# Ecology of Columbia River Redband Trout, Oncorhynchus mykiss gairdneri, in Dry Creek,

# Idaho (Lower Boise River Drainage)

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Chapter 1:

## Summer Distribution and Habitat Use of Columbia River Redband Trout, Oncorhynchus

## mykiss gairdneri, in Dry Creek, Idaho (Boise River Drainage)

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#### Abstract

The Columbia River redband trout (Oncorhynchus mykiss gairdneri) is Idaho's least studied salmonid. In this investigation, I documented summer (June-August, 2013) distribution and habitat use of redband trout in Dry Creek, a 3<sup>rd</sup> order, seasonally intermittent tributary of the Boise River, in southwestern Idaho. Forty-eight reaches throughout Dry Creek were surveyed for redband trout. Thirty-five temperature loggers were deployed to determine if summer stream temperature affected trout distribution in Dry Creek. Habitat analyses of 20 isolated pools were carried out to identify characteristics of critical summer trout habitat. A total of 425 redband trout (47-250-mm TL) were captured. Redband trout occupied 19.0-km of Dry Creek during early summer, equaling 13.8% of total projected redband trout habitat in the Lower Boise River drainage. Fish were more abundant in reaches above 1130-m (present in 89% of sites, 62 redband trout per 100-m) than below (present in 70% of sites, 32 redband trout per 100-m). Redband trout were absent within 12-stream-km of the Boise River, indicating redband trout distribution in Dry Creek is probably shaped by seasonal drying. Average daily maximum stream temperatures were within redband trout tolerances (mean 19.4° C, range 14.1-27.1° C). Fish persisted longest (>3weeks) in larger isolated pools (>495-cm channel width) at moderate elevation (1042—1051-m). Redband trout were observed living at dissolved oxygen levels as low as 1.8-mg/l, which suggests adaptations to hypoxic conditions. Dry Creek redband trout survival during summer drought may be enhanced by preservation of deep pool habitats with riparian vegetation cover.

## Introduction

The Columbia River redband trout Oncorhynchus mykiss gairdneri (hereafter referred to as redband trout) is native to the Columbia River Basin east of the Cascade Range below barrier falls (e.g. Shoshone Falls) (Wishard et al. 1984; Behnke 2002; Meyer et al. 2010). Redband trout display a diversity of life history strategies including resident (remain in small streams and rivers throughout their life), anadromous, and adfluvial (Anderson 2007; Thurow et al. 2007). The species also occupies a variety of habitats—from low elevation sage-brush desert systems to high elevation forest montane streams (Wishard et al. 1984; Allen et al. 1998; Meyer et al. 2010; Narum et al. 2010). Redband trout tolerate large fluctuations in temperature (at least 11°C daily (Zoellick 1999)) and flow associated with desert streams (Gamperl et al. 2002; Schill et al. 2004; Zoellick 2004; Dambacher et al. 2009). The species has been observed in streams with temperatures as high as 28.3°C (Behnke 1992). Stream shading, cobble/boulder substrate, and deep pools are considered among the most important habitat requirements for redband trout (Behnke 1992; Meyer et al. 2010). Redband trout have received little attention among fisheries managers because of low commercial value and minimal recreational fishing pressure (Schill et al. 2004; Dambacher et al. 2009). Insufficient knowledge concerning the status and biology of redband trout has hindered their conservation.

Petitions to place different populations of redband trout on the Endangered Species Act were filed in 1994, 1995, and 1997 (Federal Register 1995a, 1995b, 2000). The 1995 petition requested the United States Fish and Wildlife Service (USFWS) to list redband trout of the Snake River drainage between Brownlee Dam and Shoshone Falls (Rhew 2007). All petitions were declared unwarranted due to lack of evidence that populations were distinct from other rainbow, redband, and steelhead trout (Rhew 2007). Currently, redband trout are considered a species of concern by the American Fisheries Society, United States Forest Service, and Bureau of Land Management (Thurow et al. 2007).

Redband trout presently occupy about 42% (25,417-km of stream) of their historical range (May et al. 2012). Within the current redband trout range, only 32% of habitat is in good condition (May et al. 2012). Logging, mining, grazing, agriculture, impoundments, and hybridization with non-native fish all contribute to the loss of redband trout (Muhlfeld et al. 2001a; Meyer et al. 2010; May et al. 2012). The Lower Boise River drainage (Boise River downstream of Lucky Peak Dam to confluence with the Snake River, southwestern Idaho) historically held 636-km of river habitat occupied by redband trout (May et al. 2012). According to May et al. (2012), only 138-km (21.7%) is thought to currently support redband trout. Dry Creek and its tributary Shingle Creek represent 39-km of possible habitat for redband trout, which is a significant proportion of total estimated redband trout habitat in the Lower Boise River drainage.

