different ( $P > 0.05$ ).

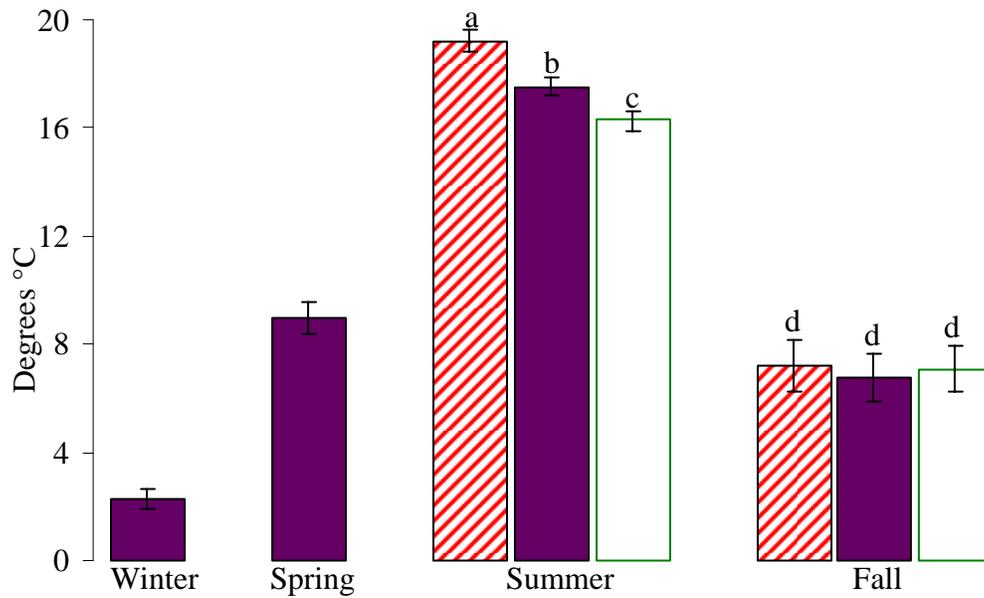


Figure 2.4. Seasonal averages of daily mean stream temperatures at Murderers Creek sentinel sites. Striped bars are reach 1, solid bars are reach 2, and open bars are reach 3. Error bars are 95% Confidence Intervals for the seasonal means. Common letters indicate sites which were not significantly different ( $P > 0.05$ ).

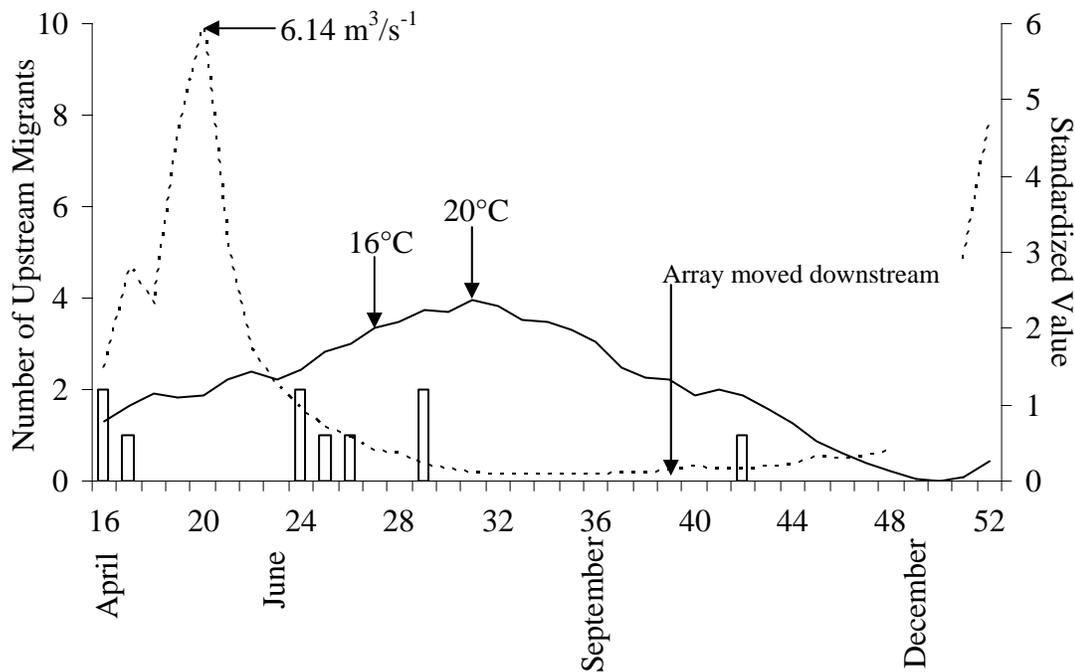


Figure 2.5. Detections of upstream migrant *O. mykiss* at fixed PIT arrays in Murderers Creek during 2005. Horizontal axis is Julian weeks, with corresponding months given for reference. Bars denote number of individuals observed making an upstream migration during each Julian week of antenna operation, and correspond to the left vertical axis. Standardized (daily mean/annual mean) weekly average stream flow (dashed line) and stream temperature (solid line), corresponding to the right vertical axis, are presented for comparison.

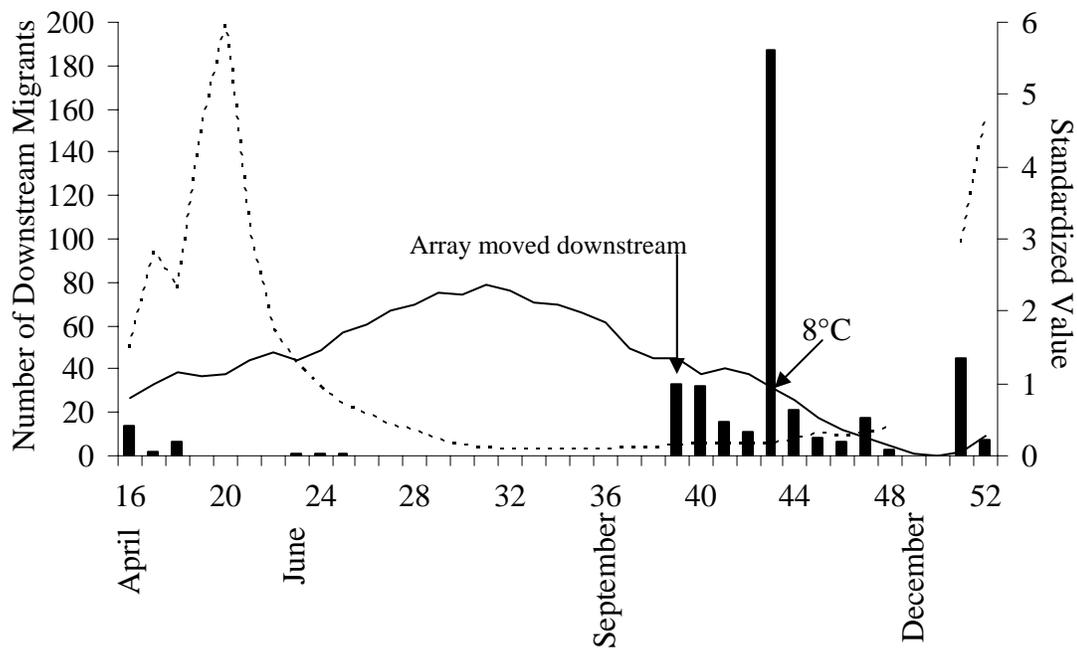


Figure 2.6. Detections of downstream migrant *O. mykiss* at fixed PIT arrays in Murderers Creek during 2005. Bars denote number of individuals observed making a downstream migration, and correspond to the left vertical axis. Horizontal axis is Julian weeks, with corresponding months given for reference. Standardized (daily mean/annual mean) weekly average stream flow (dashed line) and stream temperature (solid line), corresponding to the right vertical axis, are presented for comparison.

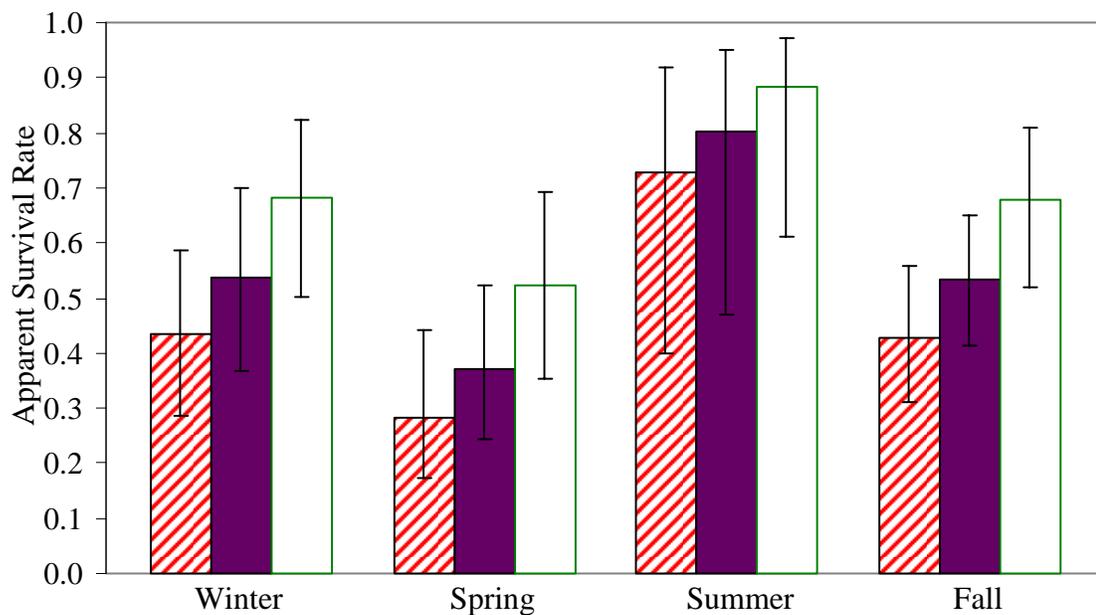


Figure 2.7. Seasonal pattern in apparent survival rate of PIT tagged *O. mykiss* in Murderers Creek. Striped bars are Reach 1, solid bars are Reach 2, and open bars represent Reach 3. Error bars are 95% Confidence Intervals. Winter encompasses survival during the period December 2004-March/April 2005. Spring is survival during the period March/April 2005-June 2005. Summer is survival from June 2005-September 2005. Fall is survival from September 2005-January 2006.

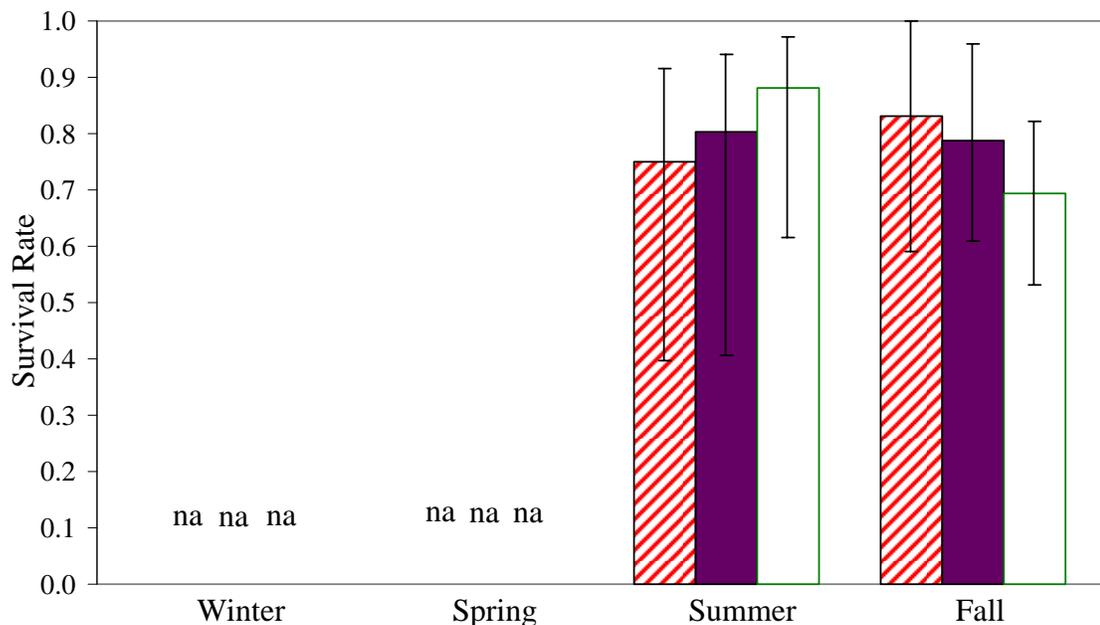


Figure 2.8. Seasonal survival rate of PIT tagged *O. mykiss* in Murderers Creek. Survival rate is apparent survival (Figure 2.7) corrected for emigration. Seasons for which emigration data were unavailable are denoted by “na.” Upstream emigration from Reach 1 was estimated during summer, emigration from other reaches was assumed to be negligible (apparent survival = survival). Emigration from Murderers Creek during fall was estimated for all reaches. Striped bars are Reach 1, solid bars are Reach 2, and open bars represent Reach 3. Error bars are 95% Confidence Intervals.

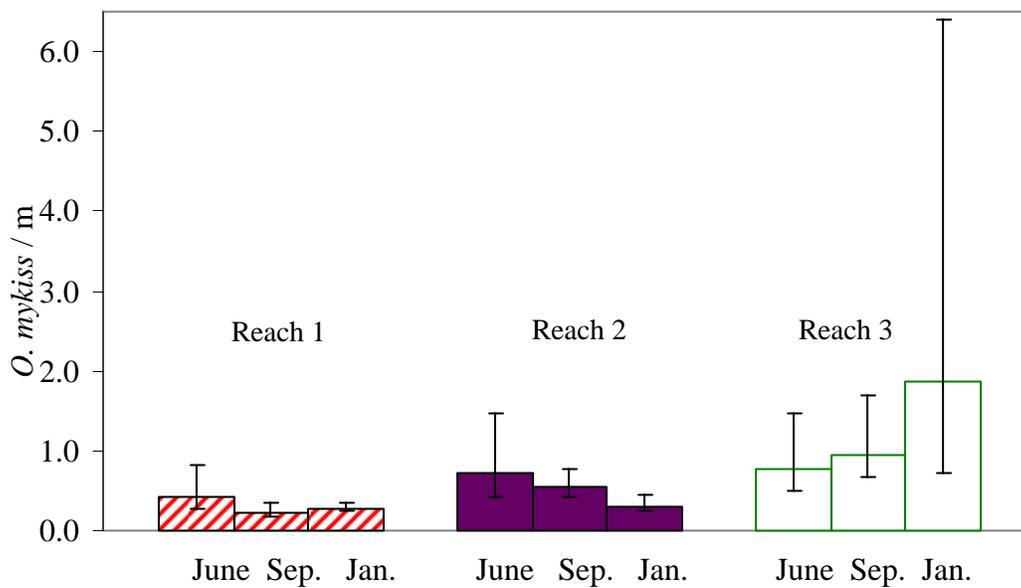


Figure 2.9. Estimated mean density (individuals per lineal stream meter) of *O. mykiss* (> 75mm fork length) at Murderers Creek sentinel sites. Sampling periods were June 2005, September 2005, and January 2006. Striped bars are Reach 1, solid bars are Reach 2, and open bars represent Reach 3. Error bars are 95% Confidence Intervals.

Table 2.1. Stream temperatures by reach and season in Murderers Creek during 2005. Degree days are the sum of the individual daily mean temperatures for each season. The 95% Confidence Interval for mean daily temperature and the maximum temperature observed in each season are also listed.

Season	Reach	Degree Days	Mean Daily	95% C.I.	Maximum Observed
Winter	1	-	-	-	-
Winter	2	182	2.3	1.9 to 2.7	9.6
Winter	3	-	-	-	-
Spring	1	-	-	-	-
Spring	2	861	9.0	8.4 to 9.5	19.8
Spring	3	-	-	-	-
Summer	1	1,382	19.2	18.8 to 19.6	27.2
Summer	2	1,316	17.5	17.2 to 17.9	25.3
Summer	3	1,236	16.3	15.9 to 16.6	24.4
Fall	1	722	7.2	6.3 to 8.2	19.7
Fall	2	663	6.8	5.9 to 7.7	20.6
Fall	3	694	7.1	6.2 to 7.9	18.9

Table 2.2. Model selection results for Cormack-Jolly-Seber apparent survival rate models fit to PIT tag mark-recapture sampling. Models were run in Program MARK. Lower AICc values indicate more plausible models, which better fit the data. Phi represents apparent survival rate, while P is probability of detection. Phi and P were, in some models, allowed to vary by Reach (sentinel site location) or season of sampling (Time). In models where Phi and P were fixed, these parameters are followed by ‘.’.

Model	AICc	Delta AICc	Weights
Phi (Reach + Time), P.	693.3	0.0	0.43
Phi (Time + (Reach 1= Reach 2) ≠ Reach 3), P.	693.8	0.5	0.34
Phi (Reach · Time) P.	695.9	2.5	0.12
Phi (Reach · Time), P (Time)	698.2	4.9	0.04
Phi (Reach · Time), P(Reach)	699.7	6.4	0.02
Phi (Time), P.	700.1	6.8	0.01
Phi.(Reach 1 = Reach 2 but not Reach 3), P.	700.1	6.8	0.01
Phi (Reach), P.	700.6	7.3	0.01
Phi (Reach · Time), P (Reach + Time)	702.1	8.8	0.01
Phi (Reach · Time), P (Reach · Time)	705.8	12.5	0.00
Phi., P.	707.7	14.4	0.00

Table 2.3. Frequency of body scars or cysts observed on *O. mykiss*. Each observation represents a visual inspection of an individual *O. mykiss* in Murderers Creek on either the first capture event, or after being at large for > 30 days. Stream reaches are sequentially numbered from downstream to upstream.

Reach	Total Observations	Body Scars		Cysts	
		Number	Proportion	Number	Proportion
1	1,070	6	0.006	5	0.005
2	1,444	9	0.006	12	0.008
3	972	1	0.001	1	0.001
Total	3,486	16	-	18	-

**CHAPTER 3**

INFLUENCES ON AND EFFECTS OF FALL MOVEMENT BY *ONCORHYNCHUS*  
*MYKISS* IN THE SOUTH FORK JOHN DAY RIVER

### **Abstract**

Life history patterns of *Oncorhynchus mykiss* during fall varied between and within tributaries to the South Fork John Day River. Differences in fall life history had lasting implications for fitness. The proportion of fall emigrants from a lower gradient, anthropogenically influenced stream (Murderers Creek) was significantly greater than the proportion emigrating from a higher gradient, wilderness stream (Black Canyon Creek) for two consecutive years ( $P < 0.001$  for both years). There were no significant differences in proportion of emigrants between years within either stream ( $P > 0.10$  for both streams). At the whole-stream scale in Murderers Creek, the odds of emigration were significantly and positively related to body length in fall and growth rate experienced during summer. This analysis suggested that the “winners” were volitionally emigrating from Murderers Creek during fall. After emigration, *O. mykiss* dispersed primarily downstream into the Mainstem John Day River. Radio-telemetry indicated that, although fall emigrants were distributed across 126 km of the stream network, the majority occupied a  $< 6$  km stream section. Fall emigration amplified phenotypic differences between emigrant and sedentary individuals, as emigrants shifted into an alternative niche where skeletal growth rates during winter were significantly ( $P < 0.001$ ) higher than for individuals remaining in tributaries.

## Introduction

Movement of fish in lotic systems has been extensively studied. Stream fishes, including salmonids, were initially thought to be sedentary, or have restricted movement (Gerking 1953; Edmundson et al. 1968). Improved tagging techniques and tracking of individuals demonstrated, however, that stream salmonids can be highly mobile (Gowan et al. 1994; Kahler et al. 2001; Baxter 2002; Bramblett et al. 2002; Gowan and Fausch 2002). However, movement is not uniform between streams, or even among individuals within the same stream. Rates of movement can vary widely between (Riddell and Leggett 1981) and within (Roni and Quinn 2001; Steingrimmson and Grant 2003) populations. Within a single population, there are typically both “movers” and “stayers” (Leider et al. 1986; Grant and Noakes 1987).

Social dominance and growth rate may influence which individuals become movers and which become stayers. Observational and experimental studies have often concluded that movement is a density dependent response wherein “losers” or smaller, less dominant individuals are forced to emigrate (Chapman 1962; Keeley 2001; Bujold et al. 2004; Imre et al. 2004). Conversely, dominant individuals, the “winners,” may volitionally emigrate in search of higher levels of resources in alternative areas (Armstrong et al. 1997; Roni and Quinn 2001; Gowan and Fausch 2002). In other instances, social hierarchies and intraspecific competition may not appreciably influence which individuals emigrate (Giannico and Healey 1998; Kahler et al. 2001). In these instances, movers and stayers may have similar growth rates and condition factors prior to the migration (Riddell and Leggett 1981).

Anadromous life history complicates the study of salmonid movement, as anadromous salmonids, by definition, will eventually emigrate to the ocean. However, given the eventuality of migration to saltwater, the timing and nature of individual development and also migration may be controlled by growth rate (Thorpe 1987a, 1987b; Thorpe and Metcalfe 1998; Cucherousset et al. 2005), and intraspecific competition (Chapman 1962; Huntingford et al. 1988) similar to resident salmonids. Movement of species displaying “partial migration” (Jonsson and Jonsson 1993) such as Atlantic salmon *Salmo salar* and steelhead *Oncorhynchus mykiss*, may be especially influenced by growth and intraspecific competition. For instance, individuals may only emigrate when their metabolic demands can no longer be supported by their current environment (Cucherousset et al. 2005).

Environmental heterogeneity further complicates comparison of migrant and sedentary individuals, as abiotic characteristics can be more influential than biotic factors. For instance, Riddell and Leggett (1981) observed different proportions of fall emigrants between two streams although growth rate and condition of Atlantic salmon parr were similar between streams. Riddell and Leggett (1981) theorized that higher fall emigration from one stream was an adaptive response to the higher gradient and cooler temperature in that stream. In experimental channels, Bjornn (1971) found fewer juvenile salmonids emigrated when rubble substrate was present, as opposed to smaller gravel substrate.

Movement during fall, typically in the form of emigration from low order tributaries to higher order streams is common among juvenile anadromous salmonid

populations (Bjornn 1971; Riddell and Leggett 1981). Fall emigration can be a precursor to smoltification (McCormick et al. 1998) and dictates where an individual overwinters. Overwintering survival may be a key factor limiting smolt recruitment (Seelbach 1987; Everest et al. 1988; Solazzi et al. 2000) and overwintering location may also influence growth rate. Downstream rearing areas typically have warmer winter stream temperatures, which may increase growth rate (Higgins 1985; Koskela et al. 1997; Morgan and Metcalfe 2001; Murphy et al. 2006), and decrease mortality (Smith and Griffith 1994). Higher growth rates may trigger individuals to smolt at younger ages (Duston and Saunders 1997), and the seasonal timing of smoltification may be advanced by increased temperature accumulation (Zydlewski et al. 2005) in higher order streams.

We studied the individual and population scale ecology of fall emigration in the South Fork John Day River basin. Specifically, we investigated the prevalence and potential correlates of fall emigration in two major tributaries to the South Fork John Day; Black Canyon Creek and Murderers Creek. These creeks support populations of *Oncorhynchus mykiss gairdneri* which have both resident and anadromous adult forms. These populations are further comprised of individuals that, during any given season, may be sedentary or migrant. The relative influence of biotic or environmental characteristics on life history expression in these populations remains unclear, and is an important avenue of research (Leider et al. 1986; Rodriguez 2002). The objectives of this study were to: i) determine if there were differences in the proportion of individuals in these two creeks emigrating during fall; ii) determine the spatial distribution of

emigrants; iii) compare the prior physical characteristics and growth histories of individuals in Murderers Creek with their subsequent fall life history strategy and; iv) evaluate the long-term influence that fall life history has on individuals.

## **Methods**

### *Study Location Description*

The South Fork John Day River basin is a fifth order watershed in Northeast Oregon, bounded on the west by the Ochoco Mountains and on the east by the Aldrich Mountains. A falls at river kilometer 45 restricts anadromous fish downstream and to four principal tributaries (Figure 3.1). This study focused on two of the four tributaries, Black Canyon and Murderers creeks, and the South Fork downstream from the confluence with Murderers Creek (Figure 3.1). Black Canyon Creek is a short, high gradient stream characterized by confined canyons. Murderers Creek, conversely, is longer and lower gradient, with broad alluvial valleys allowing for channel meander.

Environmental stability is greater in Black Canyon than in Murderers Creek. Temperature in Black Canyon ranged from minima of approximately 1 °C to maxima of approximately 20 °C. Ice formation during winter is typically minimal. Conversely, water temperatures in Murderers Creek ranged from summer maxima of 26 °C to winter minima of 0 °C. Ice formation during winter time can be extensive, with surface ice as thick as 15 cm covering pools and glides. Stream flow regimes also differed between these two tributaries, with flows during the study period in Murderers Creek ranging from 0.071 m<sup>3</sup>/s during summer to 14.2 m<sup>3</sup>/s during winter (a 200-fold range). This

contrasted with observed flows in Black Canyon that had a 23-fold range from 0.25 to 5.66 m<sup>3</sup>/s.

The South Fork John Day River is a fifth-order stream downstream from the confluence with Murderers Creek. Stream flows here ranged from summer baseflow of 0.48 m<sup>3</sup>/s<sup>-1</sup> to peak flow estimated at 70.8 m<sup>3</sup>/s<sup>-1</sup> during winter 2005. Stream temperatures were similar to Murderers Creek and ranged from 0 °C to 26 °C. Ice formation occurred in slow flowing sections and in shaded canyon sections.

#### *Prevalence of Fall Migration and subsequent Winter Distribution*

*O. mykiss* were captured using either a snorkeler or a backpack electrofisher (Smith-Root 12-B POW Electrofisher, Vancouver WA<sup>1</sup>) to herd fish from pools into a bag seine (3.7 m wide and 1.2 m deep, with 6.4 mm mesh) located in the thalweg of the pool. Flyfishing with barbless hooks was also used to collect fish from pool habitats. In riffles less than 40 cm deep, we used either pulsed-DC electrofishing and dipnetted fish, or used an electrofisher to herd *O. mykiss* into a bag seine located in the thalweg of the riffle. In the former case, we electrofished upstream, capturing fish as possible, whereas in the latter case we electrofished downstream into the bag seine, with sets of the seine progressing upstream at discrete intervals.

Captured *O. mykiss* were anesthetized (MS-222) and individually marked with a Passive Integrated Transponder (PIT, 12 mm long) tag injected into the peritoneal cavity (e.g., Prentice et al. 1990, PTSC 1999). Fork length (nearest millimeter), location of capture (to the channel unit scale, i.e., pool or riffle specific), and conditional status were recorded on each individual capture occasion. Fish were

allowed sufficient recovery in a dark, well oxygenated container before being returned to the channel unit of capture. Tagging effort was systematically distributed between Black Canyon and Murderers creeks during summer 2004 and summer 2005 (Figure 3.1). During summer 2004 (early July through late September), we PIT tagged 1,203 *O. mykiss* in Black Canyon Creek, and 1,125 in Murderers Creek. During summer 2005 (late June through early September) we PIT tagged 1,449 *O. mykiss* in Black Canyon Creek, and 1,521 in Murderers Creek. Mean fork length of tagged *O. mykiss* in Black Canyon during these two summers was 113 mm (range: 62 to 229 mm). Mean fork length of tagged *O. mykiss* in Murderers Creek during these summers was 124 mm (range: 65 to 248 mm). Emigration and distribution of these marked *O. mykiss* was monitored with four methods: i) recapture of tagged individuals by night snorkel and dip-net; ii) recapture in a rotary screw trap; iii) PIT arrays and; iv) radio-telemetry.

We recaptured tagged individuals to determine distribution in the South Fork John Day River between river kilometer 28 (confluence of Murderers Creek) and river kilometer 10 (screw trap location). This section was subdivided into 5 contiguous stream reaches, based on stream power and valley topography. We randomly selected 5 pools from each reach (Figure 3.2). These 25 pools were night snorkeled during December 2004 and early January 2005. One to two snorkelers worked upstream through the pool with dive lights (C-8 UK Sunlight, with a white LED bulb<sup>1</sup>), searching for *O. mykiss*. Fish were chased into a handheld dip-net positioned vertically, but in contact with the substrate, immediately downstream of the fish. Captured *O. mykiss* were interrogated for PIT tags to identify emigrants.

A 1.52 m diameter rotary screw trap (E.G. Solutions Inc., Corvallis, OR<sup>1</sup>) captured emigrant *O. mykiss* at river kilometer 10 of the South Fork John Day (Figure 3.2). This trap was operated nearly continuously during fall 2004 and fall 2005. During fall 2004 (October 10 through December 31), we operated a weir 78 m upstream of the screw trap (Figure 3.2), which directed migrant fish past a rectangular PIT tag detection antenna (30.5 cm by 80.0 cm inner diameter antenna coupled to a Destron-Fearing<sup>1</sup> 2001F transceiver). During fall 2005 we operated an array consisting of six 3.3 m wide PIT antennas (similar in components to those described by Axel et al. 2005, coupled to a Destron-Fearing<sup>1</sup> 1001M transceiver) 90 m upstream of the screw trap (Figure 3.3).

Differences in type and temporal scope of PIT array operations between years precluded using solely PIT tag detections to compare fall emigration rates between years. During fall 2005 (October 8 through December 15), we estimated capture efficiency of the screw trap through PIT tagging, upstream release, and recapture of *O. mykiss* captured in the trap (Thedinga et al. 1994). We used logistic regression to model trap efficiency and generate daily capture efficiency estimates (described in detail in Appendix B), which were then used to estimate total fall emigration of PIT tagged individuals. During limited periods when the trap was not operated, we used the PIT arrays to detect and quantify tagged emigrants. The proportion of PIT tagged *O. mykiss* from Murderers and Black Canyon creeks migrating past this site during fall was compared within and between years with chi-square tests.

Radio-telemetry was used to determine distribution downstream of river-kilometer 10. We opportunistically radio-tagged individuals captured in the screw trap

that were >160 mm fork length on a weekly basis from early October through December 2004. When possible, we radio-tagged previously PIT tagged *O. mykiss*.

Radio tags were surgically implanted (1.7 and 2.1 gram pulsed radio transmitters with external whip antennas, Advanced Telemetry Systems, Isanti, MN<sup>1</sup>) into the peritoneal cavity of *O. mykiss*. Equipment was sterilized (95% ethanol) and air-dried while fish were being anesthetized (MS-222 buffered 2:1 with sodium bicarbonate). Fish were irrigated throughout surgery with 50% concentration MS-222 solution (also buffered) delivered continually via gravity feed. A canula was inserted through an incision anterior and dorsal to the pelvic girdle and exited the body cavity anterior and dorsal to the anal fin. The canula was used to thread the radio tag wire through the body cavity, after which the canula was posteriorly. The radio tag was then inserted into the body cavity, after which the anterior incision was closed by two 5/0 acrylic sutures. Both wounds were dabbed with Nexaband<sup>1</sup> veterinary glue to aid in tag retention. The radio tag antenna was trimmed to extend less than 4 cm beyond the caudal fork.

Radiotagged fish were ground tracked once or twice per week through mid-January when fish were migrating to wintering areas, then less frequently from mid-January through late March. We used an ATS scanning telemetry receiver (Advanced Telemetry Systems, Isanti MN<sup>1</sup>) and four-element Yagi antenna to locate radio tags from the ground. On two occasions we used fixed wing aircraft, with attached H or monopole antennas coupled to an ATS receiver. On the first flight we searched the South Fork from 1 km upstream of the screw trap (where tagging occurred) downstream

to the confluence with the Mainstem John Day, and the Mainstem John Day from 10 km upstream of the confluence with the South Fork downstream to the confluence of the North Fork, 43 km downstream of the South Fork. On the second flight we repeated this search area, and also searched from the confluence of the North Fork downstream to the confluence of the John Day and Columbia rivers. Signal locations were triangulated, then referenced by landmarks and GPS coordinates.

We tested whether distribution of radio-tagged individuals occurred at random. Since the capacity to detect fish during telemetry flights over the South Fork and Mainstem was excellent (i.e., there was never ground cover sufficient to impede signal propagation, and interference was negligible) we suspect that the observed range in overwintering locations was equal to the actual spatial extent of radio tagged *O. mykiss* in the South Fork and Mainstem. We used a chi-square analysis to determine if distribution occurred randomly (Neu et al. 1974) within this observed range. Relationships between overwintering location and physical variables such as size at tagging and date tagged were evaluated with Pearson correlation.

#### *Individual Based Monitoring of Fall Life History*

We investigated individual correlates of *O. mykiss* fall life history at sentinel sites in Murderers Creek during fall 2005. Previously PIT-tagged *O. mykiss* (age 1+ and older only) were recaptured in September and measured for summer growth. Individuals were recaptured from both pools and riffles. For individuals captured in a riffle, we assumed they were likely to move downstream into pools during the fall-winter transition (Muhlfeld et al. 2001), thus, the nearest downstream pool was used to

represent available habitat. During fall (September 27 to December 27), we used a PIT array consisting of two antennas (each 30.5 cm by 80.0 cm inner diameter) separated by >100 m to detect *O. mykiss* emigrating from Murderers Creek. The array was located 0.9 kilometers upstream of the South Fork John Day (Figure 3.3).

Monitoring movement of recaptured *O. mykiss*, for which size and prior growth rate was known, allowed us to examine the influence of several variables on fall life history. Biotic variables such as growth rate (Cucherousset et al. 2005) and body size (Keeley 2001; Imre et al. 2004) may influence the likelihood of emigration. Thus, we incorporated summer relative growth rate ( $\text{mm}\cdot\text{mm}^{-1}\cdot\text{d}^{-1}$ ), and fork length at the beginning of fall. The availability of winter concealment habitat also influences emigration rates (Bjornn 1971). Overwintering *O. mykiss* are often associated with deep pools and large substrate (Johnson and Kucera 1985; Muhlfeld et al. 2001). We quantified concealment habitat of each pool via maximum depth, and substrate size. Substrate size (sand/silt, gravel, cobble, boulder) and relative composition (dominant or subdominant) were visually estimated. Numeric values were assigned, increasing with particle size (1 for sand/silt to 4 for boulder), then a weighted sum ( $(1.25 \cdot \text{dominant}) + (0.75 \cdot \text{subdominant})$ ) was calculated for each pool. Higher scores hence indicated larger substrate and presumably more attractive overwintering locations.

We tested for correlation among potential explanatory variables. Length and growth rate were not correlated ( $r = 0.06$ ,  $P = 0.67$ ). There was evidence of correlation between pool depth and substrate size ( $r = -0.59$ ,  $P = 0.02$ ). However, this relationship was influenced by one outlier, and when removed, there was no significant correlation

( $r = -0.41$ ,  $P = 0.15$ ). There were no significant correlations between biotic (length, growth rate) and abiotic (depth, substrate size) variables ( $r < 0.25$  in all cases).

The outcome of each PIT tagged individual was scored as either: (1) it passed the array near the mouth of Murderers Creek during fall (emigrant), or (2) it was not detected at this array and hence assumed to remain in Murderers Creek (sedentary). These events can be treated as binary responses, which if individuals are presumed to behave independently, can be modeled as a Bernoulli distribution which is a special case of the binomial distribution. This response was modeled with logistic regression as a function of the aforementioned explanatory variables. We first jointly analyzed all three sentinel sites in Murderers Creek. At this whole-stream scale, significant serial autocorrelation was present among model residuals. Thus we grouped individuals by spatial location (by pool-riffle unit within each sentinel site) and then by length. We increased length groupings until no significant autocorrelation was present among residuals. As a result of this grouping procedure, we used binomial logistic regression for the whole-stream analysis. This process modeled the number of emigrants as a function of the number released in each group, as influenced by group means of each explanatory variable. When analyzing sentinel sites individually (reach scale), we did not find significant serial autocorrelation, thus, we used binary logistic regression for individual sentinel sites.

At the whole-stream scale, we used small-sample corrected Akaike Information Criterion (AICc) selection to select a model which explained the greatest amount of life history variation with the fewest parameters. For individual sentinel sites, we used

drop-in-deviance tests to compare reduced models with a full model which contained all explanatory variables and first order interactions. This process identified explanatory variables which had a significant influence on fall life history.

### *Influence of Fall Life History*

We measured relative growth rates of individuals at sentinel sites in Black Canyon and Murderers creeks during fall 2004 and fall 2005. Date of entry into the South Fork John Day prior to recapture (primarily at the screw trap) was unknown for many individuals. Thus, during fall, we only compared growth rates of sedentary individuals, since with emigrants we were unable to distinguish between growth occurring in tributaries versus growth occurring in the South Fork John Day. Reaches were combined to increase sample size, and we subsequently used two-way analysis of variance (ANOVA) and multiple comparisons (Bonferroni adjustment) to compare growth rates between Black Canyon and Murderers creeks during 2004 and 2005.

We measured winter relative growth rate at three sentinel sites in Murderers Creek, and two sentinel sites in Black Canyon Creek during winter 2004-2005. In the South Fork John Day, we measured growth at two locations that were representative of available habitat conditions, and in proximity to overwintering locations of emigrants from Murderers and Black Canyon creeks. At these two locations, we also conducted repeated sampling of PIT tagged individuals between December 2004 and March 2005 to establish a temporal chronology of skeletal growth. Sites were aggregated at the stream scale to increase sample size. We then used one-way ANOVA and multiple

comparisons (Bonferroni adjustment) to compare growth rates between Black Canyon Creek, Murderers Creek and the South Fork John Day.

Migration of *O. mykiss* through the Columbia River during spring 2005 was monitored via stationary PIT arrays at John Day (Columbia River rkm 347) and Bonneville (Columbia River rkm 234) dams, and a mobile PIT array in the Columbia River estuary (Columbia River rkm 75; Ledgerwood et al. 2004). We compared median date of detection at John Day Dam (4.0 km downstream of the John Day River mouth) between *O. mykiss* tagged in Murderers Creek during summer 2004 which overwintered in Murderers Creek (sedentary life history), and those which emigrated during fall (emigrant life history). Sedentary individuals were either captured in the creek during winter, or detected emigrating (with a PIT array at Murderers Creek rkm 7.4) during spring 2005. Fall emigrants were detected when migrating past the South Fork rotary screw trap during fall 2004. Sample sizes of emigrant ( $n = 8$ ) and sedentary ( $n = 30$ ) groups were unequal, thus, to err on the side of caution we used a Wilcoxon rank-sum test to compare groups. We used Pearson correlation to evaluate the relationship between migration timing at John Day Dam and the Columbia River estuary.

## **Results**

### *Prevalence of Fall Migration and subsequent Winter Distribution*

Fall emigrants from Black Canyon and Murderers creeks were recaptured during winter 2004 throughout the South Fork John Day between their respective tributary junctions and river kilometer 10 (Figure 3.1). Individuals dispersed primarily in a

downstream direction (Figure 3.4). However, upstream dispersal also occurred during fall. *O. mykiss* emigrating from Black Canyon Creek were recaptured in the South Fork John Day upstream of Black Canyon Creek and also in Murderers Creek (Figure 3.4). Prevalence of fall migration was higher among individuals tagged in Murderers Creek than those tagged in Black Canyon Creek (Table 3.1). The proportion of *O. mykiss* emigrating from Murderers Creek in comparison to Black Canyon was significantly different for both fall 2004 ( $\chi^2 = 78.5$ ,  $df = 1$ ,  $P < 0.001$ ), and fall 2005 ( $\chi^2 = 68.9$ ,  $df = 1$ ,  $P < 0.001$ ). The proportion of *O. mykiss* emigrating during fall did not differ significantly between years within either Black Canyon ( $\chi^2 = 0.0$ ,  $df = 1$ ,  $P = 0.97$ ) or Murderers creeks ( $\chi^2 = 2.2$ ,  $df = 1$ ,  $P = 0.14$ ).

Migrants captured at the screw trap primarily overwintered in the Mainstem John Day River (Table 3.2). Of individuals for which we determined overwintering location (10 of the 12 individuals radio-tagged), 80% emigrated from the South Fork and entered the Mainstem John Day River (Figure 3.5). Mean fall migration distance of radio-tagged *O. mykiss* was 26 km (Range: 1.6 to 126.2 km). We found no relationship between fork length at tagging and overwintering location (i.e., distance moved;  $r = 0.29$ ,  $P = 0.40$ ,  $n = 10$ ), nor between date of tagging and overwintering location ( $r = 0.35$ ,  $P = 0.33$ ,  $n = 10$ ). Fifty percent of radio-tagged *O. mykiss* (5 of 10) overwintered in a 6 kilometer section of the total stream network occupied by any of the radio-tagged *O. mykiss*. This section comprised 4.4% of the total area used by radio-tagged *O. mykiss*. Radio-tagged individuals were thus non-randomly distributed throughout the 126 stream kilometers ( $\chi^2 = 13.61$ ,  $df = 1$ ,  $P < 0.001$ ) occupied by *O. mykiss* tagged at

the South Fork screw trap. The principal overwintering area was near the western end of the Upper John Day Valley, upstream of a confined canyon segment of the river. A sixth radio-tagged *O. mykiss* overwintered in a tributary to the Mainstem John Day in this area. Individuals exhibited a typical migration pattern of rapid downstream movement during fall and early winter, followed by primarily holding behavior during winter. Fish continued to explore throughout winter however, as evidenced by localized migrations (in both upstream and downstream directions) occurring sporadically during winter.

#### *Individual Based Monitoring of Fall Life History*

At the whole-stream scale (all three sentinel sites) in Murderers Creek, two models were within a single AICc unit of each other (Table 3.3). However, since the model with the lowest AICc value included an additional first-order interaction term, we selected the second model, as the addition of the second interaction term was not warranted given the negligible decrease in AICc score (Table 3.3). The “Fork Summer Reach Fork·Reach” model (Table 3.3) explained the greatest amount of variation with the fewest explanatory variables. This model indicated that odds of emigration increased with fork length at the end of summer, and also increased with summer growth rate (Table 3.4). Reach was not a significant term in the model, but was included because the Fork·Reach interaction was significant. The significant interaction between fork length and reach (Table 3.4) indicates that the influence of body length on emigration is spatially dependent in Murderers Creek.

Emigration from Reach 3 of Murderers Creek was negligible, and hence we did not detect sufficient PIT tagged emigrants for regression models. Therefore, we only analyzed reaches 1 and 2 at the individual sentinel site scale. Drop-in-deviance tests of regression models at this scale found one significant explanatory variable in each reach. In reach 1 ( $n = 20$  individuals), fork length in early September best explained differences in fall life history. There was evidence ( $P = 0.096$ ) of a positive relationship between the probability of fall emigration and fork length in September. The odds of an individual emigrating during fall were 1.04 (95% Confidence Interval: 0.99 to 1.09) times greater with each unit increase in fork length. Conversely, in reach 2 ( $n = 18$  individuals) summer growth rate best explained the probability of fall emigration. There was a significant relationship between summer growth rate of each individual and odds of emigration ( $P = 0.04$ ). Odds of emigration during fall were 1.37 times greater (95% Confidence Interval: 1.01 to 1.86) with each unit increase in summer growth rate.