The purpose of this investigation is to describe the summer (June—August, 2013) distribution and habitat use of redband trout in Dry Creek, a 3<sup>rd</sup> order tributary of the Boise River drainage in southwestern Idaho (Fig. 1). The Dry Creek watershed includes both montane and desert reaches. Many of the lower elevation (<1310-m) desert reaches experience complete and regular drying during the summer. The role of drought (Dekar & Magoulick 2007) and habitat use of fish in intermittent streams is not well understood (Seilheimer & Fisher 2010), even though these streams provide important spawning and rearing habitat for salmonids (Wigington et al. 2006). Our investigation is the first to describe the distribution and document habitat use of redband trout in Dry Creek. Moreover, this is one of only a few studies describing redband trout ecology in the Boise River drainage (but see Meyer et al. 2010, Neville & Dunham 2011). The majority of studies on redband trout have focused on tributaries of the Snake and Owyhee Rivers

(Allen et al. 1996; Zoellick 1999, 2004, Zoellick & Cade 2006; Meyer et al. 2010) and only Zoellick (2004) investigated a system (Big Jacks Creek) similar in hydrology to Dry Creek. The Land Trust for the Treasure Valley has recently expressed interest in securing a conservation easement for portions of the Dry Creek watershed (personal communication, Tim Breuer, Land Trust for the Treasure Valley). Determining the distribution and habitat use of redband trout in Dry Creek will help managers decide if a redband trout watershed conservation plan is needed.

## **Study Area**

Dry Creek is a 3<sup>rd</sup> order tributary of the Boise River drainage approximately 10-km north of Boise, Idaho (Fig. 1). It flows through montane forest, sagebrush-steppe, rangeland, and urban areas before meeting the Lower Boise River in Eagle, Idaho (Fig. 1). For the purposes of this study, Dry Creek was divided into upper and lower reaches. Upper reaches (>1130-m in elevation) occur above the Shingle Creek and Dry Creek confluence, which is approximately 24.5-stream-km from the mainstem Boise River. Areas downstream of the Shingle and Dry Creek confluence were classified as lower reaches. The Dry Creek watershed encompasses approximately 169-km<sup>2</sup> and ranges in elevation from 775-m to 2100-m (Gould et al. 2013). The watershed consists of private and public lands, with the USFS (United States Forest Service) and BLM (Bureau of Land Management) holding the majority of land in the upper reaches of Dry Creek. Lower reaches are held primarily by private land owners. Below the tree line (1310-m) the watershed is dominated by bunch grasses (Poaceae) and sagebrush (Artemisia sp.) with a riparian community of mountain alder (Alnus sp.), black cottonwood (Populus trichocarpa), willow (Salix sp.) and wild rose (Rosa acicularis). Douglas fir (Pseudotsuga menziesii) and ponderosa pine (Pinus ponderosa) comprise much of the upland and riparian communities above 1310-m, although alder, willow, and wild rose are also present. Soils are typically shallow (< 2-m) and consist of gravelly sands and gravelly loams; bedrock is granitic, derived from the Idaho Batholith (Gerov et al. 2011, Smith et al. 2011). Annual air temperatures range from -14° C to 33° C (Smith et al. 2011). Approximately 57-cm of precipitation falls in the upper reaches of the drainage while the lower reaches receive an average of 37-cm (Kelleners et al. 2009; Williams et al. 2009; Gould et al. 2013). Most precipitation falls during winter; with snowfall accounting for 77% of annual precipitation (Smith et al. 2011). Flows in the upper reaches of Dry Creek rise in early February and peak in April at 0.6-m<sup>3</sup>/s to 1.8-m<sup>3</sup>/s (Fig. 2; Dry Creek Experimental Watershed, Department of Geosciences, Boise State University). Springs near the headwaters of Dry Creek provide perennial flow, but reaches of Dry Creek below the Bogus Basin Rd. crossing are often dry by mid-summer. Flows to these areas typically return in late September as air temperatures cool and evapotranspiration by the riparian vegetation decreases (Fig. 2) (personal communication, James McNamara, Boise State University). Much of the Dry Creek watershed is disturbed by cattle grazing and the area above Bogus Basin Rd. supports a network of mountain biking and hiking trails. A culvert (first constructed in 1941) and artificial waterfall (rock-debris from construction of Bogus Basin Rd) are present at the intersection of Dry Creek and Bogus Basin Rd. (Fig. 1).

## **Materials and Methods**

## Redband Trout Distribution

From May to July 2013, I surveyed 35 reaches (10 to 118-m in length) via electrofishing across the Dry Creek watershed for redband trout (Fig. 1). Sample reaches were approximately 20 times the active channel width as recommended by Dambacher et al. (2009). For 25 reaches, I used the two pass depletion method to assess redband trout density. I used a Smith-Root Model 15-D backpack electrofisher set at 200-volts and 20-Hz. Due to lack of time, only one pass was used for the remaining 10 sites. Redband trout captured were held in a 5-gallon bucket and anesthetized

with 50-ppm MS-222. All trout were measured (mm total length-TL) but only the first 143 fish were weighed (g) with an O-Haus portable balance. A second order regression equation of fish length vs. mass for these fish was used to estimate the mass of any additional trout captured given their length. ( $R^2$ =0.930, p<0.05) (Fig. 3).

In addition to the 35 reaches electrofished, I randomly selected 15 (30-m long) reaches for a visual assessment of redband trout occurrence (Fig. 1). Five locations within each of three elevation classes (775–913-m, 913–1226-m, and 1226–1832-m) were randomly generated from a stratified elevation map of Dry Creek using the Create Random Points function in ArcGIS (v 10.0). Due to private property restrictions, I was only able to assess three of the five low-elevation locations. I walked each 30-m reach in June—July, 2013 and recorded redband trout occurrence. Stream flows were low and water was clear during the visual surveys. Furthermore, no other fish species are present in Dry Creek (except at the creek's confluence with the Boise River) and thus the visual survey was deemed a reliable method for assessing occurrence of redband trout.