#### *Influence of Fall Life History*

Individuals that stayed in Black Canyon or Murderers creeks during fall experienced different growth rates. Growth rates differed significantly between streams ( $F_{1,94} = 32.03$ ,  $P < 0.0001$ ), even though degree days of stream temperature were similar for both streams during that period. Growth rates also differed between years ( $F_{1,94} = 5.91$ ,  $P = 0.02$ ) with evidence of an interaction between stream and year ( $F_{1,94} = 3.57$ ,  $P = 0.06$ ). During the fall of 2004, the mean relative growth rate in Black Canyon was significantly lower ( $t = -2.37$ ,  $df = 94$ ,  $P = 0.02$ ) than the mean relative growth rate in Murderers Creek (Figure 3.6). A similar pattern emerged in fall of 2005; the mean

relative growth rate in Black Canyon was significantly lower ( $t = -6.24$ ,  $df = 94$ ,  $P < 0.0001$ ) than Murderers Creek (Figure 3.6). Growth rate in Murderers Creek was significantly higher ( $t = -2.65$ ,  $df = 94$ ,  $P = 0.01$ ) in fall 2005 than fall 2004 (Figure 3.6). Unlike Murderers Creek, relative growth rate in Black Canyon was not significantly different ( $t = -0.47$ ,  $df = 94$ ,  $P = 0.64$ ) between years (Figure 3.6).

There was a strong temporal pattern to winter growth. Individual *O. mykiss* in recaptured multiple times during winter showed similar patterns in skeletal growth. Little to no growth occurred during December and January (Figure 3.7). Between late January and mid-March, however, all individuals showed substantial positive changes in fork length (Figure 3.7).

Fall life history pattern created spatial differences in winter growth rate. Relative growth rates were significantly different among Murderers Creek, Black Canyon Creek and the South Fork John Day River ( $F_{2,44} = 42.2$ ,  $P < 0.001$ ). Mean relative growth rate in the South Fork John Day River (Figure 3.8) was significantly higher than mean relative growth rate in either Murderers ( $P < 0.0001$ ) or Black Canyon ( $P < 0.0001$ ) creeks. Evidence suggested a difference ( $P = 0.08$ ) in relative growth rate between Black Canyon and Murderers creeks.

Fall life history patterns were associated with differences in migration timing at John Day Dam. *O. mykiss* from Murderers Creek had significantly different dates of detection based on fall life history pattern (Wilcoxon rank sum test,  $z = -2.89$ ,  $df = 37$ ,  $P = 0.01$ ). Fall emigrants from Murderers Creek had a median detection date at John Day Dam of Julian day 122 (May 2, 2005). Conversely, sedentary *O. mykiss* which

overwintered in Murderers Creek had a median detection date at John Day Dam of julian day 130 (May 10, 2005). Detection date in the Columbia River estuary (of PIT tagged *O. mykiss* originating from the South Fork John Day River) was correlated (Figure 3.9) with detection date at John Day Dam ( $r = 0.92$ ,  $P < 0.001$ ,  $n = 13$ ). Thus, life history based differences in migration timing at John Day Dam were likely maintained through entry into the Columbia River estuary.

## Discussion

### *Prevalence of Fall Migration and subsequent Winter Distribution*

The proportion of individuals emigrating during fall varied between streams, but not between years. Interestingly, little inter-annual variation occurred despite more extensive ice formation in 2005 (both anchor and surface ice) than 2004 (none observed). This finding is at variance with those of Cucherousset et al. (2005), who found life history traits to vary between cohorts, but not between streams. Furthermore, the observed relationship between stream gradient and emigration was opposite that reported by Riddell and Leggett (1981). We observed fewer emigrants from high gradient areas (all reaches of Black Canyon Creek, and reach 3 of Murderers Creek) and more emigrants from low gradient areas (reaches 1 and 2 of Murderers Creek). However, in contrast to the streams studied by Riddell and Leggett (1981), higher gradient areas (especially Black Canyon) also had less severe winter temperatures (lower occurrence of 0°C temperatures) than low gradient areas (e.g., reaches 1 and 2 of Murderers Creek). Temperature and stream gradient co-varied, and may interactively influence emigration.

Higher fall emigration rates from Murderers Creek may be due to several factors. Higher sedimentation rates in Murderers than Black Canyon may reduce interstitial space (Cunjak 1996) and hence concealment habitat. Lack of concealment cover may create higher rates of fall emigration (Bjornn 1971). As sediment deposition is influenced by stream gradient, sedimentation rates may be highest in the low gradient reaches 1 and 2 of Murderers Creek. The highest proportions of emigration also occurred in these reaches. Alternatively, individual growth rate is higher in Murderers Creek than Black Canyon Creek during all seasons (Tattam, unpublished data). This may cause some individuals to reach length or growth rate thresholds which put them on a higher growth rate trajectory (Metcalf et al. 1988; Metcalfe 1998), and they are hence more liable to emigrate (Cucherousset et al. 2005) from small tributaries. Both biotic and abiotic factors likely interact in a complicated fashion (see schematic in Figure 3.10) to create different emigration rates between Murderers and Black Canyon creeks. Influence of these factors is difficult to discern in observational studies, however, we were able to identify key biotic factors related to emigration of Murderers Creek *O. mykiss* (see discussion of “Individual Based Monitoring of Fall Life History”).

*O. mykiss* mainly migrated into higher order streams during winter. This is consistent with other movement studies in the interior Columbia basin (Bjornn 1971; Reischauer et al. 2003). In contrast, *O. mykiss* in coastal systems typically migrate into lower order tributaries to overwinter (Everest 1973; Bramblett et al. 2002). However, we observed one individual which displayed both of these patterns by emigrating from the South Fork into the Mainstem John Day, then shortly thereafter immigrating into a

low-order tributary (Cottonwood Creek) to the Mainstem. Temperature based selection may influence emigration into higher order (typically warmer) streams in the interior Columbia Basin where ice formation in small tributaries is more prevalent than in coastal systems.

Emigrants radio-tagged at the South Fork John Day screw trap typically migrated only a short distance upon entry into the Mainstem John Day. This migration pattern is similar to that observed by Bjornn (1971), with many individuals migrating into higher order streams, but not making long migrations once in the larger stream. As in other telemetry studies of salmonid movement (Schrank and Rahel 2004), we found the frequency of shorter distance migrations to be much greater than the frequency of ones of longer distance (Figure 3.5). Although we surveyed the entire Mainstem John Day downstream of the confluence with the South Fork, the majority of individuals migrated less than 20 km. This suggests that fish are moving downstream until suitable overwintering habitat is located (ranging behavior, as described by Schrank and Rahel 2004). Emigrants may be seeking areas of groundwater input, or upwelling. The dominant overwintering location (Figure 3.5) is at the downstream end of a long alluvial valley, immediately upstream of a confined canyon segment. This pattern of topography typically creates an upwelling zone where groundwater is forced to rejoin surface water. Thus, warmer water in this area may limit the distance that emigrants need to migrate.

### *Individual Based Monitoring of Fall Life History*

Fall emigration compounded pre-existing phenotypic differences between emigrant and sedentary *O. mykiss*. Fall emigrants from Murderers Creek were larger than and/or growing faster than, sedentary individuals at the time of emigration. Emigration shifted these individuals into an alternative realized niche where winter growth was higher. The size and growth rate differential was hence increased by emigration. This is consistent with a growing body of literature suggesting that “movers” or migrant fish experience better growth than “stayers” (Kahler et al. 2001). However, our conclusion that “winners,” defined as the larger and faster growing individuals, are volitionally emigrating seemingly contradicts typical experimental results (Chapman 1962; Keeley 2001; Bujold et al. 2004; Imre et al. 2004). When viewed in the context of a species which exhibits partial migration, however, it is logical for the larger and faster growing individuals to emigrate in preparation for either smolting or shifting into a potamodromous life history. This niche shift opens new trophic opportunities, while relaxing density dependence for the remaining smaller and slower growing portion of the population. In Atlantic salmon populations an “upper modal” or faster growing group is triggered to begin smolting in late summer or early fall, whereas the “lower modal” or slower growing group may not be triggered to smolt until the following spring (Huntingford et al. 1988; Whitesel 1993; Jonsson et al. 1998). *O. mykiss* in the South Fork John Day basin appear to express this pattern, in part, through different fall migration patterns.

Growth in early fall may influence emigration. We observed significantly higher growth rates among sedentary individuals in Murderers Creek during fall 2005 as compared to fall 2004 (Figure 3.6). We sampled an average of 9 days earlier in 2005 (September 7) than in 2004 (September 16). Thus, a substantial amount of growth may occur in the first 1-2 weeks of September, perhaps initiating some individuals to emigrate from the creek shortly thereafter. On the other hand, we also sampled Black Canyon 9 days earlier in 2005 (September 9) than 2004 (September 18), yet found no difference in fall growth rate between years (Figure 3.6). The apparent lack of growth during early September may contribute to low emigration rates from Black Canyon. If individual growth rate remains low during summer and early fall, then individuals may not become limited by the growth opportunities in the creek (Cucherousset et al. 2005). Metcalfe et al. (1988) found upper modal Atlantic salmon parr (which are more likely to become emigrants) increased feeding intensity and hence growth rate during fall. Growth opportunity influences life history (Metcalfe 1998), hence limited growth opportunities in Black Canyon may restrict the number of individuals emigrating in fall. Conversely, Bacon et al. (2005) found no difference in fall growth between future emigrants and future residents, however. Thus, the influence of fall growth on emigration is still open to question.

#### *Influence of Fall Life History*

Fall emigration does not appear to be an evolutionarily stable strategy, but rather a facultative tactic in response to the constraints of the rearing environment. Although fall migrants grow faster, and presumably reach a larger size at smoltification, this life

history has not taken over the population as a whole. Mortality due to predation may be greater for migratory individuals in larger streams than for sedentary individuals in small streams. Moreover, fall emigration created different smolt migration timing that may differentially influence survival of each group among years. Although higher growth rates during winter and hence larger size at smoltification may confer higher marine survival (Bilton et al. 1982; Ward et al. 1989; Tipping 1997), peak marine survival rates occur at different ocean entry times in different years (Lundqvist et al. 1994). Thus, inter-annual variation may create asynchronous productivity among different life history types (Hilborn et al. 2003), and prevent any single life history from establishing population level dominance.

### **Acknowledgements**

We thank S. White, F. Madrinan, J. Feldhaus, S. Heppell, P. Bayley, J. Davis, B. Kingsley, V. Mueller, J. Togstad, J. Silbernagel, D. Myers, N. Weber, B. Tattam, and T. Tattam for assistance collecting data in Murderers and Black Canyon creeks. We thank J. Schricker, T. Goby, T. Schultz, R. Lamb, D. Bondurant, T. Hartill, and L. Hewlett for their diligent work operating the South Fork screw trap. We thank C. Moore of ODFW for providing us a field camp at the Murderers Creek Ranch. The involvement of I.A.T., H.W.L., and G.R.G. was funded by the U.S. Bureau of Reclamation, Pacific Northwest Region through M. Newsom. The work of J.R.R. and W.H.W. was supported by the Bonneville Power Administration (Project Number 199801600) through J. Baugher. C. Jordan of the NOAA Northwest Fisheries Science Center provided PIT tags. P. Bayley provided extensive comments which greatly improved the manuscript.

## References

- Armstrong, J. D., V. A. Braithwaite, and F. A. Huntingford. 1997. Spatial strategies of wild Atlantic salmon parr: exploration and settlement in unfamiliar areas. *Journal of Animal Ecology* 66:203-211.
- Bacon, P. J., W. S. C. Gurney, W. Jones, I. S. McLaren, and A. F. Youngson. 2005. Seasonal growth patterns of wild juvenile fish: partitioning variation among explanatory variables, based on individual growth trajectories of Atlantic salmon (*Salmo salar*) parr. *Journal of Animal Ecology* 74:1-11.
- Baxter, C. V. 2002. Fish movement and assemblage dynamics in a Pacific Northwest riverscape. Doctoral dissertation, Oregon State University, Corvallis.
- Bilton, H. T., D. F. Alderdice, and J. T. Schnute. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. *Canadian Journal of Fisheries and Aquatic Sciences* 39:426-447.
- Bjornn, T. C. 1971. Trout and salmon movement in two Idaho streams as related to temperature, food, stream flow, cover and population density. *Transactions of the American Fisheries Society* 100:423-438.
- Bramblett, R. G., M. D. Bryant, B. E. Wright, and R. G. White. 2002. Seasonal use of small tributary and main-stem habitats by juvenile steelhead, coho salmon, and dolly varden in a Southeastern Alaska drainage basin. *Transactions of the American Fisheries Society* 131:498-506.
- Bujold, V., R. A. Cunjak, J. P. Dietrich, and D. A. Courtemanche. 2004. Drifters versus residents: assessing size and age differences in Atlantic salmon (*Salmo salar*) fry. *Canadian Journal of Fisheries and Aquatic Sciences* 61:273-282.
- Chapman, D. W. 1962. Aggressive behavior in juvenile coho salmon as a cause of emigration. *Journal of the Fisheries Research Board of Canada* 19:1047-1080.
- Cucherousset, J., D. Ombredane, K. Charles, F. Marchand, and J. Bagliniere. 2005. A continuum of life history tactics in a brown trout (*Salmo trutta*) population. *Canadian Journal of Fisheries and Aquatic Sciences* 62:1600-1610.
- Duston, J., and R. L. Saunders. 1997. Life histories of Atlantic salmon altered by winter temperature and summer rearing in fresh- or sea-water. *Environmental Biology of Fishes* 50:149-166.

- Edmundson, E., F. E. Everest, and D. W. Chapman. 1968. Permanence of station in juvenile Chinook salmon and steelhead trout. *Journal of the Fisheries Research Board of Canada* 25:1453-1464.
- Everest, F. H. 1973. Ecology and management of summer steelhead in the Rogue River. Fishery Research Report Number 7. Oregon State Game Commission, Corvallis.
- Everest, F. H., G. H. Reeves, J. R. Sedell, D. B. Hohler, and T. C. Cain. 1988. Changes in habitat and populations of steelhead trout, coho salmon, and Chinook salmon in Fish Creek, Oregon, 1983-1987, as related to habitat improvement. Final Report to the Bonneville Power Administration, Project Number 84-11, Portland, Oregon.
- Gerking, S. D. 1953. Evidence for the concepts of home range and territory in stream fishes. *Ecology* 34:347-365.
- Giannico, G. R., and M. C. Healey. 1998. Effects of flow and food on winter movements of juvenile coho salmon. *Transactions of the American Fisheries Society* 127:645-651.
- Gowan, C., M. K. Young, K. D. Fausch, and S. C. Riley. 1994. Restricted movement in resident stream salmonids: A paradigm lost? *Canadian Journal of Fisheries and Aquatic Sciences* 51:2626-2637.
- Gowan, C., and K. D. Fausch. 2002. Why do foraging stream salmonids move during summer? *Environmental Biology of Fishes* 64:139-153.
- Grant, J. W. A., and D. L. G. Noakes. 1987. Movers and stayers: Foraging tactics of young-of-the-year brook charr, *Salvelinus fontinalis*. *Journal of Animal Ecology* 56:1001-1013.
- Higgins, P. J. 1985. Metabolic differences between Atlantic salmon (*Salmo salar*) parr and smolts. *Aquaculture* 45:33-53.
- Hilborn, R., T. P. Quinn, D. E. Schindler, and D. E. Rogers. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Science* 100:6564-6568.
- Huntingford, F. A., N. B. Metcalfe, and J. E. Thorpe. 1988. Choice of feeding station in Atlantic salmon, *Salmo salar*, parr: effects of predation risk, season and life history strategy. *Journal of Fish Biology* 33:917-924.
- Imre, I., J. W. A. Grant, and E. R. Keeley. 2004. The effect of food abundance on

- territory size and population density of juvenile steelhead trout (*Oncorhynchus mykiss*). *Oecologia* 138:371-378.
- Johnson, J. H., and P. A. Kucera. 1985. Summer-autumn habitat utilization of subyearling steelhead trout in tributaries of the Clearwater River, Idaho. *Canadian Journal of Zoology* 63:2283-2290.
- Jonsson, B., and N. Jonsson. 1993. Partial migration: niche shift versus sexual maturation in fishes. *Reviews in Fish Biology and Fisheries* (3):348-365.
- Jonsson, N., B. Jonsson, and L. P. Hansen. 1998. Long-term study of the ecology of wild Atlantic salmon smolts in a small Norwegian river. *Journal of Fish Biology* 52:638-650.
- Kahler, T. H., P. Roni, and T. P. Quinn. 2001. Summer movement and growth of juvenile anadromous salmonids in small western Washington streams. *Canadian Journal of Fisheries and Aquatic Sciences* 58:1947-1956.
- Keeley, E. R. 2001. Demographic responses to food and space competition by juvenile steelhead trout. *Ecology* 82:1247-1259.
- Koskela, J., J. Pirhonen, and M. Jobling. 1997. Growth and feeding responses of a hatchery population of brown trout (*Salmo trutta*) at low temperatures. *Ecology of Freshwater Fish* 6:116-121.
- Ledgerwood, R. D., B. A. Ryan, E. M. Dawley, E. P. Nunnallee and J. W. Ferguson. 2004. A surface trawl to detect migrating juvenile salmonids tagged with Passive Integrated Transponder tags. *North American Journal of Fisheries Management* 24:440-451.
- Leider, S. A., M. W. Chilcote, and J. J. Loch. 1986. Movement and survival of presmolt steelhead in a tributary and the main stem of a Washington river. *North American Journal of Fisheries Management* 6:526-531.
- Lundqvist, H., S. McKinnell, H. Fangstam, and I. Berglund. 1994. The effect of time, size and sex on recapture rates and yield after river releases of *Salmo salar* smolts. *Aquaculture* 121:245-257.
- McCormick, S. D., L. P. Hansen, T. P. Quinn, and R. L. Saunders. 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 55(Suppl. 1):77-92.
- Metcalf, N. B., F. A. Huntingford, and J. E. Thorpe. 1988. Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon

*Salmo salar*. Journal of Animal Ecology 57:463-474.

- Metcalf, N. B. 1998. The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 55(Suppl. 1):93-103.
- Morgan, I. J., and N. B. Metcalfe. 2001. The influence of energetic requirements on the preferred temperature of overwintering juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 58:762-768.
- Muhlfeld, C. C., D. H. Bennett, and B. Marotz. 2001. Fall and winter habitat use and movement by Columbia River redband trout in a small stream in Montana. North American Journal of Fisheries Management 21:170-177.
- Murphy, M. H., M. J. Connerton, and D. J. Stewart. 2006. Evaluation of winter severity on young-of-the-year Atlantic salmon. Transactions of the American Fisheries Society 135:420-430.
- Neu, C. W., C. R. Byers, and J. M. Peek. 1974. A technique for analysis of utilization-availability data. Journal of Wildlife Management 38:541-545.
- Paulsen, C. M., and T. R. Fisher. 2001. Statistical relationship between parr-to-smolt survival of Snake River Spring-summer Chinook salmon and indices of land use. Transactions of the American Fisheries Society 130:347-358.
- Prentice, E. F., T. A. Flagg, C. S. McCutcheon, D. F. Brastow, and D. C. Cross. 1990. Equipment, methods, and an automated data entry station for PIT tagging. Pages 335-340 in N. C. Parker, A. E. Giorgi, R. C. Hedinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. Fish-marking techniques. American Fisheries Society, Symposium 7, Bethesda, Maryland.
- PTSC (Pit Tag Steering Committee). 1999. PIT Tag Marking Procedures Manual. Pacific States Marine Fisheries Commission, Gladstone, Oregon.
- Reischauer, A. G., F. R. Monzyk, E. S. Van Dyke, B. C. Jonasson, and R. W. Carmichael. 2003. Investigations into the early life history of naturally produced spring chinook salmon and summer steelhead in the Grande Ronde River basin. 2001 annual report to the Bonneville Power Administration, Project Number 199202604, Portland, Oregon.
- Riddell, B. E., and W. C. Leggett. 1981. Evidence of an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 38:308-320.

- Rodriguez, M. A. 2002. Restricted movement in stream fish: the paradigm is incomplete, not lost. *Ecology* 83(1):1-13.
- Roni, P., and T. P. Quinn. 2001. Effects of wood placement on movements of trout and juvenile coho salmon in natural and artificial stream channels. *Transactions the American Fisheries Society* 130:675-684.
- Schrank, A. J., and F. J. Rahel. 2004. Movement patterns in inland cutthroat trout (*Oncorhynchus clarki utah*): management and conservation implications. *Canadian Journal of Fisheries and Aquatic Sciences* 61:1528-1537.
- Seelbach, P. W. 1987. Effect of winter severity on steelhead smolt yield in Michigan: An example of the importance of environmental factors in determining smolt yield. *American Fisheries Society Symposium* 1:441-450.
- Solazzi, M. F., T. E. Nickelson, S. L. Johnson, and J. D. Rodgers. 2000. Effects of increasing winter rearing habitat on abundance of salmonids in two coastal Oregon streams. *Canadian Journal of Fisheries and Aquatic Sciences* 57:906-914.
- Smith, R. W., and J. S. Griffith. 1994. Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. *Transactions of the American Fisheries Society* 123:747-756.
- Steingrimsson, S. O., and J. W. A. Grant. 2003. Patterns and correlates of movement and site fidelity in individually tagged young-of-the-year Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 60:193-202.
- Thedinga, J. F., M. L. Murphy, S. W. Johnson, J. M. Lorenz, and K. V. Koski. 1994. Determination of salmonid smolt yield with rotary-screw traps in the Situk River, Alaska, to predict effects of glacial flooding. *North American Journal of Fisheries Management* 14:837-851.
- Thorpe, J. E. 1987a. Environmental regulation of growth patterns in juvenile Atlantic salmon. Pages 463-474 *in*: R. C. Summerfeldt and G. E. Hall, editors. *Age and Growth of Fish*. Iowa State University Press, Ames, Iowa.
- Thorpe, J. E. 1987b. Smolting versus residency: Developmental conflict in salmonids. *American Fisheries Society Symposium* 1:244-252.
- Thorpe, J. E., and N. B. Metcalfe. 1998. Is smolting a positive or a negative developmental decision? *Aquaculture* 168:95-103.