I used ArcGIS (v 10.0) and the results of our field sampling (48 reaches—35 electrofished and 13 visually inspected reaches) to create a distribution map for redband trout in Dry Creek. The distribution map also includes locations of redband trout occurrence made while preforming other aspects of this study (*e.g.* temperature logger deployment). Finally, I estimated the population density of redband trout within each of the 25 two-pass electrofished reaches using the methods of Lockwood and Schneider (2000).

### Stream Temperature

To determine if the distribution of redband trout in Dry Creek is shaped by summer stream temperatures, I deployed 35 pairs of HOBO tidbit temperature loggers throughout Dry Creek. Loggers were programmed to record stream temperature (° F and ° C) every 30-min until their

retrieval in mid to late October, 2013. At each site, a set of two loggers was fastened with plastic zip ties to the inside of a perforated PVC pipe (2.5-cm in diameter x 8-cm in length) which was secured to a 25-cm long, 1-cm wide wooden dowel. Each dowel was secured to the stream bed under large rocks and/or small mesh bags filled with sand and gravel. Two loggers were deployed at each site in case one logger experienced battery failure or otherwise became inoperable. One set of loggers was deployed on March 31, 2013, sixteen on April 13, 2013, eight on May 24, 2013, three on May 31, 2013, five on June 6, 2013, and two on June 12, 2013. Logger deployment was staggered due to the amount of time it took to access remote locations. Due to unusually low summer flows, 18 sets of loggers were collected between July 29<sup>th</sup> and August 2<sup>nd</sup>, 2013. Data were off-loaded and loggers were redeployed (using the same data recording parameters) to isolated pools (see *Pool Habitat* sub-section).

Temperature data from one logger at each site was off-loaded using BoxCar Pro 4.3. Mean daily average stream temperature, mean daily maximum stream temperature, and mean daily minimum stream temperature (° C) were calculated for July and August 2013 (hereafter referred to as summer stream temperature). Warm temperatures are known to limit the distribution of trout so analysis was limited to months with the warmest air temperatures. A graph of summer stream temperature versus time was examined to determine whether a logger was ever exposed to air during deployment (i.e. site became dry). Exposure to air was indicated by a sudden spike in temperature and increase in daily temperature fluctuations. If determined that the logger recorded summer air temperature, these data were excluded from the analyses. Mean daily, mean daily minimum, and mean daily maximum summer stream temperatures were graphed against distance from mouth using ArcGIS (v 10.0).

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## Pool Habitat

A particularly severe drought in Dry Creek during 2013 (See Fig. 2) provided the unique opportunity to study habitat characteristics critical to redband trout survival within isolated pools. Twenty pools from sections of creek with intermittent flow were surveyed for presence or absence of redband trout from July 29<sup>th</sup> to August 2<sup>nd</sup>, 2013 (Fig. 4). Redband trout movement between isolated pools was either highly unlikely or impossible due to extremely limited flow and dry streambeds. Six of the pools were located in upper reaches and fourteen were located within lower reaches. Pools were chosen to represent a wide range of habitat types and included pools with and without redband trout. Redband trout occurrence and estimated number was visually determined for each pool. A HOBO tidbit temperature logger was placed in each pool (as described earlier). Pool location and elevation (m) were recorded with a GPS (Garmin GPSMAP 62). Pool length (cm), wetted width (cm) at upstream, downstream, and widest portion of reaches, maximum wetted depth (cm), and max bank full width and depth (cm) were measured. A horizontal level was used to ensure the measuring tape was level while measuring bankfull width. Maximum bankfull depth was measured from the deepest section of the pool to the plane of maximum bankfull width. Dominant substrate type at the furthest upstream, furthest downstream, and widest sections was recorded as sand-silt (<0.2-cm in diameter, rank 1), gravel (0.2-7.5-cm, rank 2), rubble (7.5-15.0-cm, rank 3), cobble (15.0—30.0-cm, rank 4), boulder (>30-cm, rank 5), or bedrock (rank 6) (Muhlfeld et al. 2001b). At the middle of the furthest upstream, furthest downstream, and widest sections of pool the presence or absence of woody debris in the stream was recorded. At the same locations, presence or absence of overhead cover was determined using a densitometer. I also recorded the presence or absence of undercut banks. Stream velocity (cm<sup>3</sup>/s) was measured using a Swoffer Model 2100 Current Meter in the thalweg at 60% depth. Lastly, daytime dissolved

oxygen (mg/l), conductivity (ms), and total dissolved solids (mg/l) were measured for each pool. I also measured dissolved oxygen concentrations of pools containing redband trout one week post the initial measurement. Pools were revisited weekly from July 29<sup>th</sup> to August 30<sup>th</sup>, 2013 to determine if redband trout continued to persist.

Temperature data from pools were analyzed as previously presented. The relationship between redband trout persistence and pool habitat data were assessed using principal components analysis (PCA). PCA is a statistical technique used to reduce the number of variables in a multivariate dataset to those that explain the most amount of information in the original set of variables (Dunteman 1989). The goal is to reduce the dimensionality of the original data set. I used the PCA function (bootstrap value of 10000) in PAST (Paleontological /Statistics Version 3.0) to calculate the number of principal components which accounted for most of the variation in the original 16 habitat measures. I also used PAST to calculate the strength of association between each habitat variable and component score (based upon factor loadings).