- Tipping, J. M. 1997. Effect of smolt length at release on adult returns of hatchery-reared winter steelhead. *The Progressive Fish Culturist* 59:310-311.
- Ward, B. R., P. A. Slaney, A. R. Facchin, and R. W. Land. 1989. Size-biased survival in steelhead trout (*Oncorhynchus mykiss*): Back-calculated lengths from adult's scales compared to migrating smolts at the Keogh River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1853-1858.
- Whitesel, T. A. 1993. Comparison of juvenile Atlantic salmon (*Salmo salar*) reared in a hatchery and introduced into a stream: A two-size-threshold model of smoltification. Pages 239-247 in: R. J. Gibson and R. E. Cutting, editors. *Production of juvenile Atlantic salmon, Salmo salar, in natural waters*. Canadian Special Publication of Fisheries and Aquatic Sciences 118.
- Zydlewski, G. B., A. Haro, and S. D. McCormick. 2005. Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences* 62:68-78.

### **Endnotes**

<sup>1</sup>Reference to trade names does not imply endorsement by the United States Geological Survey, Oregon Cooperative Fishery Research Unit, Oregon State University, or the Oregon Department of Fish and Wildlife.

## Figures and Tables

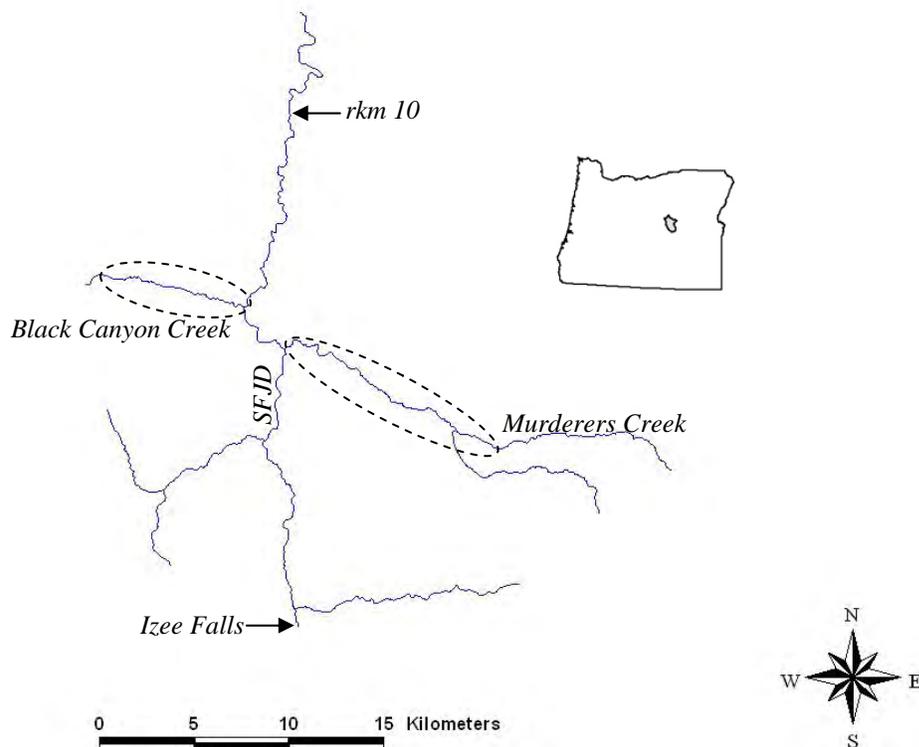


Figure 3.1. Location of the South Fork John Day River basin, including Black Canyon and Murderers creeks. The South Fork John Day River (SFJD) flows from South to North, and is a fifth-order stream downstream (North) of the confluence with Murderers Creek. Inset shows the position of the South Fork basin within Oregon. Dashed circles denote areas where *O. mykiss* were captured and PIT tagged during summer 2004 and summer 2005. A rotary screw trap and PIT arrays were located at rkm 10 during fall 2004 and fall 2005.

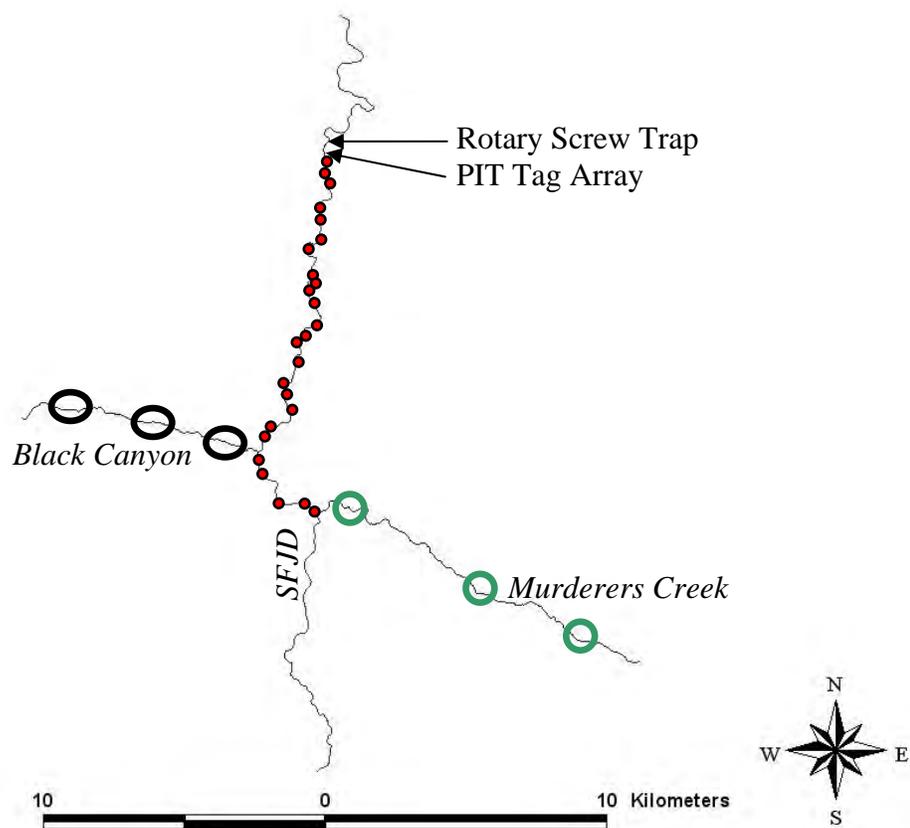


Figure 3.2. Locations of recapture and redetection of PIT tagged *O. mykiss* during fall-winter 2004. Open circles in Black Canyon and Murderers creeks denote sentinel sites composed of 5 pool-riffle units. Closed circles represent single pools in the South Fork John Day River (SFJD) that were randomly selected and sampled.

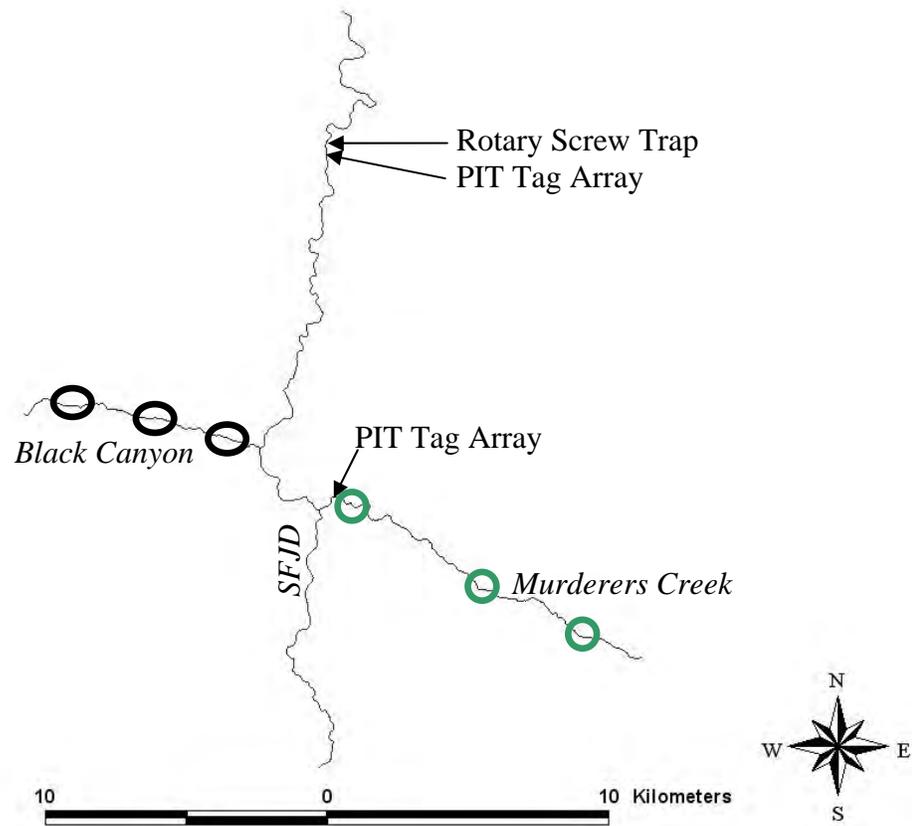


Figure 3.3. Location of recapture and redetection of PIT tagged *O. mykiss* in the South Fork John Day River (SFJD) and tributaries during Fall-Winter 2005. Open circles in Black Canyon and Murderers creeks denote sentinel sites composed of 5 pool-riffle units.

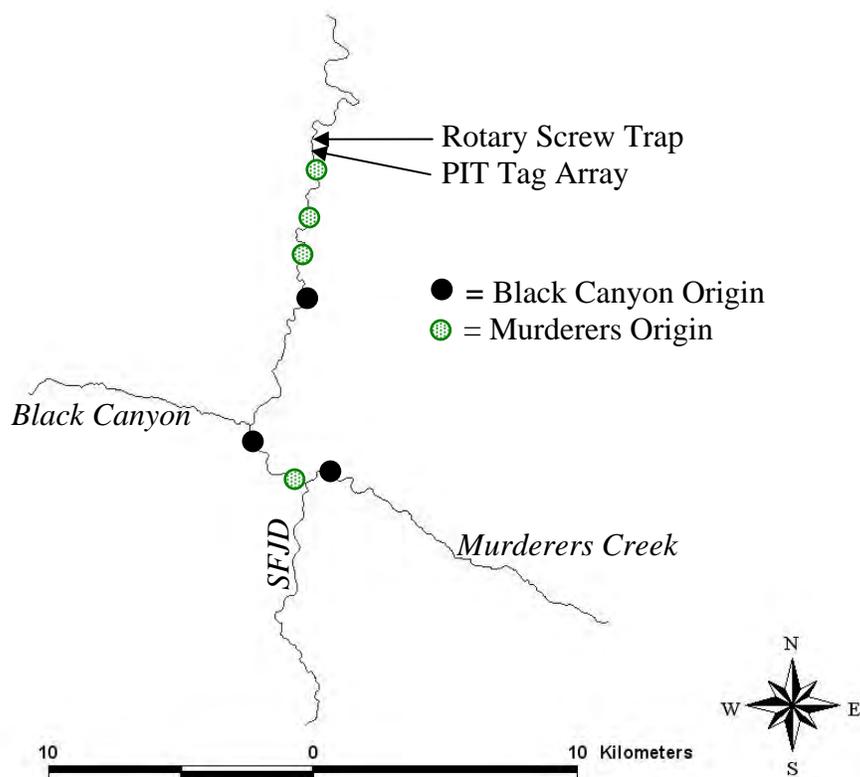


Figure 3.4. Recapture location of PIT tagged *O. mykiss* in the South Fork John Day river (SFJD). These individuals emigrated from the stream in which they were tagged during summer 2004. Recaptures were made via night dipnetting during December 2004 or January 2005.

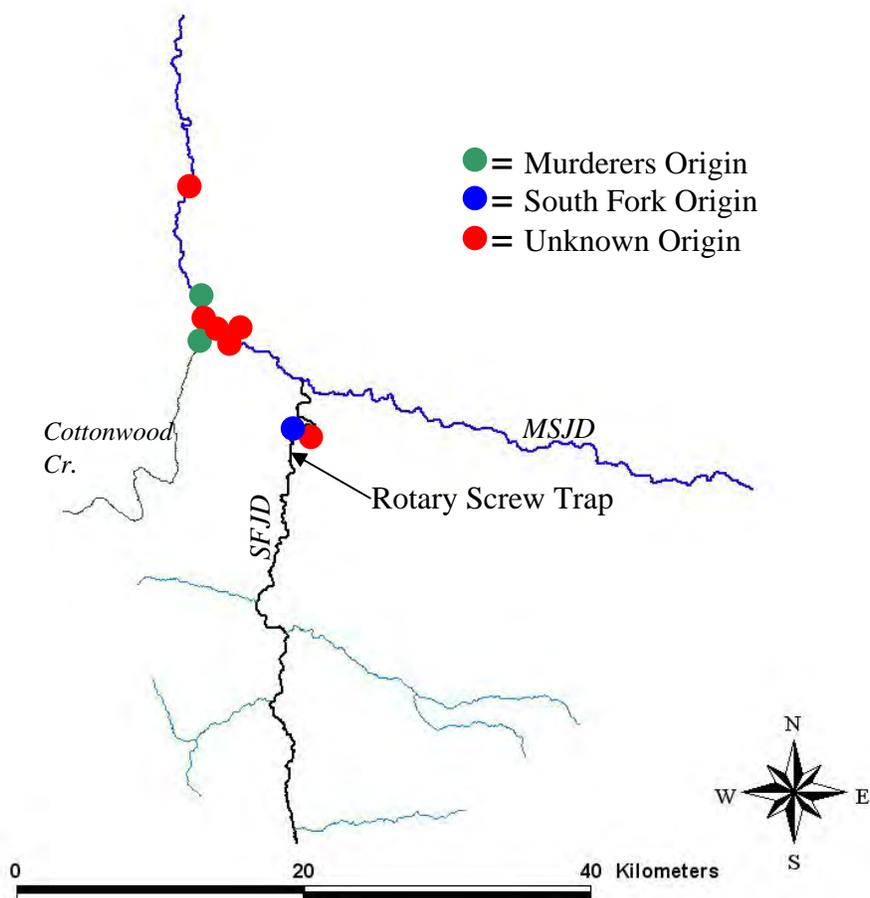


Figure 3.5. Winter distribution of radio-tagged *O. mykiss* downstream from the South Fork John Day River screw trap. Each dot represents the overwintering location of an individual that was relocated multiple times between fall 2004 and spring 2005. Aerial telemetry surveys provided the capacity to detect radio-tagged *O. mykiss* in the South Fork (SFJD) and Mainstem John Day (MSJD) downstream of the screw trap location, and further downstream than the extent of this map.

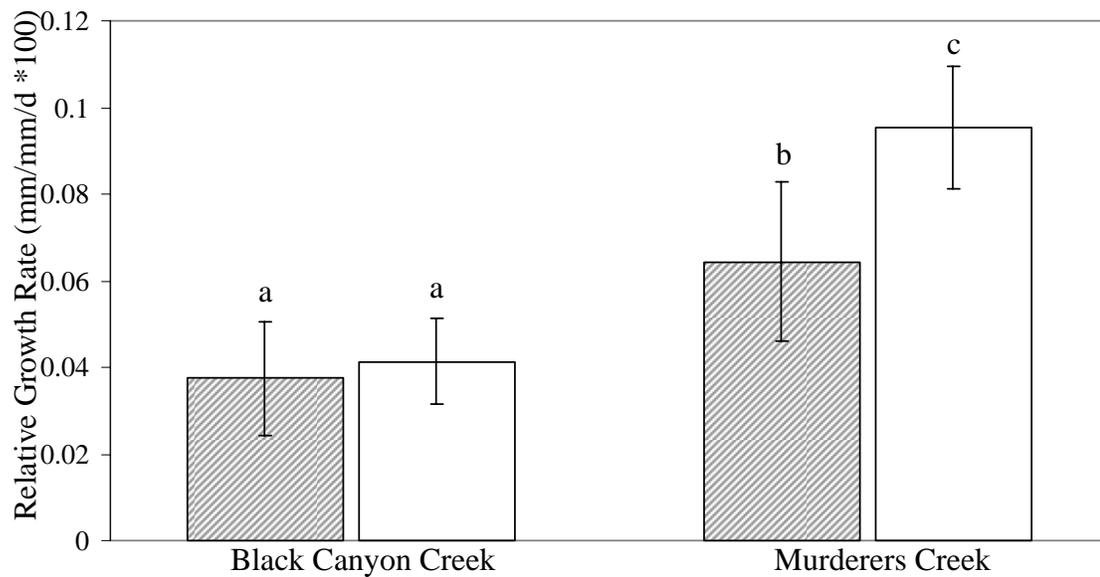


Figure 3.6. Comparison of individual growth rates experienced during fall by sedentary *O. mykiss* in Black Canyon and Murderers creeks. Shaded bars are fall 2004, and open bars are fall 2005. Error bars are 95% Confidence Intervals. Common letters indicate groups which were not significantly ( $P > 0.05$ ) different.

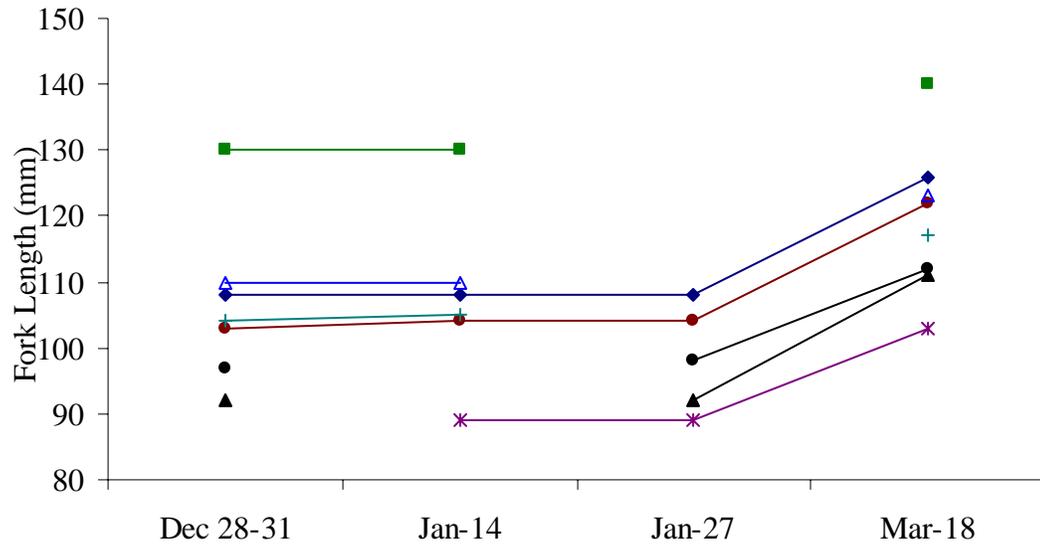


Figure 3.7. Chronology of skeletal growth of individually marked *O. mykiss* in the South Fork John Day River. Fish were repeatedly captured from December 2004 through March 2005 by night snorkel and dipnet. Marking and recapture occurred at two sites (rkm 12-16) in proximity to the rotary screw trap (rkm 10).

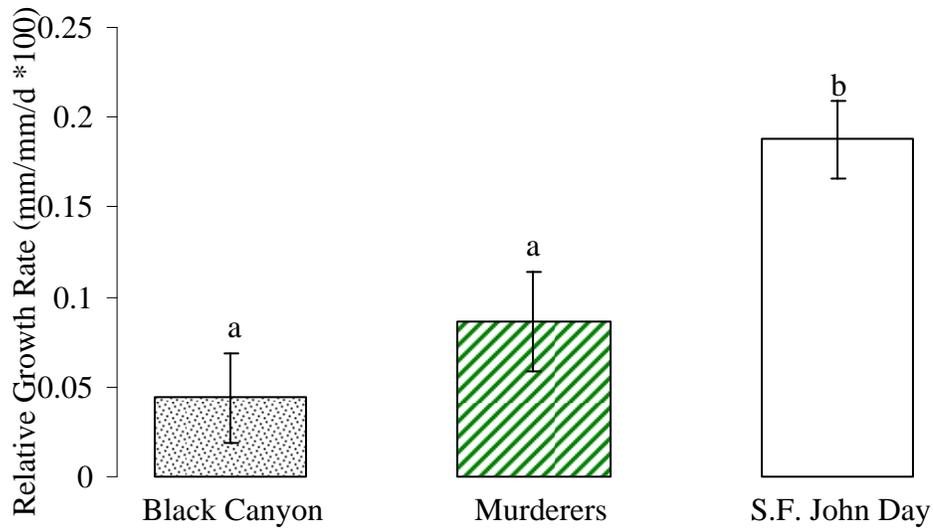


Figure 3.8. Comparison of average individual growth rates during winter 2005. *O. mykiss* were individually marked in December 2004, and recaptured in late-March 2005. Sample sites were Black Canyon Creek (shaded bar), Murderers Creek (striped bar), and the South Fork John Day River (open bar). Common letters indicate groups which are not significantly ( $P > 0.05$ ) different. Error bars are 95% Confidence Intervals.

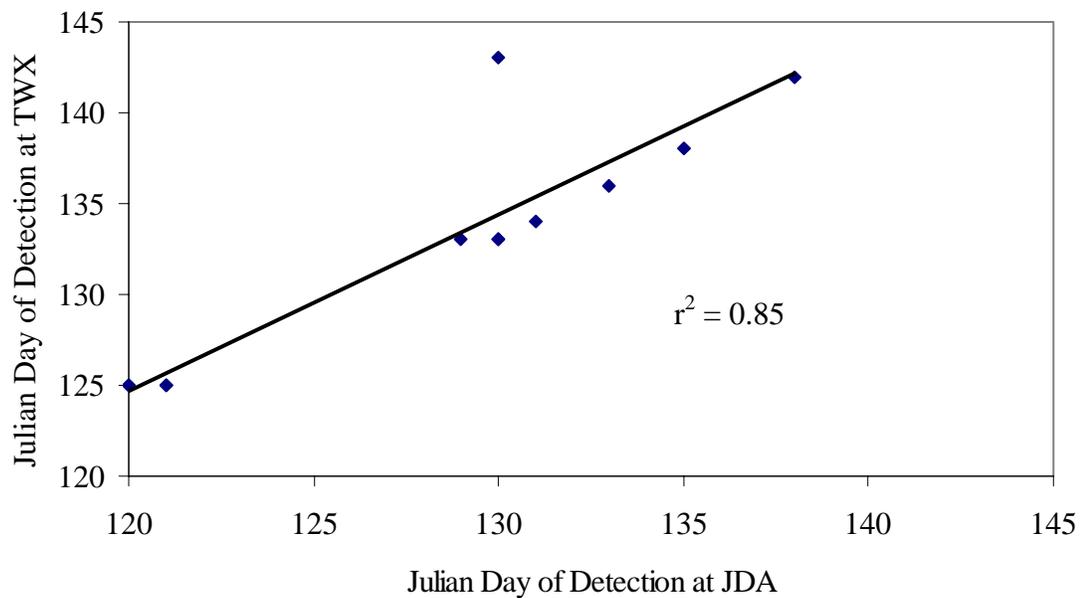


Figure 3.9. Correlation between julian day of detection at John Day Dam (JDA) and julian day of detection in the Columbia River estuary (TWX). Thirteen PIT tagged *O. mykiss* originating from the South Fork John Day River were used in this regression.

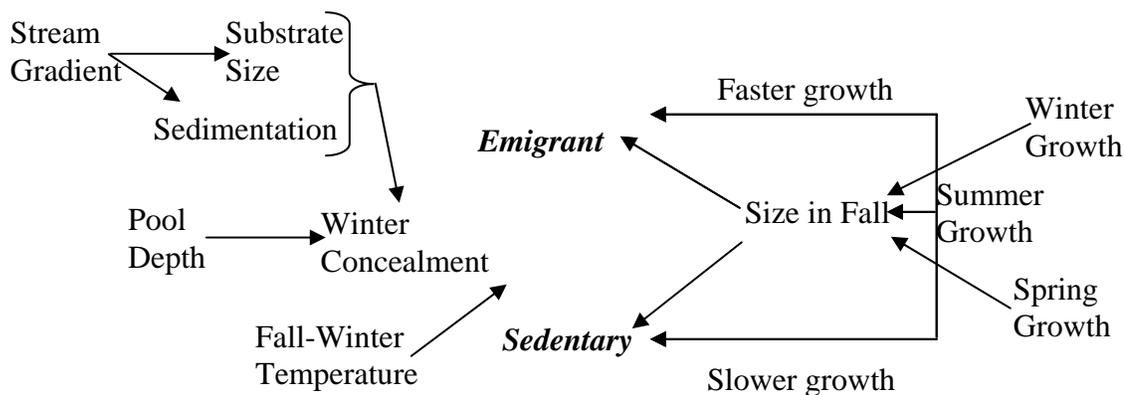


Figure 3.10. Influence diagram of factors affecting the fall life history of an individual *O. mykiss*. Growth rate during the previous winter, spring, and summer can interactively or independently influence body size in fall. However, high growth rate during summer also may trigger smaller individuals to emigrate, thus bypassing the influence of fall size. Stream gradient controls substrate size, and also influences sedimentation rates, both of which interact to determine (in concert with depth) the quality of winter concealment habitat. High quality winter concealment habitat, coupled with warm fall-winter temperatures may create a more sedentary population. Conversely, low quality winter concealment habitat may create a fall emigrant dominated population, provided that growth rate and body size are above the threshold needed for emigration.

Table 3.1. Estimated fall emigration of PIT tagged *O. mykiss* from Black Canyon and Murderers creeks. This estimate includes all *O. mykiss* captured in either tributary during summer 2004 or summer 2005 which emigrated past the South Fork John Day rotary screw trap (river kilometer 10). The time period of fall migration was defined as October 1-December 31.

Location	Year	n Tagged	$\hat{n}$ Emigrants	% Emigrants	95% C.I.
Murderers	2004	1,125	154	13.7%	9.6% to 19.6%
Black Canyon	2004	1,203	37	3.1%	2.2% to 4.2%
Murderers	2005	1,521	176	11.6%	8.5% to 16.5%
Black Canyon	2005	1,449	46	3.2%	2.5% to 3.8%

Table 3.2. Radio-telemetry monitoring of fall migrating *O. mykiss* in the John Day River basin. All fish were captured in the South Fork John Day River screw trap (river kilometer 10). The stream of origin, and stream kilometer (in parentheses), are given when known from PIT tag identification. Telemetry surveys began October 9, 2004 and continued through March 20, 2005.

Date Tagged	Length (mm)	Origin	Number of Relocations	Wintering Location
Oct-9	214	Unknown	22	Mainstem John Day River
Oct-10	201	Unknown	20	Mainstem John Day River
Oct-23	219	Unknown	8	South Fork John Day River
Oct-31	193	Unknown	18	Mainstem John Day River
Nov-6	228	Unknown	11	Mainstem John Day River
Nov-7	198	Unknown	16	Mainstem John Day River
Nov-20	197	Unknown	13	Tagging Mortality
Dec-11	193	South Fork (38)	3	Unknown
Dec-16	177	Murderers (13)	2	Mainstem John Day River
Dec-18	192	Murderers (7)	8	Cottonwood Creek
Dec-18	162	Murderers (14)	8	Mainstem John Day River
Dec-18	166	South Fork (32)	7	South Fork John Day River

Table 3.3. Model selection results for AICc analysis of fall emigration from Murderers Creek. Binomial logistic regression was used to model the probability of emigration per number released in each “group”. Individuals were grouped by location of release, and within each location by fork length. Explanatory variables are: (Fork) is average fork length of each group in September 2005, (Summer) is average growth rate of each group during the June-September period prior to emigration, (Depth) is the maximum depth of the nearest pool, and (Substrate) is an index of streambed particle size in the nearest pool. The (Null) model has no explanatory variables and serves as a check on the power of the explanatory variables. Product dots indicate first order interactions.

Model	AICc	Delta AICc
(Fork) + (Summer) + (Reach) + (Fork·Reach) + (Summer·Reach)	132.1	0.0
(Fork) + (Summer) + (Reach) + (Fork·Reach)	132.4	0.3
(Fork) + (Reach) + (Fork·Reach)	134.0	1.9
(Fork) + (Summer) + (Reach)	134.7	2.6
(Summer) + (Reach) + (Summer·Reach)	135.0	2.9
(Summer) + (Reach)	135.1	3.0
(Fork) + (Reach)	136.2	4.1
(Reach)	137.4	5.3
(Fork) + (Summer) + (Depth) + (Reach)	137.7	5.7
(Fork) + (Summer) + (Depth) + (Substrate) + (Reach) + (Fork·Reach)	138.0	5.9
(Substrate) + (Reach)	138.8	6.8
(Depth) + (Reach)	139.1	7.1
(Fork) + (Summer) + (Depth) + (Substrate) + (Reach) + (Fork·Reach) + (Summer·Reach)	140.5	8.5
(Depth) + (Reach) + (Depth·Reach)	140.6	8.5
(Fork) + (Summer) + (Depth) + (Substrate) + (Reach)	141.3	9.2
(Substrate) + (Reach) + (Substrate·Reach)	141.8	9.7
(Fork) + (Summer) + (Depth) + (Substrate) + (Reach) + (Fork·Reach) + (Summer·Reach) + (Depth·Reach)	145.0	12.9
(Fork) + (Summer) + (Depth) + (Substrate) + (Reach) + (Fork·Reach) + (Summer·Reach) + (Depth·Reach) + (Substrate·Reach)	150.4	18.3
(Fork) + (Summer)	155.5	23.4
(Summer)	157.3	25.2
(Depth)	161.9	29.9
(Fork)	164.1	32.0
(Null)	165.1	33.0
(Substrate)	167.4	35.3

Table 3.4. Parameter estimates for a logistic regression model of the probability of fall emigration from Murderers Creek. Estimates and confidence intervals have been back-transformed. Fork is fork length at the end of summer (September). Summer is relative growth rate during summer (June-September). Reach denotes sentinel site location (ascending order proceeding upstream from the mouth). Sample size was 54 individuals binned into 27 different length-area groups.

Parameter	Coefficient Estimate	95% Confidence Bounds		P-Value
		Lower	Upper	
Fork	1.09	1.02	1.16	0.01
Summer	1.16	1.01	1.34	0.04
Reach	1.34	0.80	2.25	0.26
Fork·Reach	0.96	0.92	0.99	0.02

## CHAPTER 4

### CONCLUSIONS AND IMPLICATIONS

The relative quality of different reaches of Murderers Creek, as indexed by individual growth rate, was heterogeneous in time and space. Growth rates in Reach 1 were high during winter, but lower than other reaches during summer (Figure 2.3). Conversely, growth rates in Reach 3 were lower during winter and higher during summer (Figure 2.3). This seasonal switching of relative habitat quality within Murderers Creek emphasizes the importance of monitoring these populations on a year-round basis. Stream reaches which have low relative habitat quality during summer may still be important to overall annual production (Ebersole et al. 2006). Furthermore, management and recovery actions may only impact individual growth or survival rates during a single season (Solazzi et al. 2000). However, changes in growth or survival during that single season may be substantial enough to impact smolt production and population viability.

We observed that *O. mykiss* in Murderers Creek were able to survive peak stream temperatures of up to 27 °C with no evidence of reduced survival during summer or subsequent seasons. However, individuals in the hottest stream reach had reduced individual growth rates relative to cooler upstream reaches. Maximum temperatures in the range of 27-28 °C are the “threshold temperature” beyond which *O. mykiss* cannot survive (Rodnick et al. 2004). Given this threshold, the current thermal regime is not maximally productive for *O. mykiss*. Peak summer stream temperatures of 27-28 °C are currently observed in upper portions of the stream network. For instance, this

temperature threshold currently occurs in the lower few kilometers of Murderers Creek (Table 2.1), a fourth-order stream. This “temperature threshold” would have historically occurred further down the stream network, in higher order streams. When temperatures were homogeneous across the stream network (fall and winter, Table 2.1), we found individual growth rates (Figure 3.8) were significantly greater in higher-order streams. Higher-order reaches may be inherently more productive than lower order reaches. If the point at which the summer “temperature threshold” occurred was further down the stream network, *O. mykiss* would be able to utilize more productive stream reaches. Shifting the “temperature-threshold” point downstream into higher-order streams by maintaining tributary stream temperatures further down the stream network (e.g., through more extensive shading and stream narrowing) is likely to increase the production capacity of the basin.

Apparent survival rates in reaches 1 and 2 of Murderers Creek were consistently lower than those in reach 3 (Table 2.2, Figure 2.7). Coincident with lower survival rates, we observed greater evidence of body scars (indicative of attempted predation) in the lower 2 reaches of the creek (Table 2.3). Percent canopy coverage is significantly higher in Reach 3 than either Reach 1 or Reach 2. Increased canopy coverage apparently reduces the effectiveness of aerial predators, and hence increases survival. Although the riparian area of Murderers Creek has been protected from domestic livestock grazing for over three decades, canopy coverage remains sparse in the downstream reaches (1 and 2). Bank armoring and stabilization may be precluding recovery of the riparian canopy. Bank armoring in key locations has eliminated or

reduced channel meander, which is a key process for recruitment of cottonwood (*Populus* spp.) stands (Friedman and Lee 2002). Cottonwood stands are typically even-aged, with little recruitment occurring in locations with established stands (Friedman and Lee 2002). The only location in Murderers Creek with a recently established cottonwood stand is in an area of extensive natural channel meander (personal observation). Thus, bank armoring appears to be limiting the natural recruitment of cottonwood trees. Low cottonwood recruitment appears to indirectly influence *O. mykiss* survival rate via reduced canopy coverage which, arguably, may allow more effective aerial predation.

We found a diversity of migratory strategies expressed by *O. mykiss* during fall, but not during spring-summer. At a coarse scale during fall, there were emigrants which left tributary streams, and sedentary fish which remained at their stations (Table 3.1). Within the emigrants, however, some moved only a few kilometers (e.g., movements documented in Figure 3.4). Conversely, other *O. mykiss* adopted an alternate pattern, and migrated many kilometers into the Mainstem John Day (Figure 3.5). Fall emigration divided the tributary populations into several spatially discrete subpopulations, which may reduce risk to the population as a whole. However, the extant migration strategies may be a remnant of the strategies historically present. For instance, we found little evidence of upstream migration during spring-summer within Murderers Creek (Figure 2.5), and no evidence of migration during summer from the South Fork into Murderers Creek. This long-distance migrant, or potamodromous component to the population (Armstrong et al. 1994) was not detected in our study.

This life history type may only exist in higher-order portions of the basin.

Alternatively, historic seasonal dams in the South Fork John Day which blocked upstream migration (passage barriers were present from May-September in some years; J. Neal, ODFW, personal communication) may have reduced the prevalence of migratory life histories. Continued monitoring would determine i.) how current fall migration strategies respond to short-term (e.g., drought years) and long-term (e.g., potential wide-scale climate changes) environmental changes, and ii.) whether a potamodromous component of the population can be detected in Murderers Creek following elimination of seasonal dams in the South Fork John Day.

## BIBLIOGRAPHY

- Armstrong, J. D., V. A. Braithwaite, and F. A. Huntingford. 1997. Spatial strategies of wild Atlantic salmon parr: exploration and settlement in unfamiliar areas. *Journal of Animal Ecology* 66:203-211.
- Averett, R. C. 1969. Influence of temperature on energy and material utilization by juvenile coho salmon. Doctoral dissertation, Oregon State University, Corvallis.
- Bacon, P. J., W. S. C. Gurney, W. Jones, I. S. McLaren, and A. F. Youngson. 2005. Seasonal growth patterns of wild juvenile fish: partitioning variation among explanatory variables, based on individual growth trajectories of Atlantic salmon (*Salmo salar*) parr. *Journal of Animal Ecology* 74:1-11.
- Baxter, C. V. 2002. Fish movement and assemblage dynamics in a Pacific Northwest riverscape. Doctoral dissertation, Oregon State University, Corvallis.
- Behnke, R. J. 2002. Trout and salmon of North America. Free Press: New York.
- Belanger, G. and M. A. Rodriguez. 2002. Local movement as a measure of habitat quality in stream salmonids. *Environmental Biology of Fishes* 64:155-164.
- Berg, O. K., and G. Bremset. 1998. Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. *Journal of Fish Biology* 52:1272-1288.
- Bilton, H. T., D. F. Alderdice, and J. T. Schnute. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. *Canadian Journal of Fisheries and Aquatic Sciences* 39:426-447.
- Biro, P. A., A. E. Morton, J. R. Post, and E. A. Parkinson. 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 61:1513-1519.
- Bjornn, T. C. 1971. Trout and salmon movement in two Idaho streams as related to temperature, food, stream flow, cover and population density. *Transactions of the American Fisheries Society* 100:423-438.
- Bramblett, R. G., M. D. Bryant, B. E. Wright, and R. G. White. 2002. Seasonal use of small tributary and main-stem habitats by juvenile steelhead, coho salmon, and dolly varden in a Southeastern Alaska drainage basin. *Transactions of the American Fisheries Society* 131:498-506.
- Bujold, V., R. A. Cunjak, J. P. Dietrich, and D. A. Courtemanche. 2004. Drifters

- versus residents: assessing size and age differences in Atlantic salmon (*Salmo salar*) fry. *Canadian Journal of Fisheries and Aquatic Sciences* 61:273-282.
- Bull, C. D., N. B. Metcalfe, and M. Mangel. 1996. Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. *Proceedings of the Royal Society of London* 263:13-18.
- Carlson, S. M., and B. H. Letcher. 2003. Variation in brook and brown trout survival within and among seasons, species and age classes. *Journal of Fish Biology* 63:780-794.
- Chapman, D. W. 1962. Aggressive behavior in juvenile coho salmon as a cause of emigration. *Journal of the Fisheries Research Board of Canada* 19:1047-1080.
- Contor, C. R., and J. S. Griffith. 1995. Nocturnal emergence of juvenile rainbow trout from winter concealment relative to light intensity. *Hydrobiologia* 299:179-183.
- Cucherousset, J., D. Ombredane, K. Charles, F. Marchand, and J. Bagliniere. 2005. A continuum of life history tactics in a brown trout (*Salmo trutta*) population. *Canadian Journal of Fisheries and Aquatic Sciences* 62:1600-1610.
- Cunjak, R. A., R. A. Curry, and G. Power. 1987. Seasonal energy budget of brook trout in streams: implications of a possible deficit in early winter. *Transactions of the American Fisheries Society* 116: 817-828.
- Cunjak, R. A. 1996. Winter habitat of selected stream fishes and potential impacts from land-use activity. *Canadian Journal of Fisheries and Aquatic Sciences* 53(Suppl. 1):267-282.
- Duston, J., and R. L. Saunders. 1997. Life histories of Atlantic salmon altered by winter temperature and summer rearing in fresh- or sea-water. *Environmental Biology of Fishes* 50:149-166.
- Ebersole, J. L., P.J. Wigington Jr., J. P. Baker, M. A. Cairns, M. R. Church, B. P. Hansen, B. A. Miller, H. R. LaVigne, J. E. Compton, S. G. Leibowitz. 2006. Juvenile coho salmon growth and survival across stream network seasonal habitats. *Transactions of the American Fisheries Society* 135:1681-1697.
- Edmundson, E., F. E. Everest, and D. W. Chapman. 1968. Permanence of station in juvenile Chinook salmon and steelhead trout. *Journal of the Fisheries Research Board of Canada* 25:1453-1464.
- Everest, F. H. 1973. Ecology and management of summer steelhead in the Rogue

River. Fishery Research Report Number 7. Oregon State Game Commission, Corvallis.

- Everest, F. H., G. H. Reeves, J. R. Sedell, D. B. Hohler, and T. C. Cain. 1988. Changes in habitat and populations of steelhead trout, coho salmon, and Chinook salmon in Fish Creek, Oregon, 1983-1987, as related to habitat improvement. Final Report to the Bonneville Power Administration, Project Number 84-11, Portland, Oregon.
- Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. *BioScience* 52: 483-498.
- Feldhaus, J. W. 2006. Heat shock proteins and physiological stress in redband rainbow trout (*Oncorhynchus mykiss gairdneri*) in the South Fork John Day River, Oregon. Masters thesis, Oregon State University, Corvallis.
- Filbert, R. B., and C. P. Hawkins. 1995. Variation in condition of rainbow trout in relation to food, temperature, and individual length in the Green River, Utah. *Transactions of the American Fisheries Society* 124:824-835.
- Finstad, A. G., O. Ygedal, T. Forseth, and T. F. Naesje. 2004. Energy-related juvenile winter mortality in a northern population of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 61:2358-2368.
- Friedman, J. M., and V. J. Lee. 2002. Extreme floods, channel change, and riparian forests along ephemeral streams. *Ecological Monographs* 73:409-425.
- Frissell, C. A., W. J. Liss, C. E. Warren, and M. D. Hurley. 1986. A hierarchical framework for stream habitat classification. *Environmental Management* 10:199-214.
- Gamperl, A. K., K. J. Rodnick, H. A. Faust, E. C. Venn, M. T. Bennett, L. I. Crawshaw, E. R. Keeley, M. S. Powell, and H. W. Li. 2002. Metabolism, swimming performance and tissue biochemistry of high desert redband trout (*Oncorhynchus mykiss ssp.*): evidence for phenotypic differences in physiological function. *Physiological and Biochemical Zoology* 75:413-431.
- Gerking, S. D. 1953. Evidence for the concepts of home range and territory in stream fishes. *Ecology* 34:347-365.
- Giannico, G. R., and M. C. Healey. 1998. Effects of flow and food on winter movements of juvenile coho salmon. *Transactions of the American Fisheries Society* 127:645-651.