### Results

### Redband Trout Distribution

Four-hundred twenty-five redband trout were captured during the electrofishing survey. Mean TL of captured redband trout was 199-mm and sizes ranged from 60—250-mm (Fig. 5). Mass of captured fish ranged from 4 to 140-g, with a mean of 20-g. The total estimated early summer range of redband trout in Dry Creek is 19.0-stream-km. Redband trout were present in 88.9% (16/18) of the upper reach sites and 70.0% (21/30) of the lower reach sites of Dry Creek (Fig. 6). Average density of redband trout in the upper reaches of Dry Creek was 62 redband trout per 100-m (range 41—95, n=3) while in the lower reaches I found an average of 32 per 100-m (range 0—100, n=22). Redband trout were only absent from two upper reach sites—both of which were non-flowing tributaries to Dry Creek. Redband trout were found in Shingle Creek at several locations (Fig. 6). The most downstream occurrence of redband trout in Dry Creek was just below the town of Hidden Springs, 12-stream-km away from the confluence with the Boise River (Fig. 6). No other fish species were detected in Dry Creek except at the creek's confluence with the Boise River. At this site, I captured northern pikeminnow (*Ptychocheilus oregonensis*), bluegill (*Lepomis macrochirus*), speckled dace (*Rhinichthys osculus*), common carp (*Cyprinus carpio*), redside shiner (*Richardsonius balteatus*), longnose dace (*Rhinichthys cataractae*), largemouth bass (*Micropterus salmoides*), largescale sucker (*Catostomus macrocheilus*), and oriental weatherfish (*Misgurnus anguillicaudatus*).

## Stream Temperature

Summer stream temperature results are summarized in Table 1 and presented graphically in Figure 7 and 8. Mean daily summer stream temperature was 15.4° C (range 13.5—17.0° C, n=7) in the upper reaches and 18.6° C (range 16.8—22.7° C, n=18) in the lower reaches while mean daily minimum summer temperature was 13.5° C (range 12.3—14.6° C) in upper reaches and 16.4° C (range 14.7—19.0° C) in lower reaches. Upper Dry Creek had a mean daily maximum summer temperature of 18.0° C (range 15.0—21.0° C) while sites in lower Dry Creek had an average daily max of 21.5° C (range 18.1—27.1° C). Average daily summer stream temperature fluctuation was 4.5° C in upper Dry Creek and 5.12° C in lower Dry Creek. In upper Dry Creek, 42.9% (3/7) of logger sites dried up before the end of summer, while 88.9% (16/18) of logger sites in lower Dry Creek were dry before August 31<sup>st</sup>, 2013.

Summer stream temperature in Shingle Creek had a mean daily temperature of 14.4° C (range 12.4—16.8° C, n=10), a mean daily minimum temperature of 12.9° C (range 11.2—14.3° C), and a mean daily maximum temperature of 16.3° C (range 14.1—19.3° C) (Table 1). Average

daily summer temperature fluctuation was 3.4° C in Shingle Creek. Complete drying occurred at 70.0% (7/10) of temperature logger sites in Shingle Creek by August 31<sup>st</sup>, 2013.

## Pool Habitat:

Mean daily isolated pool temperature was 17.1° C (range 13.5—17.0° C, n=20) while mean daily minimum temperature was 15.4° C (range 12.3—14.6° C) and mean daily maximum temperature was 19.3° C (Table 1, Fig. 7, Fig. 8). Average daily isolated pool temperature fluctuation was 3.9° C.

PCA analysis revealed that two principal components accounted for over 95% of the variation in the original 16 habitat variables. The first principal component explained 89.1% of the variation in the data and the second principal component explained 6.7% of the variation in the data. Channel width loaded strongly on the first principal component and elevation loaded strongly on the second principal component (Fig. 9). Thus, the first principal component was interpreted as a function of channel size while the second principal component described pool elevation Temperature, substrate type, average cover, daytime dissolved oxygen, conductivity, and woody debris loaded weakly on both the first and second principle component. Before August 31<sup>st</sup>, 2013, 25.0% (5/20) of the isolated study pools were dry (Table 1). Pools that contained fish for more than one week were large in size and located at moderate elevations (1042—1051-m) (Fig. 9). The only pool that contained fish for all four weeks was the largest pool sampled (bankfull width 815cm). Dissolved oxygen levels were notably low in many pools containing redband trout. Redband trout were present in pools with daytime dissolved oxygen levels ranging from 1.8 to 7.8-mg/l (mean 2.7-mg/l, n=12) during the first week of sampling. In the second week, redband trout were observed at dissolved oxygen levels 2.0 to 4.4-mg/l (mean 4.5-mg/l, n=5). In the most hypoxic

conditions (<2.0-mg/l) redband trout gilled quickly near the surface, remained stationary unless disturbed, and possessed white discoloration especially around the head.

## Discussion

## Redband Trout Distribution

Redband trout were distributed in Dry Creek above the town of Hidden Springs, Idaho, and were more abundant in upper reaches (>1310-m). Based on the distribution map, the estimated early summer range of redband trout in Dry Creek is 19.0-total-stream-km. May et al. (2012) estimated that the Lower Boise River drainage contained only 138 km of habitat occupied by redband trout. Dry Creek therefore equals 13.8% of total projected redband trout habitat in the Lower Boise River drainage. In Dry Creek, most of the stream sections that did not contain redband trout were dry during July and August. 88.9% (16/18) of temperature logger sites in lower Dry Creek dried up by August 31<sup>st</sup>, 2013, compared to of 42.9% (3/7) temperature logger sites in upper Dry Creek. The stream normally dries downstream of Bogus Basin Rd. during the summer, but flows were extremely low in summer 2013 (personal communication, James McNamara, Boise State University, Fig. 2). Even if some water is retained in intermittent streams, low flows may eliminate fish from streams indirectly through increases in water temperature or decreases in dissolved oxygen levels (Caruso 2002; Moyle & Cech 2004). Low flow also reduces water quality (Schindler 1997; Caruso 2002; van Vliet & Zwolsman 2008) through the concentration of inorganic solutes (Boar et al. 1995; Reynolds & Edwards 1995) and fecal coliforms originating from cattle (Caruso 2002). In addition, low flow intensifies eutrophication and prevents the washing out of fine and organic sediments (Matono et al. 2012). Thus, seasonal drying of the lower Dry Creek reaches most likely contributes to the absence of redband trout in these areas.