- Girard, I. L., J. W. A. Grant, and S. O. Steingrimsson. 2004. Foraging, growth, and loss rate of young-of-the-year Atlantic salmon (*Salmo salar*) in relation to habitat use in Catamaran Brook, New Brunswick. *Canadian Journal of Fisheries and Aquatic Sciences* 61:2339-2349.
- Gowan, C., M. K. Young, K. D. Fausch, and S. C. Riley. 1994. Restricted movement in resident stream salmonids: A paradigm lost? *Canadian Journal of Fisheries and Aquatic Sciences* 51:2626-2637.
- Gowan, C., and K. D. Fausch. 1996. Mobile brook trout in two high-elevation Colorado streams: re-evaluating the concept of restricted movement. *Canadian Journal of Fisheries and Aquatic Sciences* 53:1370-1381.
- Gowan, C., and K. D. Fausch. 2002. Why do foraging stream salmonids move during summer? *Environmental Biology of Fishes* 64:139-153.
- Grant, J. W. A., and D. L. G. Noakes. 1987. Movers and stayers: Foraging tactics of young-of-the-year brook charr, *Salvelinus fontinalis*. *Journal of Animal Ecology* 56:1001-1013.
- Grunbaum, J. B. 1996. Geographical and seasonal variation in diel habitat use by juvenile (age 1+) steelhead trout (*Oncorhynchus mykiss*) in coastal and inland Oregon streams. Master's thesis, Oregon State University, Corvallis.
- Higgins, P. J. 1985. Metabolic differences between Atlantic salmon (*Salmo salar*) parr and smolts. *Aquaculture* 45:33-53.
- Hilborn, R., T. P. Quinn, D. E. Schindler, and D. E. Rogers. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Science* 100:6564-6568.
- Hughes, N. F., and T. C. Grand. 2000. Physiological ecology meets the ideal-free distribution: predicting the distribution of size-structured fish populations across temperature gradients. *Environmental Biology of Fishes* 59:285-298.
- Huntingford, F. A., N. B. Metcalfe, and J. E. Thorpe. 1988. Choice of feeding station in Atlantic salmon, *Salmo salar*, parr: effects of predation risk, season and life history strategy. *Journal of Fish Biology* 33:917-924.
- Imre, I., J. W. A. Grant, and E. R. Keeley. 2004. The effect of food abundance on territory size and population density of juvenile steelhead trout (*Oncorhynchus mykiss*). *Oecologia* 138:371-378.

- Juanes, F., B. H. Letcher, and G. Gries. 2000. Ecology of stream fish: insights gained from an individual-based approach to juvenile Atlantic salmon. *Ecology of Freshwater Fish* 9:65-73.
- Johnson, J. H., and P. A. Kucera. 1985. Summer-autumn habitat utilization of subyearling steelhead trout in tributaries of the Clearwater River, Idaho. *Canadian Journal of Zoology* 63:2283-2290.
- Jonsson, B., and N. Jonsson. 1993. Partial migration: niche shift versus sexual maturation in fishes. *Reviews in Fish Biology and Fisheries* (3):348-365.
- Jonsson, N., B. Jonsson, and L. P. Hansen. 1998. Long-term study of the ecology of wild Atlantic salmon smolts in a small Norwegian river. *Journal of Fish Biology* 52:638-650.
- Kahler, T. H., P. Roni, and T. P. Quinn. 2001. Summer movement and growth of juvenile anadromous salmonids in small western Washington streams. *Canadian Journal of Fisheries and Aquatic Sciences* 58:1947-1956.
- Keeley, E. R. 2001. Demographic responses to food and space competition by juvenile steelhead trout. *Ecology* 82:1247-1259.
- Koskela, J., J. Pirhonen, and M. Jobling. 1997. Growth and feeding responses of a hatchery population of brown trout (*Salmo trutta*) at low temperatures. *Ecology of Freshwater Fish* 6:116-121.
- Larsson, S., T. Forseth, I. Berglund, A. J. Jensen, I. Naslund, J. M. Elliott, and B. Jonsson. 2005. Thermal adaptation of Arctic charr: experimental studies of growth in eleven charr populations from Sweden, Norway and Britain. *Freshwater Biology* 50:353-368.
- Ledgerwood, R. D., B. A. Ryan, E. M. Dawley, E. P. Nunnallee and J. W. Ferguson. 2004. A surface trawl to detect migrating juvenile salmonids tagged with Passive Integrated Transponder tags. *North American Journal of Fisheries Management* 24:440-451.
- Leider, S. A., M. W. Chilcote, and J. J. Loch. 1986. Movement and survival of presmolt steelhead in a tributary and the main stem of a Washington river. *North American Journal of Fisheries Management* 6:526-531.
- Letcher, B. H., G. Gries, and F. Juanes. 2002. Survival of stream-dwelling Atlantic salmon: effects of life history variation, season and age. *Transactions of the American Fisheries Society* 131:838-854.

- Lichtowich, J. A. 1999. Salmon without rivers: A history of the Pacific salmon crisis. Island Press, Covelo, California.
- Lovtang, J. C. 2005. Distribution, habitat use, and growth of juvenile Chinook salmon in the Metolius River Basin, Oregon. Masters thesis, Oregon State University, Corvallis.
- Lundqvist, H., S. McKinnell, H. Fangstam, and I. Berglund. 1994. The effect of time, size and sex on recapture rates and yield after river releases of *Salmo salar* smolts. *Aquaculture* 121:245-257.
- Marine, K. R., and J. J. Cech Jr. 2004. Effects of high water temperature on growth, smoltification, and predator avoidance in juvenile Sacramento River Chinook salmon. *North American Journal of Fisheries Management* 24:198-210.
- Martel, G., and L. M. Dill. 1995. Influence of movement by coho salmon (*Oncorhynchus kisutch*) parr on their detection by common mergansers (*Mergus merganser*). *Ethology* 99:139-149.
- McCormick, S. D., L. P. Hansen, T. P. Quinn, and R. L. Saunders. 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 55(Suppl. 1):77-92.
- McIntosh, B. A. 1996. Historical changes in stream habitats in the Columbia River Basin. Dissertation, Oregon State University, Corvallis.
- Metcalf, N. B., F. A. Huntingford, and J. E. Thorpe. 1988. Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon *Salmo salar*. *Journal of Animal Ecology* 57:463-474.
- Metcalf, N. B., and J. E. Thorpe. 1992. Anorexia and defended energy levels in over-wintering juvenile salmon. *Journal of Animal Ecology* 61:175-181.
- Metcalf, N. B. 1998. The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 55(Suppl. 1):93-103.
- Metcalf, N. B., N. H. C. Fraser, and M. D. Burns. 1999. Food availability and the nocturnal vs. diurnal foraging trade-off in juvenile salmon. *Journal of Animal Ecology* 68:371-381.
- Miller, B. A., J. D. Rodgers, and M. F. Solazzi. 2000. An automated device to release marked juvenile fish for measuring trap efficiency. *North American Journal of Fisheries Management* 20:284-287.

- Morgan, I. J., and N. B. Metcalfe. 2001. The influence of energetic requirements on the preferred temperature of overwintering juvenile Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 58:762-768.
- Morinville, G. R., and J. B. Rasmussen. 2003. Early juvenile bioenergetic differences between anadromous and resident brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* 60:401-410.
- Muhlfeld, C. C., D. H. Bennett, and B. Marotz. 2001. Fall and winter habitat use and movement by Columbia River redband trout in a small stream in Montana. *North American Journal of Fisheries Management* 21:170-177.
- Murphy, M. H., M. J. Connerton, and D. J. Stewart. 2006. Evaluation of winter severity on young-of-the-year Atlantic salmon. *Transactions of the American Fisheries Society* 135:420-430.
- Myrick, C. A., and J. J. Cech Jr. 2000. Temperature influences on California rainbow trout physiological performance. *Fish Physiology and Biochemistry* 22:245-254.
- Nehlsen, W., J. E. Williams, and J. A. Lichatowich. 1991. Pacific salmon at the crossroads: Stocks at risk from California, Oregon, Idaho and Washington. *Fisheries* 16(2):4-21.
- Neu, C. W., C. R. Byers, and J. M. Peek. 1974. A technique for analysis of utilization-availability data. *Journal of Wildlife Management* 38:541-545.
- Nicola, G. G., and A. Almodovar. 2004. Growth pattern of stream-dwelling brown trout under contrasting thermal conditions. *Transactions of the American Fisheries Society* 133:66-78.
- National Marine Fisheries Service (NMFS). 2000. Federal Columbia River Power System Biological Opinion: Reinitiation of consultation on operation of the Federal Columbia River Power System, including the juvenile fish transportation program, and 19 Bureau of Reclamation projects in the Columbia Basin. NMFS-NWR, Seattle, WA.
- Paulsen, C. M., and T. R. Fisher. 2001. Statistical relationship between parr-to-smolt survival of Snake River Spring-summer Chinook salmon and indices of land use. *Transactions of the American Fisheries Society* 130:347-358.
- Peven, C. M., R. R. Whitney, and K. R. Williams. 1994. Age and length of steelhead

smolts from the mid-Columbia River Basin, Washington. *North American Journal of Fisheries Management* 14:77-86.

Prentice, E. F., T. A. Flagg, C. S. McCutcheon, D. F. Brastow, and D. C. Cross. 1990. Equipment, methods, and an automated data entry station for PIT tagging. Pages 335-340 in N. C. Parker, A. E. Giorgi, R. C. Hedinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. *Fish-marking techniques*. American Fisheries Society, Symposium 7, Bethesda, Maryland.

PTSC (Pit Tag Steering Committee). 1999. *PIT Tag Marking Procedures Manual*. Pacific States Marine Fisheries Commission, Gladstone, Oregon Available on-line at: [http://www.psmfc.org/pittag/Software\\_and\\_Documentation/](http://www.psmfc.org/pittag/Software_and_Documentation/).

Reeves, G. H., F. H. Everest, and J. D. Hall. 1987. Interactions between the redband shiner (*Richardsonius balteatus*) and the steelhead trout (*Salmo gairdneri*) in western Oregon: the influence of water temperature. *Canadian Journal of Fisheries and Aquatic Sciences* 44:1603-1613.

Reischauer, A. G., F. R. Monzyk, E. S. Van Dyke, B. C. Jonasson, and R. W. Carmichael. 2003. Investigations into the early life history of naturally produced spring chinook salmon and summer steelhead in the Grande Ronde River basin. 2001 annual report to the Bonneville Power Administration, Project Number 199202604, Portland, Oregon.

Richter, A., and S. A. Kolmes. 2005. Maximum temperature limits for Chinook, coho, and chum salmon, and steelhead trout in the Pacific Northwest. *Reviews in Fisheries Science* 13:23-49.

Riddell, B. E., and W. C. Leggett. 1981. Evidence of an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 38:308-320.

Riehle, M. D., and J. S. Griffith. 1993. Changes in habitat use and feeding chronology of juvenile rainbow trout (*Oncorhynchus mykiss*) in fall and the onset of winter in Silver Creek, Idaho. *Canadian Journal of Fisheries and Aquatic Sciences* 50:2119-2128.

Rodnick, K. J., A. K. Gamperl, K. R. Lizars, M. T. Bennett, R. N. Rausch, and E. R. Keeley. 2004. Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. *Journal of Fish Biology* 64:310-335.

Rodriguez, M. A. 2002. Restricted movement in stream fish: the paradigm is incomplete, not lost. *Ecology* 83(1):1-13.

- Roni, P., and T. P. Quinn. 2001. Effects of wood placement on movements of trout and juvenile coho salmon in natural and artificial stream channels. *Transactions of the American Fisheries Society* 130:675-684.
- Schrank, A. J., and F. J. Rahel. 2004. Movement patterns in inland cutthroat trout (*Oncorhynchus clarki utah*): management and conservation implications. *Canadian Journal of Fisheries and Aquatic Sciences* 61:1528-1537.
- Seelbach, P. W. 1987. Effect of winter severity on steelhead smolt yield in Michigan: An example of the importance of environmental factors in determining smolt yield. *American Fisheries Society Symposium* 1:441-450.
- Smith, R. W., and J. S. Griffith. 1994. Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. *Transactions of the American Fisheries Society* 123:747-756.
- Solazzi, M. F., T. E. Nickelson, S. L. Johnson, and J. D. Rodgers. 2000. Effects of increasing winter rearing habitat on abundance of salmonids in two coastal Oregon streams. *Canadian Journal of Fisheries and Aquatic Sciences* 57:906-914.
- Steingrimsson, S. O., and J. W. A. Grant. 2003. Patterns and correlates of movement and site fidelity in individually tagged young-of-the-year Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 60:193-202.
- Thedinga, J. F., M. L. Murphy, S. W. Johnson, J. M. Lorenz, and K. V. Koski. 1994. Determination of salmonid smolt yield with rotary-screw traps in the Situk River, Alaska, to predict effects of glacial flooding. *North American Journal of Fisheries Management* 14:837-851.
- Thorpe, J. E. 1987a. Environmental regulation of growth patterns in juvenile Atlantic salmon. Pages 463-474 *in*: R. C. Summerfeldt and G. E. Hall, editors. *Age and Growth of Fish*. Iowa State University Press, Ames, Iowa.
- Thorpe, J. E. 1987b. Smolting versus residency: Developmental conflict in salmonids. *American Fisheries Society Symposium* 1:244-252.
- Thorpe, J. E., and N. B. Metcalfe. 1998. Is smolting a positive or a negative developmental decision? *Aquaculture* 168:95-103.
- Tipping, J. M. 1997. Effect of smolt length at release on adult returns of hatchery-reared winter steelhead. *The Progressive Fish Culturist* 59:310-311.

- Van den Avyle, M. J., and R. S. Hayward. 1999. Dynamics of exploited fish populations. Pages 127-166 in: C. C. Kohler and W. A. Hubert, editors. *Inland Fisheries Management in North America*. American Fisheries Society, Bethesda, Maryland.
- Ward, B. R., P. A. Slaney, A. R. Facchin, and R. W. Land. 1989. Size-biased survival in steelhead trout (*Oncorhynchus mykiss*): Back-calculated lengths from adult's scales compared to migrating smolts at the Keogh River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1853-1858.
- White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory LA-8787-NERP. 235 pp.
- White, G. C. and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. *Bird Study* 46 Supplement, 120-138.
- Whitesel, T. A. 1993. Comparison of juvenile Atlantic salmon (*Salmo salar*) reared in a hatchery and introduced into a stream: A two-size-threshold model of smoltification. Pages 239-247 in: R. J. Gibson and R. E. Cutting, editors. *Production of juvenile Atlantic salmon, Salmo salar, in natural waters*. Canadian Special Publication of Fisheries and Aquatic Sciences 118.
- Wright, K. K. 2000. From continua to patches: Longitudinal patterns in the Middle Fork of the John Day River, Oregon. Doctoral dissertation, Oregon State University, Corvallis.
- Zimmerman, C. E., and G. H. Reeves. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. *Canadian Journal of Fisheries and Aquatic Sciences* 57:2152-2162.
- Zydlewski, G. B., A. Haro, and S. D. McCormick. 2005. Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences* 62:68-78.
- Zydlewski, G. B., G. Horton, T. Dubreuil, B. Letcher, S. Casey, and J. Zydlewski. 2006. Remote monitoring of fish in small stream: A unified approach using PIT tags. *Fisheries* 31:492-502.

**Appendix A**

POPULATION ABUNDANCE ESTIMATION

We conducted population estimates for each sentinel site in June 2005, September 2005, and January 2006. Capture techniques, number of samples within each sampling event, and sampling conditions varied between these occasions. We used program Capture (White et al. 1982) to simulate some of the potential biases within sampling events, and thus determine an appropriate mark-recapture model.

June and September sampling events were two-sample mark-recapture trials. During January, we conducted three-sample mark-recapture trials. With two-sample events, we were unable to conduct mark-recapture model simulations in program Capture. Therefore, we used the January three-sample events to conduct model simulations in program Capture. The mark-recapture model selected from these simulations was then applied to the June and September samples.

For the January 2006 sampling events, we conducted closed population estimates with all available mark-recapture models. In addition to the Murderers Creek sampling sites, we also included sampling events from Black Canyon Creek (South Fork John Day River basin) and Bridge Creek (Lower Mainstem John Day River basin) during December 2005. These sites were added to encompass a broader range of possible variation in mark-recapture estimates during winter. We then used typical results (i.e., normal numbers of marked and recaptured individuals on each event) from these sampling events to run simulations in program Capture. Twelve simulations (each with 1,000 iterations) were run with parameter values suggested from the data. Heterogeneity of capture probability (based on fish length), temporal variation in capture probability, and behavioral response (only trap-shy behavior was simulated)

were evaluated at various levels in these simulations. The simulation included models which assumed: constant capture probability (model M(o)), individual variability in capture probability (model M(h)), constant capture probability but variable behavioral response to capture (model M(b)), individual variability in capture probability and behavioral response to capture (model M(bh)), temporal changes in capture probability with constant capture probability across the population (model M(t)), and temporal changes in capture probability with individual variation in capture probability (model M(th)). Results (Table A1) suggested that both models M(o) and M(t) performed well, with relatively little statistical bias and good confidence interval coverage across all simulated values. These simulations, however, do not account for potential biases in field operation, such as lack of population closure, and thus likely underestimate the true bias in population estimates.

It is possible that size-based heterogeneity in capture probability exists in the December/January sampling, with smaller ( $< 100$  mm) fish having lower capture rates. However, the simulations suggest that heterogeneity in capture probability is not pervasive enough to dictate a model accounting for heterogeneity. For example, in reach 2 of Murderers Creek, for PIT tagged individuals, *O. mykiss*  $\geq 140$  mm ( $n = 3$ ) had 3 recapture events out of 6 possible (50%), and *O. mykiss*  $< 140$  mm ( $n = 11$ ) had 10 recapture events out of 22 possible (45%). This suggests similarity of recapture rates, at least for this site and size stratification. Furthermore, the majority of PIT tagged *O. mykiss*, for which we are most concerned with efficiency estimates, are in the

larger size class ( $> 100$  mm) as most fish were over 80 mm when tagged, and subsequently added length before recapture events.

It is likely that there are some time effects on capture efficiency during the December/January sampling. The number of new fish captured on each successive day did not always decline, as expected under constant capture probability. Total number of captures typically varied in a decreasing trend across the three sampling events, but occasionally total captures increased on night 2 or 3. We attempted to maintain equal effort on each sampling event (during all seasons). Therefore, variation in captures during winter sampling is likely due either to change in capture efficiency between nights, or movement. The former seems more likely, as movement rates of *O. mykiss* through PIT tag antennas during sampling periods in December and January were generally low.

Diel activity patterns of *O. mykiss* during winter may influence capture efficiency. *O. mykiss* conceal in the substrate during winter daylight hours (Grunbaum 1996) and emerge into the water column after the end of civil twilight (Contor and Griffith 1995; Grunbaum 1996). Emergence rates vary between nights, based on several environmental factors (Contor and Griffith 1995). If a fish failed to emerge from the substrate on a given evening, it would be unavailable for capture during that nights' sampling. Thus, diel variation in emergence may have created temporal variation in capture efficiency.

Considering the results of model simulation and the aforementioned factors, we used the  $M(t)$  model to account for potential time variation. Furthermore, we used the

modified  $M(t)$  Chao version of the model. This version is better suited for small capture numbers observed during some sampling events.

Table A.1. Selected simulations conducted in Program Capture to determine an appropriate estimator for mark-recapture population estimates. "Population" is the prescribed known population size. "Occasions" indicates the number of sampling events. "Heterogeneity" describes the capture probability of groups of individuals, relative to the mean value. "Behavior" apportions a trap-shy response to a specified portion of the population. "Time" allows for capture probability to decrease or increase on successive days, relative to a standard of 1.0. Percent coverage indicates the portion of simulations in which the estimated confidence interval included the fixed population size.

Population = 170. Occasions = 3. Heterogeneity = 10, 0.67, 140 , 0.50, 20, 0.39 Behavior = 145, 1, 25, 0.73. Time = 1.0, 0.9, 0.8.				
Model	Simulated N	Fixed N	% Bias	%CI Coverage
M(o)	170	170	0	93
M(h)	205	170	20	12
M(b)	151	170	-11	35
M(bh)	154	170	-10	72
M(t)	170	170	0	100
M(th)	216	170	27	52
Population = 170. Occasions = 3. Heterogeneity = 10, 0.67, 140 , 0.50, 20, 0.39 Behavior = 145, 1, 25, 0.73. Time = 0.8, 1.0, 0.90.				
Model	Simulated N	Fixed N	% Bias	%CI Coverage
M(o)	170	170	0	93
M(h)	201	170	19	14
M(b)	220	170	30	47
M(bh)	200	170	18	80
M(t)	169	170	-1	93
M(th)	219	170	29	56
Population = 170. Occasions = 3. Heterogeneity = 10, 0.67, 140 , 0.50, 20, 0.39 Behavior = 140, 1, 30, 0.53. Time = 1.0, 0.9, 0.8.				
Model	Simulated N	Fixed N	% Bias	%CI Coverage
M(o)	172	170	1	93
M(h)	208	170	22	6
M(b)	151	170	-11	43
M(bh)	155	170	-9	79
M(t)	171	170	1	100
M(th)	218	170	28	48

Table A.2. Summary of *O. mykiss* captures during mark-release-recapture sampling events in Murderers Creek. Sampling occurred in June 2005, September 2005, and January 2006. The sentinel site in each reach was the sample location. All individuals captured for the first time within any given event were marked (M), and assumed available for recapture (R) during subsequent samples within that event. *O. mykiss* which had been PIT tagged in a prior season were considered “marked” when first captured within a sampling event. Capture probability estimates were derived with the M(t) Chao model in Program Capture.

Reach	Month	Sample 1		Sample 2		Sample 3		Capture Probability		
		M	R	M	R	M	R	1	2	3
1	June	43	0	18	6	-	-	0.28	0.15	-
2	June	42	0	33	5	-	-	0.15	0.14	-
3	June	22	0	13	5	-	-	0.31	0.25	-
1	September	31	0	16	10	-	-	0.40	0.33	-
2	September	69	0	39	18	-	-	0.32	0.27	-
3	September	25	0	20	7	-	-	0.28	0.30	-
1	January	45	0	13	19	17	18	0.46	0.33	0.36
2	January	38	0	20	15	14	13	0.32	0.29	0.22
3	January	18	0	6	0	8	1	0.10	0.03	0.05

**Appendix B**

2005 SOUTH FORK JOHN DAY RIVER SCREW TRAP CALIBRATION

## Introduction

The South Fork John Day River (SFJD) screw trap was used to capture emigrating juvenile salmonids during fall 2004 and fall 2005. The screw trap is a moored, floating device driven by water flow (Figure B1). It is positioned close to the thalweg and collects a portion of fish moving downstream. Emigrant abundance estimates relied on a mark-recapture protocol to determine the efficiency of capture. Abundance of migrant salmonids was estimated with the equation:

$$\hat{N} = C * \hat{E}^{-1}$$

where N is the total number of migrants, C is the total number of unmarked fish captured in the trap, and E is trap efficiency, which is the proportion of fish moving downstream past the trap that is captured.

Typical protocol for mark-recapture calibration of trap efficiency dictates marking fish captured in the trap, releasing the fish upstream of the trap, and using the proportion of these marked fish recovered in the trap as an estimate of E (Thedinga et al. 1994). Pilot data from fall 2004 monitoring of the SFJD screw trap suggested, however, that the common release protocol may induce behavioral changes in released *Oncorhynchus mykiss*. These changes in behavior may cause them to be less vulnerable to recapture in the trap than juvenile steelhead naturally migrating through the trap area.

Fall 2004 monitoring with Passive Integrated Transponder (PIT) tag detection antennas found 40% of naturally migrating fish (which had been PIT tagged upstream of the trap several months earlier) were captured in the screw trap whereas only 28% of fish captured in the trap, tagged and then released 1.6 km upstream of the trap during

daylight hours (later referred to as release strategy “A”) were recaptured. Bias of this magnitude could reduce our ability to detect differences in tributary emigration rates. Therefore, we conducted an experiment in fall 2005 to compare recapture rates at the SFJD screw trap resulting from three different protocols for time and location of upstream release. A meaningful response for the efficiency estimate of the SFJD screw trap would be a  $\geq 10\%$  difference in the odds of recapture between any of the three different release groups during the range of stream flow conditions experienced.

### **Methods**

We used a 1.52 m diameter rotary screw trap at river kilometer 10 of the SFJD. Streamflow at this location during trap operation varied from  $\sim 0.85 \text{ m}^3/\text{s}$  during fall to  $> 56 \text{ m}^3/\text{s}$  during winter. The trap was situated in a fast water glide downstream of a long riffle (Figure B1), and was cabled such that it could be adjusted both longitudinally and laterally to remain in the thalweg as streamflow changes. Our protocol involved adjusting the trap so that it remained in the thalweg, the location of which varied with major changes in flow. We investigated four potential explanatory variables for capture efficiency: stream height (height), trap rotation speed (speed), distance upstream of release of marked fish, diel release time of marked fish, and size of marked fish (length). Height was measured with a staff gauge adjacent to the trap. Speed was the number of rotations by the cone of the trap (the mechanism by which fish are captured) per second. The time per three full rotations of the cone was measured to reduce variation from periodic fluctuations in cone rotation speed. Height and speed were recorded daily.

All unmarked *O. mykiss* captured in the screw trap were tagged with intraperitoneally injected 12 mm long PIT tags. It is common practice to tag juvenile salmonids in this fashion and release them upstream from a screw trap to determine the recapture rate and hence efficiency of the trap. From October 14, 2005 through December 15, 2005; on each day that three or more unmarked *O. mykiss* were captured in the trap; all unmarked fish were tagged and systematically assigned to one of three release strategies. “A” was transported 1.6 kilometers upstream of the trap and released during daylight hours. “B” was transported 4.8 kilometers upstream of the trap and released during daylight hours. “C” was transported 1.8 kilometers upstream of the trap, and placed into a holding device equipped with a timer (see description in Miller et al. 2000) which was set to release the fish at civil twilight (the time when the sun is 6 degrees below the horizon line, and full darkness is imminent) on the same day (Figure B2). A total of 910 *O. mykiss* were PIT tagged and released upstream of the SFJD screw trap on 38 different days (daily release by strategy ranged from 1 to 51 individuals) during fall 2005.

We tested for differences in recapture frequency by fish length with a chi-square goodness-of-fit test. We divided all released individuals into 3 groups ( $\leq 140$ , 141-170,  $\geq 171$ ) based on naturally occurring breaks in length distribution. There were significant differences in recapture rate among released fish in these length categories ( $\chi^2 = 18.8$ ,  $P < 0.001$ ). *O. mykiss* less than 140 mm were recaptured more frequently than expected, while those greater than 140 mm were recaptured less frequently than expected. Therefore, we added a categorical length variable (“small” or  $\leq 140$ , and “large” or  $\geq$

141) to our initial binomial logistic regressions. To ensure that we had suitable individuals of each length category in each release group, we included only days on which 10 or more individuals were released in each group. This resulted in a total sample size of 646 individuals distributed among 3 release strategies (A, B, C) and 2 size groups on 9 different days.

We used Pearson correlation to test for correlation between explanatory variables. Height and speed were correlated ( $r = 0.85$ ,  $n = 38$ ,  $P < 0.0001$ ). We chose to therefore eliminate height, and analyze speed, since we have some measure of operational control over speed. That is, trap location can be adjusted to maximize speed. We used logistic regression with a binomial distribution and logit link function (SAS Procedure GenMod) to model daily recapture proportion. The generalized linear model was:

$$\text{logit}(p) = \log(p/1-p) = B_0 + B_1 \cdot I_b + B_2 \cdot I_c + B_3 \cdot I_{\text{large}} + B_4 \cdot \text{speed} + B_5 \cdot I_b \cdot \text{speed} + B_6 \cdot I_c \cdot \text{speed} + B_7 \cdot I_{\text{large}} \cdot \text{speed} + B_8 \cdot I_{\text{large}} \cdot I_b + B_9 \cdot I_{\text{large}} \cdot I_c \quad (1)$$

where  $p$  is the number of *O. mykiss* recaptured per the number released on any given day,  $I_b$  is the indicator for release strategy B,  $I_c$  is the indicator for release strategy C (these strategies were compared against strategy A, which was held constant),  $I_{\text{large}}$  is the indicator for the large size group, and speed is the number of cone rotations per second. Product signs denote first order interactions. B's are fitted coefficients. This regression procedure tested for differences in the odds of recapture between different release groups and lent more weight to days with larger numbers of marked fish

released. We used drop-in-deviance tests to sequentially compare reduced models to the initial full model.

This initial analysis found that all terms contributed significantly to the model, with the exception of the size-speed interaction term. The scale parameter for this model was 1.41, indicating that extra-binomial variation was present. In comparisons of release strategies, this model found diel time of release had a significant effect on recapture rate (Figure B3), as release strategy C was significantly different from A ( $P = 0.03$ ). There was some evidence ( $P = 0.06$ ) that releasing fish further upstream (strategy B) resulted in higher recapture rates than strategy A. The model also found fish size ( $P = 0.03$ ) and trap speed ( $P = 0.01$ ) had significant effects on recapture rate.

Only release strategy C was significantly different from strategy A. Pilot data from fall 2004 indicated that release strategy A underestimates actual capture efficiency of the trap. Thus, to accurately represent the efficiency with which the trap captures *O. mykiss* migrating through for the first time (some of which have previously been tagged in upstream tributaries), we used only release strategy C in our final modeling process. Given the influence of length on recapture rate, we initially used a binary logistic model for the C release strategy which used length of each fish released as an individual explanatory variable. For the C release strategy, 293 individuals were released, and 98 recaptured. The binary regression model was:

$$\text{logit}(p) = \log(p/1-p) = B_0 + B_1 \cdot \text{Length} + B_2 \cdot \text{speed} + B_3 \cdot \text{Length} \cdot \text{speed} \quad (2)$$

where  $p$  is the fate of each *O. mykiss* released (i.e., recapture or escape), Length is fork length of each individual, and speed is the number of cone rotations per second.

Product signs denote first order interactions. B's are fitted coefficients.

Analysis of deviance for this model found that length was the only significant explanatory variable. Thus, speed, and the speed·length interaction term were dropped. Analysis of residuals found significant lag-1 autocorrelation in this model however. Therefore, we iteratively grouped individuals by length until lag-1 autocorrelation was no longer significant, and ran the selected model as a binomial logistic regression. This model used three length groups: 86-115 mm, 116-145 mm, and 146-230 mm. The final generalized linear model was:

$$\text{logit}(p) = \log(p/1-p) = B_0 + B_1 \cdot I_{\text{small}} + B_2 \cdot I_{\text{med}} \quad (3)$$

where  $p$  is the number of juvenile steelhead recaptured per the number released on each day,  $I_{\text{small}}$  is an indicator for the 86-115 mm group, and  $I_{\text{med}}$  is an indicator for the 116-145 mm size group. B's are fitted coefficients. An over-dispersion parameter was included to account for extra-binomial variation.

### Results

The final model (3) was over-dispersed, as indicated by an estimated scale (over-dispersion) parameter of 1.32. There was no significant lag-1 or lag-2 autocorrelation present among the residuals in this model. The final model was:

$$\text{logit}(p) = \log(p/1-p) = -1.51 + 0.95 \cdot I_{\text{small}} + 1.26 \cdot I_{\text{med}}$$

SE	0.33	0.53	0.40
----	------	------	------

The model found evidence that the small size group (86-115 mm) was recaptured at a higher rate than the large (146-230 mm) size group ( $P = 0.07$ ). Furthermore, odds of recapture for an individual in the medium size group (116-145 mm) were significantly higher ( $P = 0.002$ ) than odds of recapture for an individual in the large size group (146-230 mm). There was no significant difference in odds of recapture between the small and medium size groups ( $P = 0.51$ ).

The model predicted probability of recapture varied with size group (Figure B4). Probability of recapture for the small size group was 0.36. Probability of recapture for the medium size group was 0.44. Probability of recapture for the largest size group declined to 0.18.

### **Discussion**

Location and diel timing of release influenced the odds of *O. mykiss* being recaptured in the SFJD screw trap. Releasing fish 1.6 km upstream of the trap, during daylight hours (strategy A), significantly reduced the odds of recapture (equation 1), as compared to releases made at civil twilight in nearly the same location (strategy C). Thus, as the release location of A and C were similar, we infer that different diel release time was the primary influence on odds of recapture. As noted in the methods, daylight releases in close proximity to the trap are inappropriate, and should not be employed for trap calibration.

Transporting juvenile steelhead further upstream prior to a daylight release (strategy B), did not result in recapture rates equal to that of strategy C (equation 1). The odds of recapture for B were lower than the odds of recapture for C (Figure B3).

Although this difference was not significant at an alpha of 0.05, the chi-square P-value of 0.09 for the comparison of odds of recapture between B and C is suggestive of a difference in recapture rates between these two groups. Longer migration distances back to the screw trap location (as is the case for strategy B) may increase the probability of mortality via predation, or decrease the proportion of *O. mykiss* which again emigrate past the trap site. Either case violates one underlying assumption of the mark-recapture process that all marked individuals will be “available” for recapture. This possible effect reiterates the need to use the C release strategy for trap calibration.

The effect of fish length on recapture was significant in all 3 models. The lower odds of recapture for the large size class of fish (146 to 230 mm, equation 3) suggests that the trap is only marginally effective at capturing this size of *O. mykiss*, at least when the trap is operating at speeds typically observed during fall. Continued mark and recapture work with all size classes of *O. mykiss* is needed in order to increase sample size, and more finely partition the influence of length on recapture probability. For instance, the weak difference between probability of recapture rate for small and large size classes (equation 3) may be due to limited sample size of small *O. mykiss* ( $n = 44$ ). Additionally, the relatively poor capture efficiency of the trap for large *O. mykiss* stresses the importance of using alternate methods of detecting emigrants, such as in-stream PIT tag antenna arrays or sonar, in concert with a screw trap.



Figure B.1. South Fork John Day River screw trap during typical streamflow levels observed during the study period. The stream flows from left to right. The cone in the center of the trap is turned by the force of water pressure on a screw-shaped structure inside. The cone entrains downstream migrating fish, spins the fish around, then deposits fish into a holding tank in the middle rear of the trap.



Figure B.2. Schematic representation of release site locations and times used in the South Fork John Day River during fall 2005. The dashed arrow denotes streamflow direction.

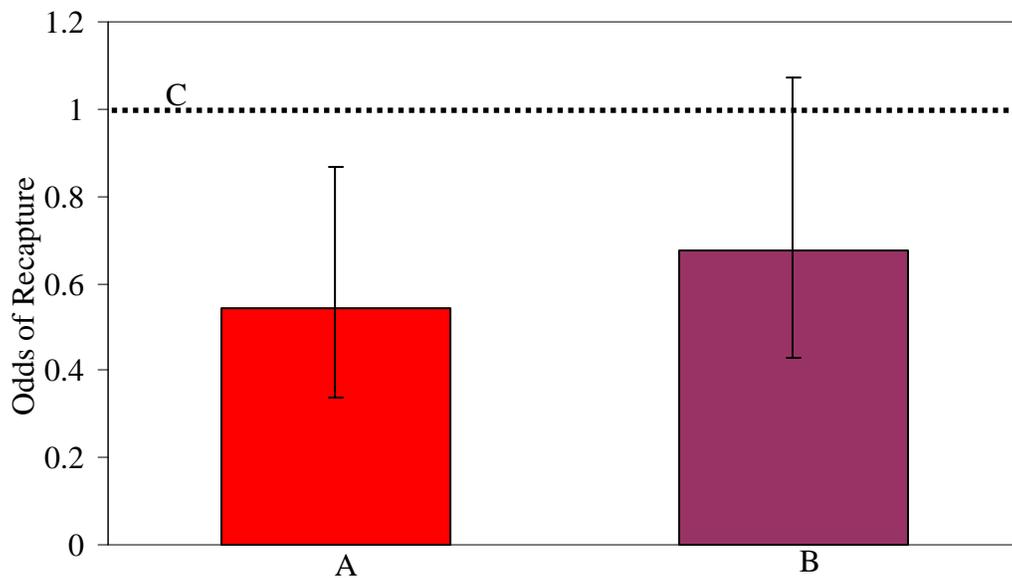


Figure B.3. Comparison of the relative odds of recapture among release strategies used during fall 2005. Strategy C (denoted by the dashed line at a value of 1.0) was the reference group. The odds of recapture for juvenile steelhead in Group A and Group B are compared to C. An odds ratio of 1.0 represents no difference. Error bars represent 95% Confidence Intervals. Odds of recapture for strategy A are significantly lower than C. There is no significant difference in odds of recapture between strategy B and C, or between strategy B and A.

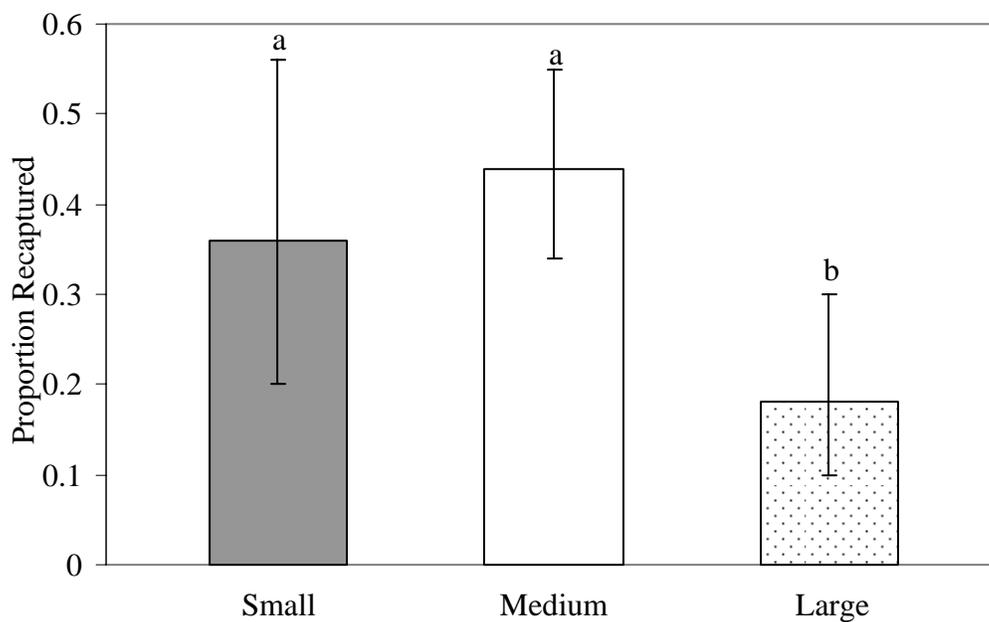


Figure B.4. Comparisons of the rotary screw trap recapture probability among size classes during fall 2005. All individuals were released following the C release strategy (released 1.8 km upstream of the trap, at civil twilight). Common letters indicate groups which are not significantly different ( $P > 0.10$ ).

**6. PROGRESS REPORT: Community Effects on the Ideal Free Distribution of Redband Trout in the South Fork John Day Basin**  
**Seth White (Lead Author)**

***Introduction***

Temperature can also have profound impacts on the biological environment (*i.e.*, community) of the redband trout. Community responses can affect the foraging base for trout, intraspecific competitive intensity, competitive relations among species, and predator-prey relations. Except for the forage base, biotic habitat factors are typically not accounted for in the IFD, but may nonetheless be very important. Within the context of physiological tolerance limits, shifts community relations can make habitat more or less suitable for *O. mykiss*. We are adapting the IFD by describing carrying capacity for redband trout to accommodate for competitive interactions among species. The practical impacts of this study lie in determining first whether food is a limiting factor for *O. mykiss* in the basin, and second how increased food production via management of riparian forests might be expected to enhance their populations. Since *O. mykiss* are a federally-listed species, information about their behavior and responses to potential limiting factors in natural streams will greatly enhance our understanding of their biology, thus providing knowledge for existing and future conservation efforts.

***Research Approach***

How can the study of fish assemblage, fish behavior, and the ideal-free distribution contribute to the modeling effort?

***I. Fish assemblage***

- A. *Standing crops*: models of fish production in the basin will include the distribution of non-salmonids. Our extensive surveys only include non-salmonids by presence and abundance, but intensive monitoring includes non-salmonid size classes, which can be related to biomass.
- B. *Biotic interactions*: how can we tell if biotic interactions are involved in structuring fish? A first shot at this involves describing distributions across the landscape and determining where species overlap, and where they do not. However, these models are correlative, and therefore a performance measure (such as HSPs, growth rates, or behavior) needs to be incorporated in order to approach a causal explanation for species distributions.

***II. Fish behavior***

- A. *The field of "behavioral ecology"*: defined as the study of how environment mediates behavioral interactions between species. This has implications for RBT production because relationships between species (competition, predation, mutualism, etc.) will change depending on environmental context.
- B. *Aggression and feeding*: from an optimal foraging theory standpoint, the relative amounts of aggression and feeding a fish does will effect energy left over for growth and reproduction. We have rates of feeding and aggression in different environmental contexts and different fish species mixes.

***III. Ideal free distribution***

- A. *Assumptions of IFD*: include organisms having the ability to track and migrate according to available resources. These assumptions are important for knowing how fish will react to stressors (e.g., high water temperatures) as well as
- B. *Resource constraints*: When resource constraints are relaxed, will fish do better? (This is essentially getting at limiting factors.) For example, when we add food, do fish grow? If not, we might conclude that in this context, food is not a limiting factor. Our experiments from 2006 indicate food is, however, a limiting factor, and we can provide back-of-the-envelope calculations for how much growth we might expect given know additions of food.

### ***Hypotheses***

Experimental work in the summer of 2006 addressed how resource constraints (i.e., food) affect growth, movement, and behavior of *O. mykiss* in context of environmental conditions and distribution of other fish species. Specifically, the experiment is designed to test the following hypotheses:

- 1) As food is a limiting factor for fish in South Fork John Day tributaries in summertime, the addition of food at amounts for optimal growth will lead to increased fish growth and/or condition factor.
- 2) As fish distributions are expected to match resource availabilitys under assumptions of the IFD, fish from pools with only baseline food will move into sites of food supplementation.
- 3) As an increase in resources should relax competitive pressure, fish in sites with food supplementation will shift from aggressive behavior to feeding.

Several studies imply that food may be a limiting factor for salmonid populations in freshwater environments (Mason 1976; Boss and Richardson 2002). Food limitation was also proposed as a potential mechanism for limiting salmonid populations in the South Fork John Day River at a recent meeting with Bureau of Reclamation officials. Results from concurrent studies in the system reveal that in South Fork tributaries, *O. mykiss* summer growth rates are low (I. Tattam, unpublished data) and invertebrate drift densities are also low (N. Weber, unpublished data). Therefore, food is a potential limiting factor to salmonid populations in this system and warrants further investigation.

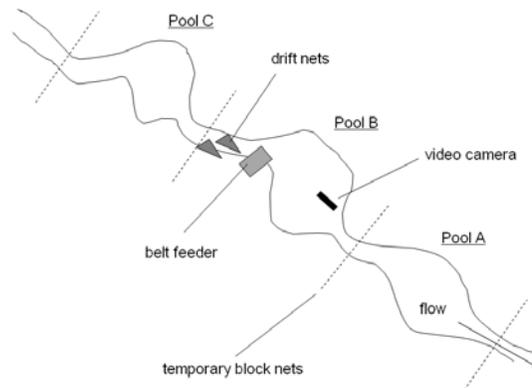
### ***Study sites***

In mid-July through late-August of 2006, we conducted experiments in two streams: Black Canyon and Deer Creek. Black Canyon and Deer Creek are similar in their fish densities, habitat characteristics, and thermal regimes; but have different fish species compositions and discharge. Deer Creek contains only *O. mykiss* and potentially sculpin (*Cottus* spp.), while Black Canyon harbors *O. mykiss*, *O. tshawytscha*, *Cottus* spp., and dace (*Rhinichthys* spp.). Observations from 2005 indicated that interactions between non-salmonids were limited or absent—the species of interest in this experiment are *O. mykiss* and *O. tshawytscha*. Black Canyon is a protected wilderness area in the Ochoco National Forest, while Deer Creek is in the Malheur National Forest.