Channelization of some sections of lower Dry Creek near Eagle, Idaho may worsen the impacts of summer drying. Altering the natural channel changes seasonal drying patterns (Beugly & Pyron 2010). Channelization results in elevated drying rates in late summer and early fall due to higher water temperatures and faster flow rates (Beugly & Pyron 2010). Moyle (2002) found stream channelization to result in great declines in populations of two trout species in California. Channelization of Dry Creek may contribute to stream drying in July and August and restrict the distribution of redband trout.

Summer water temperature is strongly related to redband trout occurrence in Idaho streams (Meyer et al. 2010), but temperature does not appear to a primary factor limiting the distribution of redband trout in Dry Creek. Mean average daily maximum stream temperature in Dry Creek during summer 2013 was 19.3° C (range 14.1-27.1° C, Table 1, Fig. 7, Fig. 8), which is below reported maximum temperature tolerances of redband trout ( $\geq 28^{\circ}$  C) (Behnke 1992; Zoellick 1999; Zoellick 2004; Meyer et al. 2010). However, Meyer et al (2010) found redband trout to be less common when mean summer stream temperature exceeded 16° C. Thus, following Meyer et al.'s (2010) findings, summer temperatures of lower Dry Creek may not be ideal for redband trout. Furthermore, to protect cold-water fishes, stream temperatures should not exceed 22° C with a maximum daily average of 19° C (Idaho Department of Water Quality 2000). Zoellick (2004) supported the appropriateness of this criteria for redband trout populations by finding significant correlation (R = -0.67, p<0.03) between trout density and maximum stream temperature. Sites with maximum temperature above 22° C had lower abundance of redband trout (Zoellick 2004). In July and August, 2013, Dry Creek exceeded the IDWQ recommendations for cold-water fishes. Sites in lower Dry Creek had average daily maximums up to 27.1° C (Fig. 7, Fig. 8).

Though average summer stream temperatures in Dry Creek were above ideal for redband trout, thermally appropriate micro-habitats likely exist. Redband trout may avoid temperature stress by leaving the area or seeking local thermal refugia (Meyer et al. 2010). Coastal rainbow trout (Matthews & Berg 1997; Ebersole et al. 2001) and McCloud River redband trout (Nielsen et al. 1994) have been observed occupying areas of cooler temperatures, such as near groundwater seeps, during midday heat. Li et al. (1994) also thought that trout utilized cooler microhabitats when temperatures neared 23—25° C. Springs provide thermal refugia against high temperatures (Seilheimer & Fisher 2010). Multiple groundwater springs feed into upper Dry Creek, which could supply cool water to localized areas (personal communication, James McNamara, Boise State University).

Within desert streams, shading by riparian vegetation is important for lowering and stabilizing in-stream temperatures (Zoellick & Cade 2006). Meyer et al. (2010) found stream shading to be the most important variable in montane streams and the second most important variable in desert streams in determining the density of redband trout in the interior Columbia River Basin. Increased cover provides lower water temperatures, more protection from predation, and increased invertebrates (Meyer et al. 2010). Much of Dry Creek, especially upper Dry Creek, has a dense riparian zone. Temperatures in Dry Creek are much cooler than they would otherwise be if riparian vegetation was absent. Riparian cover is vital to keep water temperatures within isolated pools suitable for trout (Beugly & Pyron 2010). Fencing of the riparian corridor in Dry Creek and providing off-stream watering areas for cattle would help to maintain suitable summer stream temperatures for redband trout.

Except at the confluence of Dry Creek and the Boise River, redband trout are the only species of fish found in the Dry Creek watershed. Limited information exists on how commonly

redband trout are the sole fish in a stream system. In the Owyhee River drainage, Allen et al. (1998) found other fish species (*e.g.* northern pikeminnow, speckled dace, longnose dace, redside shiner, and largescale sucker) with redband trout in 11 out of 12 streams. While limited information about redband trout exists, it has been shown elsewhere that streams with more variable flow patterns generally support fewer numbers of species (Fritz et al. 2002; Moyle & Cech 2004). Only species specifically adapted to variable conditions are able to inhabit such systems (Fritz et al. 2002). Native fishes have been found to be greater adapted to conditions of intermittent streams than introduced species, and therefore have an advantage in such systems (Giddings et al. 2006; Dekar & Magoulick 2007). By tolerating variable systems such as Dry Creek, native fishes benefit from reduced predation and competition (Willians & Coad 1979).