### ***Methodology***

***General design:*** In each of the two streams, 8 control and 8 treatment sites were maintained, totaling 16 treatment and 16 control sites. In this case, a “site” refers to a cluster of three adjacent pools (Fig. 6). Before the beginning of the experiment, suitable sites were identified based on distance from downstream existing study sites, their distance from one-another, similarity of habitat characteristics, and comparable fish densities determined from snorkel surveys. Below the lowest site in each stream, we installed stationary passive integrated transponder (PIT) antennas for monitoring downstream emigration of salmonids for the duration of the experiment. Next, we conducted mark-recapture population estimates for salmonids, using block nets in each pool and a return interval of at least 24 hours for the recapture event. All salmonids caught via electrofishing and snorkel-herding into a seine net were PIT-tagged, measured for fork length, weighed to the nearest 0.1 g, and returned to the location where they were caught. After these procedures, all block nets were removed so that fish were free to migrate during the course of the experiment. At the termination of the experiment, block nets were re-installed and mark-recapture sampling was repeated. Systematic, non-lethal samples of fish gut contents were performed during the final mark-recapture event.

Figure 6. Diagram of experimental set-up.



***Food treatments:*** At each treatment and control site, a conveyer belt feeder was installed in the riffle immediately upstream of the central pool (“Pool B,” Fig. 2). At the treatment sites, soaked freeze-dried krill (acting as invertebrate drift, the predominant feeding mode of salmonids in this system) was delivered by the belt feeder in amounts equal to 5%/day of initial salmonid biomass (determined according to bioenergetic equations for optimal growth) in Pool B, for a period of 6 weeks. Food delivery was divided between two daily sessions: one hour centered at dawn and one hour centered at dusk. This occurred on a daily basis for each treatment site until termination of the experiment.

***Fish behavior:*** For the duration of the experiment, two underwater video cameras were utilized to capture fish feeding and aggression behavior in Pool B at all sites at dawn and dusk. Visitations with the video camera were scheduled so that every site was filmed the same number of times, but the filming order was randomized. Behavioral scores from films will be compared in a BACI fashion, where treatments are compared to controls and to 2005 results.

***Fish movement:*** Permanent block nets may produce artificial responses in fish growth and drift densities known to occur in fish enclosures (Zimmerman and Vondracek 2006). In this experiment, block nets were removed after initial mark-recapture event and fish were allowed to migrate in and out of the sites during the course of the experiment. Fish were individually marked (PIT-tagged) so that their movement between pools from the start to end of the experiment could be determined. The stationary PIT antennae at the downstream end of each stream recorded fish migrating to the mainstem South Fork or lower reaches of the respective tributary. Upstream and downstream movement, as well as population estimates from each pool, will be compared among treatment and control sites and between streams.

***Fish growth:*** Individually-marked fish from all sites were measured for fork length, weighed to the nearest 0.1 g, and systematically sampled for gut content analysis before and after the termination of the experiment. As in the movement analysis, fish growth will be compared among treatment and control sites and between streams.

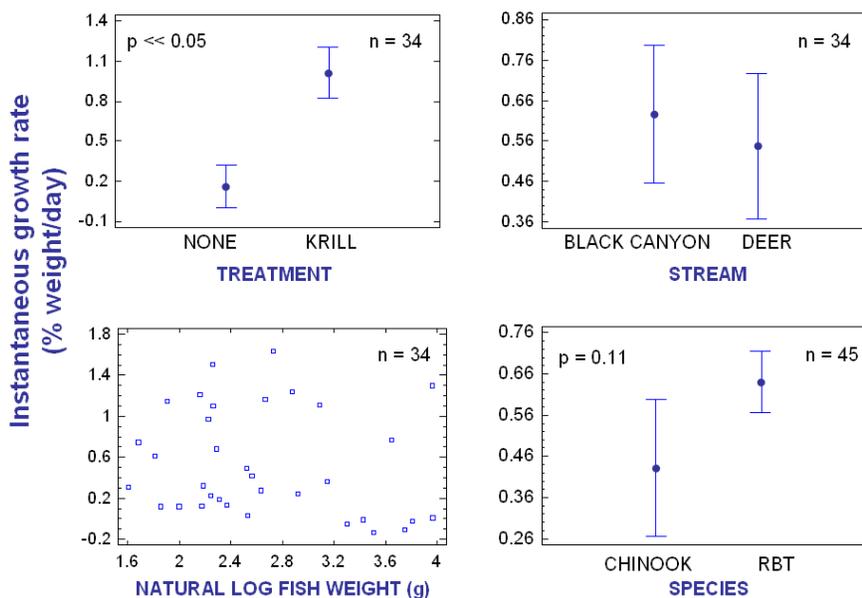
***Other habitat information:*** During the course of the experiment, we systematically monitored baseline drift densities immediately upstream and downstream of the study reaches using 250  $\mu$ m drift nets. Temperature loggers were also installed in shaded, mixed-water areas in Pool B at 16 evenly-distributed sites, recording stream temperatures every 15 minutes. Before and after the experiment, stream discharge was measured at the downstream end of the lowest site in each tributary. Habitat measures were recorded for all 3 pools in each site after the experiment was completely terminated.

## Results

2005 behavior observations: Observations from the summer of 2005 revealed that in the South Fork John Day River, juvenile *O. mykiss* aggression and feeding behaviors were relatively homogenous at the reach scale (S. White, unpublished data). Patterns of behavior were associated primarily with high abundances of juvenile *O. mykiss* and juvenile Chinook salmon (*O. tshawytscha*), habitat size as measured by pool width, and the degree of embeddedness of substrates. For instance, in the presence of high abundances of conspecifics, *O. mykiss* behavior shifted from high rates of intra- and interspecific aggression to high rates of feeding in the drift. Where substrates were embedded to a higher degree, *O. mykiss* intraspecific aggression was also more frequent. And in the presence of high abundances of *O. tshawytscha*, *O. mykiss* behavior shifted from high rates of intraspecific interactions and benthic feeding to high rates of interspecific interactions. In larger habitats as measured by pool widths, *O. mykiss* exhibited fewer interspecific bouts and fed more frequently. Although these observations yielded interesting insights into correlative reach-scale patterns associated with *O. mykiss* behavior, the underlying mechanisms remain unclear and therefore experimental manipulation was the logical next step.

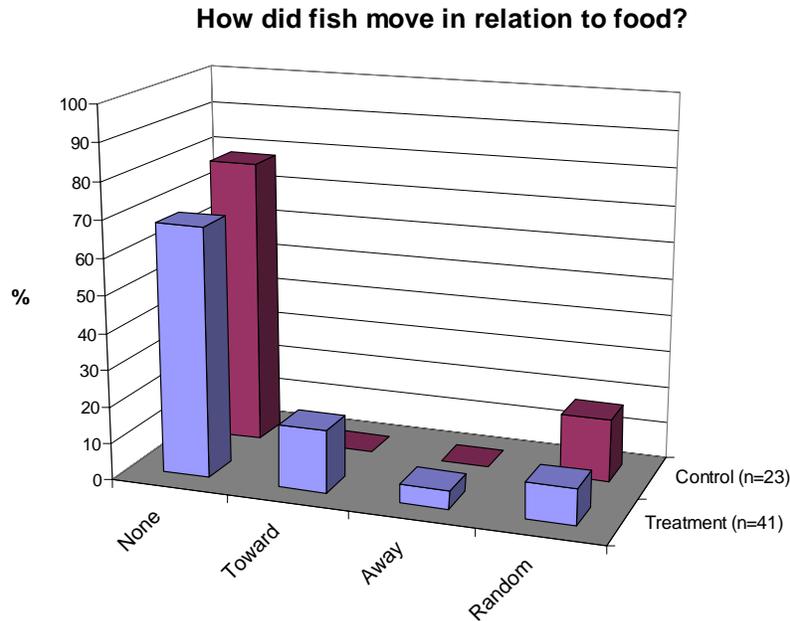
Preliminary results from 2006 field experiment: Preliminary analysis of summer 2006 data indicate that fish in treatment sites grew significantly more than fish in control sites (Fig.7). Over the 6-week period, treatment fish grew nearly 3 grams on average, while control fish grew approximately 0.5 grams.

Figure 7



Preliminary analysis of fish movement indicated that according to the predictions of the Ideal Free distribution, fish migrated to habitats where prey was augmented (Fig. 8). Most fish in treatment sites remained stationary, but also grew much faster. Density-dependent processes associated with social dominance may explain why some trout left whereas other trout were recruited. Chronological analysis of movement and time series analysis of behavior may provide insights into these patterns.

Figure 8



Drift density and videography: Results yet to be determined for this study include analysis of baseline and experimental drift densities and analysis of feeding and aggression behavior as a response to supplemental feeding. I expect that baseline drift densities will be low in both streams, comprising less food than required for optimal *O. mykiss* growth. Regarding shifts in behavior, I hypothesize that as food resources increase, so will interference competition as measured by rates of aggression. However, my observations while filming fish behavior during field experiment hinted that at least for dominant individuals, aggression increased. These results will be analyzed in a more systematic way (similar to the 2005 analysis) in order to lend confidence to these interpretations.

### References

- Boss, S.M. and J.S. Richardson. 2002. Effects of food and cover on the growth, survival, and movement of cutthroat trout (*Oncorhynchus clarki*) in coastal streams. *Can. J. Fish. Aquat. Sci.* **59**: 1044-1053.
- Marchetti, M.P. 1999. An experimental study of competition between the native Sacramento perch (*Archoplites interruptus*) and introduced bluegill (*Lepomis macrochirus*). *Biological Invasions* **1**: 55-65.
- Mason, J.C. 1976. Response of underyearling Coho salmon to supplemental feeding in a natural stream. *J. Wildl. Manage.* **40**(4): 775-788.
- Zimmerman, J.K.H. and B. Vondracek. 2006. Effects of stream enclosures on drifting invertebrates and fish growth. *J. N. Am. Benthol. Soc.* **25**(2): 453-464.

## **6. PROGRESS REPORT: Calibrating Capture Techniques for Population Estimation** **Peter Bayley (Lead Author)**

### ***Introduction***

Calibration of population counts is obviously important. It provides an unbiased estimate of the population, which of course is the fundament of natural resource management. In this report, catchability and observability are synonymous terms:

“catchability” (= observability = ‘q’):

and,

$CPUE \text{ (catch per unit effort)} = q \text{ (catchability)} \times N \text{ (abundance in a given area)}$ .

We need sufficiently unbiased estimates of  $N$  on the basis of field data based on a standardized protocol that defines the unit of effort in CPUE. CPUE is rarely proportional to abundance, because ‘ $q$ ’ is not a constant, but a random variable that is usually a function of fish species, size, and a variety of habitat properties that affect our ability to observe or catch fish. For example, in the preliminary summary of five calibrations presented in October, efficiency estimates for redband (age 1 and older) varied from 8 to 50% (6-fold) for snorkeling, and from 14 to 64% (4.5 fold) for SNERDing. Using CPUE data as a proxy for  $N$  (i.e., assuming  $q$  is constant) could easily obscure real differences in  $N$  resulting from habitat differences, including any being experimentally manipulated. Even if  $q$  does not vary according to habitat conditions encountered, in this project we need to know  $q$  to estimate  $N$ , so that we can estimate the whole population and relate it to numbers migrating and returning from salt water.

Given the range of biases, it is imperative to be able to predict  $N$  for each sample in a measured area. Any impact study or effectiveness monitoring design needs numerous samples in each watershed given the high sampling variance typical of fish sampling. However, it is prohibitively expensive if indeed feasible to obtain bias-free information by, for example, using a mark-recapture process for each sample. Therefore, we need to plan a subset of calibration samples to produce empirical models of ‘ $q$ ’ under the range of conditions expected within our survey design(s), that can subsequently be applied to any past or future standard-protocol samples satisfying the model conditions.

If any study or monitoring program requires a statement with estimated probability about a difference in absolute or relative quantities of fish, or even their presence or absence, it will typically need information that accounts for the variation in  $q$ . Exceptions are if (1) there is no physical habitat variation (which is rare), or (2) independent evidence indicates that an expected difference in catchability will not affect a qualitative conclusion that disproves the hypothesis.

For an example of (2), consider a comparison of redband density in a damaged versus a restored watershed, in which  $H_0$  represents no difference detectable with given Type II error. If the catchability in the damaged habitats is known to be higher than those in the restored habitats, yet the hypothesis is disproved (at given Type I error) in favor of more fish in the restored area, then the inference from CPUE data is acceptable. However, one cannot say by how much the population has increased. Moreover,  $q$  is often sufficiently lower in physically restored habitat that there is a danger, when using CPUE only, of failing to disprove an incorrect hypothesis or even disproving it in favor of apparently more fish in the damaged habitats.

### ***Methods and Results***

There are two issues on sampling protocols here: (a) when more than one method needs to be used, and (b) when different teams or institutions use the same nominal technique but with a different protocol.

(a) What sampling protocol(s) to use may be determined by permitting or by a consideration of observability. An experienced field biologist may not know the magnitude of ‘ $q$ ’, but knows when it is severely reduced, such

as when turbidity decreases the number of fish observed while snorkeling. Also the analyst knows the statistical nightmare that a large proportion of zero observations can produce. Calibrations can estimate low  $q$  values, but the error involved estimating  $N$  when  $q$  is very small can be huge, and zero CPUE may incorrectly indicate zero fish.

So in the case above the field biologist will sensibly change, if permitted, to a method less affected by moderate turbidity such as electrofishing. The cost is that another set of calibrations for a different model will be required, so that unbiased  $N$ -estimates can be analyzed jointly and be independent of the sampling protocol used, as well as independent of the habitat conditions that affect the  $q$  of each protocol. Therefore, the decision to change protocols should not be taken lightly, and should be taken under explicit habitat conditions.

Efficiency estimates were derived from standardized snorkel calibrations from 24 pools and ‘snorkel-herding’ (‘snerding’) calibrations from 26 pools in the South Fork John Day drainage. Variance of population estimates based on snorkel surveys from two stream accounted for variance in sampling and observation efficiency, of which the sampling variance component dominated. Population estimates for *Oncorhynchus mykiss gairdneri* based on, for example, 100 unstratified random samples from Murderers and Black Canyon creeks had estimated 95% confidence intervals that were  $\pm 24\%$  and  $\pm 15\%$ , respectively. However, snorkeling efficiency varied among snorkelers ( $P=0.0085$ ), despite the participation of experienced and equally-trained personnel. Population estimates for *O. mykiss* based on one snorkeling group were 70% higher than the other. There was also a difference for redband shiner snorkeling efficiency. Therefore calibration models should be derived for the snorkelers involved in the surveys. Conversely, snerding analysis indicated no differences among operators for redband trout, redband shiner, or speckled dace and indicated lower variances for all species (Table 2).

Table 2. Comparison of efficiencies between snorkeling and snorkel-herding (SNERDing) approaches to enumerating species of fish occupying sampled pool habitats. Species are redband trout, *Oncorhynchus mykiss gairdneri* (RBT); redband shiners, *Richardsonius balteatus* (RSS); speckled dace, *Rinichthys osculus* (SKD). Efficiency ( $q$ ) = (# observed in pool)/(estimated # vulnerable within block nets through mark-recapture). CV of observers = the Coefficient of Variation (SD/mean) of  $q$  in standard samples in calibrations. \*\* denotes significance at  $\alpha = 0.01$ , \* denotes significance at  $\alpha = 0.05$ .

	Species	Number Calibrations	Mean $q$	CV ( $q$ )	P values Observer Differences	CV of Observations
Snorkeling	RBT	24	0.31	0.49	0.008**	1.21
	RSS	17	1.41	0.89	0.05*	1.34
	SKD	15	0.26	1.21	0.76	0.70
SNERDing	RBT	26	0.30	0.45	0.67	1.05
	RSS	17	0.33	0.63	0.14	1.03
	SKD	15	0.19	1.08	0.17	1.40

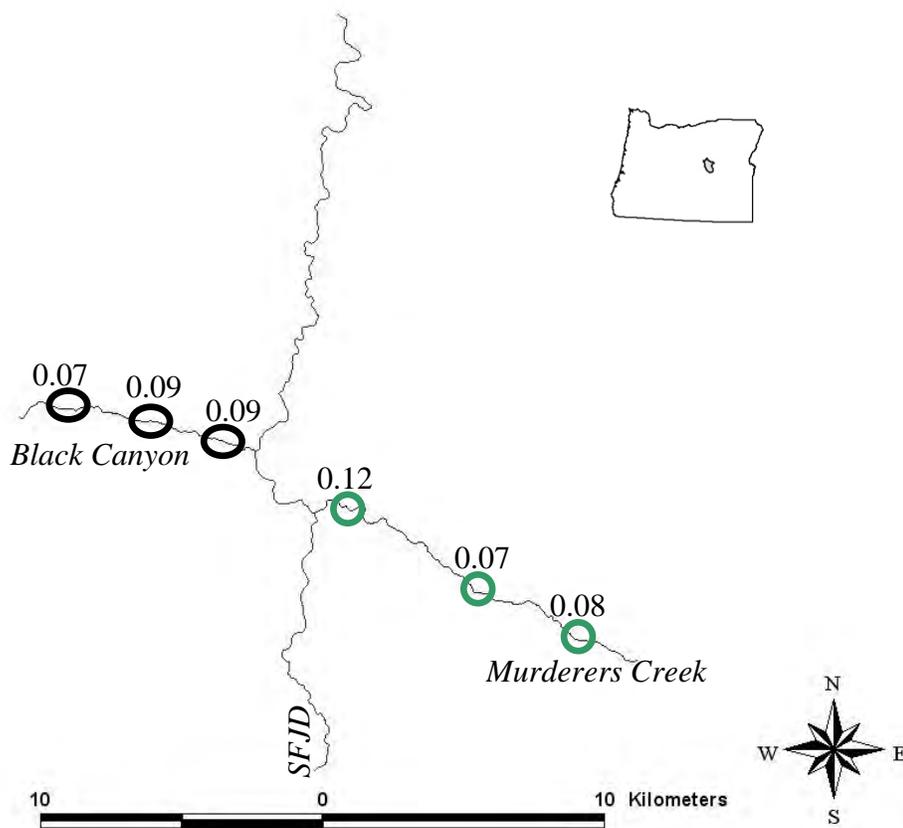
## 8. *Management Implications*

Efficiencies of detection must be conducted if we wish to compare restoration effectiveness across basins. We can reduce sampling biases among gears and effects of interpersonal differences by calibrating the two types of biases to standard mark-and-recapture estimates. Sampling different habitats requires specific sampling approaches to detect sufficient individuals for statistical purposes. However, each technique is subject to its own weaknesses and biases and by comparing each technique to a common estimate confers the ability to compare apples with oranges, we can then compare carrying capacities of different habitats using a common currency. This can be extended to comparing different watersheds among regions.

Interestingly, we found that pools in 3<sup>rd</sup> to 4<sup>th</sup> order streams can be sampled using two methods: snorkel counts and capturing fish by snorkel-herding. Snorkel-herding may be more time effective in the long run because it is not subject to interpersonal observational biases, and it has a greater detection efficiency (35-40% vs. 25%) than snorkel counts. This means that there is a savings in terms of repeated calibrations to calibrate biases among observers and to calibrate for changes in detection efficiency of an observer over time (e.g., more experience may increase detection efficiency with experience, detection efficiency may drop as a result of fatigue, etc.). We suggest that snorkel-herding be considered for adoption as a primary sampling tool, especially when PIT-tagging is needed for monitoring. Two birds can be killed with one stone. There is a need for snorkel counts, especially when conducting rapid, extensive surveys. If doing so, observer calibrations must be conducted often.

## 9. *Future Efforts:*

- (1) We will be gathering more data from the PIT-tag detectors to determine whether or not the LFSDs are potentially good tools to aid fish movement when irrigation diversion dams are required.
- (2) We will gather more data to document the productivity of cold vs. warm reaches of streams as measured by the physiological criteria for habitat quality described above.
- (3) We have been gathering data from the PIT-tag detection arrays in Columbia River dams. The hypothesis is that survival rates to the dam will be better from the coldwater reaches than those in warmer waters and as survival to this stage, largely reflects conditions of the SFJD and JD basins, it is a good biological end-point for our study (Fig. 9).
- (4) There is one caveat. Growth rate data suggests that it takes longer for redband trout to reach smolt size in coldwater streams. The ability to smolt is related to size. Therefore the data presently underestimates the contributions of cohorts from Black Canyon than Murderers Creek because of developmental time-lag (Fig. 9).
- (5) Ocean returns have started to be detected in the SFJD. 2007 and 2008 will be big return years. We will have followed the 2004 cohort through the life cycle, for the most part. This should enable us to assign spawners to their original spawning sites to determine the influence of stream temperatures from parr, to smolts, to returning adults.



**Figure 9.** The proportion of *O. mykiss* tagged during summer 2004 which were detected as smolts in the Columbia River during 2005 or 2006. Proportions were derived from raw interrogation data in the Columbia River (not corrected for detection efficiency).

### Additional References

- Bélanger, G. and M.A. Rodríguez. 2002. Local movement as a measure of habitat quality in stream salmonids. *Environmental Biology of Fishes* 64:155-164.
- Joseph W. Feldhaus. 2006 Heat Shock Proteins and Physiological Stress in Redband Rainbow Trout (*Oncorhynchus mykiss gairdneri*) in the South Fork John Day River, Oregon. MS Thesis, Oregon state University, April 27..
- Ian A. Tattam. 2006. Seasonal Life History of *Oncorhynchus mykiss* in the South Fork John Day River Basin, Oregon. MS Thesis, Oregon state University, December 22.
- Francisco Madriñan. In Progress. Estimating landscape influences on patterns of standing crop and production of *O. Mykiss* in the South Fork John Day Basin.

White, Seth. In Progress. An experimental test of the influences of interspecific competition and prey addition as influences on habitat quality and the Ideal Free Distribution for *O. mykiss* in the South Fork John Day Basin.