In Dry Creek, isolation of redband trout from other species may increase redband trout local abundance (Reeves et al. 1987; Zoellick & Cade 2006; Meyer et al. 2010). Reeves et al. (1987) found that competition between redside shiners and juvenile steelhead trout favors redside shiners and decreases steelhead trout abundance. Meyer et al. (2010) reported greater biomass of redband trout in desert streams with less piscivorous fishes such as northern pikeminnow and smallmouth bass (*Micropterus dolomieu*). Zoellick and Cade (2006) also believed that introduced smallmouth bass decreased redband trout abundance. Within the Boise River basin, isolation from exotic fishes is a major contributor to the survival of native bull trout populations (Dunham and Rieman 1999), and may contribute to the survival of Dry Creek redband trout.

Redband trout abundance in lower Dry Creek may also be impacted by poor habitat quality caused by cattle grazing and channelization. Human impacts on intermittent streams are poorly studied (Tiemann 2004), but cattle grazing is known to damage freshwater ecosystems and contribute to the decline of native trout (Kauffman & Krueger 1984; Behnke 1992; Fleishner 1994;

Kauffman et al. 2004). Redband trout select pool areas with deeper, slower moving waters and abundant cover (Muhlfeld et al. 2001b). Due to this preference, the biomass of age 1+ redband trout often increase in a downstream direction (Behnke 1992). In Dry Creek, however, seasonal drying and cattle grazing may eliminate downstream habitat. Redband density was nearly double in upper reaches (62 redband trout per 100-m) compared to lower (and more heavily grazed) reaches (32 redband trout per 100-m). Much of Dry Creek has a width of 1-m or less, making Dry Creek redband trout density values comparable to area densities reported for other streams. Within the Owyhee River drainage, Big Jacks Creek supported a redband trout density of 10 to 80 redband trout per 100-m<sup>2</sup> (Zoellick 2004). The density range in Big Jacks Creek is similar to the lower reaches of Dry Creek (32 redband trout per 100-m), which like Big Jacks Creek is strongly impacted by cattle. There is less grazing in upper Dry Creek, especially in high elevation reaches. Little Jacks Creek, which has had no cattle grazing since 1976, had a density of 60 to 130 redband trout per 100-m<sup>2</sup> (Zoellick 2004). This density range is similar to that of upper Dry Creek (62 redband trout per 100-m). The magnitude to which cattle grazing contributes to redband trout abundance in Dry Creek is difficult to discern because upper and lower reaches have many other differences, such as summer stream flow patterns. However, Meyer et al. (2010) found the percentage of fine sediment to be the second strongest contributor to redband trout density. Habitat damage such as stream sedimentation and destruction of undercut banks can be reduced by keeping cattle, as well as mountain bikers and hikers, off of banks and out of the creek.

#### Pool Habitat:

In Dry Creek, large pools at moderate elevations served as refugia for redband trout during low flow (Fig. 9). Many researchers have documented fishes utilizing isolated pools as refuges within seasonally intermittent streams (John 1964; Capone & Kushlan 1991 Taylor 1997; Fritz et al. 2002; Wigington et al. 2006; Davy & Kelly 2007; Dekar & Mogoulick 2007). Multiple studies found fish richness to increase in pools with greater depth and channel size (John 1964; Capone & Kushlan 1991; Taylor 1997). Fish are also more likely to persist through episodes of drought in larger pools (Dekar & Magoulick 2007).

Overall survival of redband trout in isolated pools during this study was low. Only one pool sheltered fish throughout the study period. Isolated pools inflict physiochemical stress and increase vulnerability to predation (Larimore et al. 1959; John 1964, Capone & Kushlan 1991; Magoulick & Kobza 2003; Dekar & Magoulick 2007). High predation by raccoons on trapped or recently deceased fish in Dry Creek is possible given numerous tracks found near isolated pools. Raccoons were also recorded (via motion activated camera) moving through the dry stream channel on a regular basis. John (1964) reported that raccoons foiled multiple experiments by catching all fish in a series of shallow, isolated pools in Arizona. Raccoons did not capture fish in deeper pools (John 1964).

While redband trout were present in isolated pools in Dry Creek, many were exposed to remarkably low dissolved oxygen concentrations (1.8—2.0-mg/l). Numerous researchers have noted the abilities of redband trout to cope with large fluctuations in temperature and stream flow (Zoellick 1999, 2004; Gamperl et al. 2002; Schill et al. 2004; Dambacher et al. 2009), but only Vinson & Levesque (1994) have examined the ability of redband trout to survive large declines in dissolved oxygen levels. Vinson and Levesque (1994) found redband trout in Sinker Creek, a 2<sup>nd</sup> order tributary of the Snake River in southwestern Idaho, living at dissolved oxygen levels as low as 2.0-mg/l. While most did not survive the duration of the study (which included weekly electrofishing), redband trout tolerance of dissolved oxygen < 2.0-mg/l up to a month was recorded (Vinson and Levesque 1994).

With the possible exception of redband trout, salmonids are among the most sensitive fish species to hypoxia (Dunn & Hochachka 1986; Wulff et al. 2012). Van Raaij et al. (1996) found 40% mortality of coastal rainbow trout following acute exposure to 3.5-mg/l dissolved oxygen for 90-min. Salmonids typically inhabit cold, flowing and well oxygenated environments, making hypoxia an uncommon stressor. However, redband trout have occupied high desert streams of the Columbia River plateau for approximately 30,000-50,000 years (Behnke 1992) and thus may have evolved behavioral, physiological, or morphological adaptations for dealing with the low and variable oxygen levels common to these stream systems. Dry Creek redband trout in hypoxic pools performed aquatic surface respiration (ASR) (i.e. rapid ventilation of their gills near the air-water interface) in the same fashion described by Vinson and Levesque (1994). With few aquatic primary producers, most of the oxygen in isolated pools diffuses from the air, so levels of oxygen are highest near the water surface. I observed fish to occupy the shallowest habitat in the pool, often with their dorsal fin exposed to the air. Fish were also very lethargic in their behavior. Gee (1978) found rainbow trout to not display ASR when exposed to hypoxic conditions (<2.0-mg/l). Gee (1978) suggested that salmonids had not evolved ASR behavior because they typically occupy aquatic habitats with high dissolved oxygen. Vinson and Levesque (1994) speculated that redband trout may be an exception to Gee's (1978) conclusion. My findings seem to agree with Vinson and Levesque (1994), suggesting that ASR may be an important adaptive behavior which allows redband trout to survive in high desert streams with seasonal hypoxia. Specific conclusions regarding redband trout physiological adaptations to hypoxia are beyond the scope of my study. Because redband trout hypoxia physiology has not been studied, rainbow trout that survive hypoxia are currently the closest studied model. Since redband trout are closely related to rainbow trout (Behnke 1992), adaptations of redband trout to hypoxia are most likely exaggerated rainbow

trout mechanisms. Based on field observations of redband behavior under hypoxia and the findings of van Raaij et al. (1996), I hypothesize that redband trout exhibit calm behavior in response to hypoxia in order to reduce lactate accumulation in the tissues. Redband trout may also have higher gluconeogenesis-abilities than rainbow trout, as indicated by cortisol and plasma free-fatty-acid levels in rainbow trout that survive hypoxia (van Raaij et al. 1996). As global climate change leads to increased summer temperatures, less snowpack, and resulting drought, adaptations to hypoxia will become even more important to redband trout survival and persistence in desert streams. Our findings regarding redband trout in low dissolved oxygen legitimize further research into redband trout hypoxia tolerance.

Dry Creek redband trout were more common and more widely distributed in upper Dry Creek than lower Dry Creek. Isolation from other fish species removes interspecific competitive pressures from redband trout of Dry Creek and may protect them from hybridization with hatchery rainbow trout. During extremely low flows of August, 2013, the largest isolated pools served as refugia for redband trout. Fish were also observed living in highly hypoxic conditions, indicating that redband trout may be adapted to low dissolved oxygen levels common to intermittent systems. Dry Creek is a unique and dynamic system that would benefit from preservation of deep pools and riparian vegetation. Climate change and groundwater draws could seriously impact systems such as Dry Creek (Seilheimer & Fisher 2010), which rely on winter snowfall and springs for perennial flows.





















**Table 1:** Temperature data collected during July—August, 2013 including average daily stream temperature (°C), average daily minimum stream temperature (°C), average daily maximum stream temperature (°C), number of temperature loggers (°C), and percent of temperature logger

Location	Mean (°C)	Average Minimum (°C)	Average Maximum (°C)	Ν	% Sites Dried
Lower Dry Creek	18.6	16.4	21.5	18	88.9%
Upper Dry Creek	15.4	13.5	18.0	7	42.9%
Shingle Creek	14.4	12.9	16.3	10	40.0%
Isolated Study Pools	17.1	15.4	19.3	20	25.0%
All	16.9	15.0	19.3	55	50.9%

sites that dried up on or before August 31<sup>st</sup>, 2013.









Fig. 9: Principal components analysis of habitat characteristics of twenty isolated pools. Open circles represent pools that did not contain redband trout July 29<sup>th</sup>-August 2<sup>nd</sup>. PC1, channel size and pool volume, explains 89.1% of the variation. PC2, elevation, explains 6.7% of the variation. Pools marked by closed circles held fish only during the initial week. Diamonds mark pools that held fish for two weeks. Squares mark pools that held fish in the third and/or fourth weeks.

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Chapter 2:

# Genetics and Summer Movement Patterns of Columbia River Redband Trout,

Oncorhynchus mykiss gairdneri, in Dry Creek, Idaho (Lower Boise River Drainage)

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#### Abstract

The Columbia River redband trout (Oncorhynchus mykiss gairdneri) is Idaho's least studied salmonid. In this investigation, I documented the genetics and summer (late May-September, 2013) movement of redband trout in Dry Creek, a 3<sup>rd</sup> order, seasonally intermittent tributary of the Boise River in southwestern Idaho. I surveyed 48 stream reaches throughout the watershed by backpack electrofisher. Four hundred seven redband trout (47–250-mm TL) were implanted with PIT tags. I collected fin clips from 136 individual fish for genetic analysis upstream and downstream of a road culvert and artificial waterfall. Fish were genotyped at 187 SNPs using Fluidigm 96.96 Dynamic Array. Redband trout movement was monitored May 30—September 30 via two 10' BIO Lite in-stream PIT Tag Antenna Systems, located upstream and downstream of a road culvert and artificial waterfall. Genetic analyses confirmed a single population ( $F_{ST}=0.015$ ) of non-introgressed Columbia River redband trout with relatively low genetic diversity (He=0.24). Principal components analysis revealed that Dry Creek redband trout appear to have genetically drifted from other populations of southern Idaho resident redband trout. Movement detected by the arrays was 91% (52/57 individual fish) upstream (median 92-m) and 9% downstream (median 53-m). 56.1% (32/57) of detections occurred in June, prior to drying of many reaches in mid-July. Maintaining isolation between redband trout of Dry Creek and non-native trout of the Boise River will help protect the genetic heritage and fitness of Dry Creek redband trout. Upstream reaches with perennial flow probably provided refugia for Dry Creek redband trout during drought.

# Introduction

The Columbia River redband trout *Oncorhynchus mykiss gairdneri* is native to the Columbia River Basin east of the Cascade Range (Wishard et al. 1984; Behnke 2002; Meyer et al. 2010). Redband trout display a diversity of life history strategies including resident (remain in small streams and rivers throughout their life), anadromous, and adfluvial (Anderson 2007; Thurow et al. 2007) The species also occupies a variety of habitats—from low elevation sage-brush desert systems to high elevation forest montane streams (Wishard et al. 1984; Allen et al. 1998; Meyer et al. 2010; Narum et al. 2010). Redband trout tolerate large fluctuations in temperature (at least 11°C daily (Zoellick 1999)) and stream flow associated with desert streams (Gamperl et al. 2002; Schill et al. 2004; Zoellick 2004; Dambacher et al. 2009). The species has been observed in streams at temperatures as high as 28.3°C (Behnke 1992). Redband trout have declined within its range due to habitat loss (the result of dams, water diversion, mining, agriculture, and cattle grazing) and hybridization with non-native fish (Muhlfeld et al. 2001a; Meyer et al. 2010; May et al. 2012).

Petitions to place different populations of redband trout on the Endangered Species Act were filed in 1994, 1995, and 1997 (Federal Register 1995a, 1995b, 2000). The 1995 petition entreated the USFWS to list redband trout of the Snake River drainage between Brownlee Dam and Shoshone Falls (Rhew 2007). All three petitions were declared unwarranted due to lack of evidence that populations were distinct from other rainbow, redband, and steelhead trout (Rhew 2007). Redband trout have received little attention among fisheries managers because of low commercial value and minimal recreational fishing pressure (Schill et al. 2004; Dambacher et al. 2009). Currently, redband trout are considered a species of concern by the American Fisheries Society, United States Forest Service, and Bureau of Land Management (Thurow et al. 2007).

Hybridization is considered one of the greatest threats to native trout species (Weigel et al. 2003; Wilson & Turner 2009; Neville & Dunham 2011). All 10 members of *Oncorhynchus* are threatened within their native ranges, and hybridization is one of the main contributors (Wilson & Turner 2009). Introgression, a type of hybridization, occurs when the genes of one species are incorporated into another (Muhlfeld et al. 2009; Meyer et al. 2010; Simmons et al. 2010, Neville and Dunham 2011). Introgression can lead to the complete replacement of parental types by hybrids (Neville and Dunham 2011). Since all offspring of hybrids are themselves hybrids, introgression permanently eliminates genetic and phenotypic heritage.

Redband trout are vulnerable to introgression with hatchery rainbow trout due to long-term evolutionary isolation of the subspecies. Redband and hatchery rainbow trout lineages diverged in the late Pliestocene when redband and rainbow trout lineages were isolated by the final glacial advance (Behnke 1992; Brown et al. 2006). This produced phylogenetically distinct coastal and inland groups of rainbow trout (Currens et al. 1990; Brown et al. 2006; Brunelli et al. 2013). When glaciers retreated 15 to 10 thousand years ago, redband trout were the dominant resident trout of the interior Columbia River Basin below barrier falls (Behnke 1992). The most ancestral rainbow trout traits belong to fish around the Gulf of California, and rainbow trout display more derived traits in a northward direction (Behnke 1992). Redband trout have higher scale counts, fewer pyloric caeca, elliptical rather than rounded parr marks, greater yellow or orange tints to the body, and lighter colored tips on dorsal, anal, and pelvic fins than coastal rainbow trout (Behnke 1992). Diploid chromosome numbers of redband trout are also 2-4 lower (Behnke 1992). Most hatchery rainbow trout are believed to be derived from coastal rainbow trout of the McCloud River or Mt. Shasta population in California between 1879 and 1888 (Thorgaard 1983; Behnke 1992; Brown et al. 2006). Hatchery rainbow trout have diverged further from redband trout due to rigorous

selection for traits beneficial to hatchery conditions (Tymchuk et al. 2009). Strong phenotypic differentiation between hatchery and wild strains of rainbow trout has been observed, such as rapid growth rates of hatchery rainbow trout (Tymchuk et al. 2009). While accelerated growth could be viewed as advantageous, hatchery selection has actually made rainbow trout poorly equipped to survive in natural conditions (Behnke 1992, Tymchuk et al. 2009).

Even though they not optimally adapted to wild habitats, hatchery rainbow trout are the most widely distributed nonnative trout in the Columbia River Basin (Weigel et al. 2003; Thurow et al. 2007). The oldest stocking records in Idaho go back to 1940, but stocking likely began earlier (Weigel et al. 2003). The Idaho Fish and Game began sterilizing hatchery rainbow trout before release in 2001, but extensive gene sharing had already occurred and fertile hybrid fish persist within the system (Meyer et al. 2010). May et al. (2012) reports that 8% of all stream dwelling populations have been shown to be genetically unaltered and that at least 54% of habitats are suspected to support introgressed populations (May et al. 2012). Redband trout in mainstem North and South Forks of the Boise River have heavily introgressed with coastal rainbow trout (Neville & Dunham 2011). Some populations existed as hybrid swarms or were almost completely replaced by hatchery coastal rainbow trout (Neville & Dunham 2011). Further genetic testing is still needed to identify and direct the preservation of pure redband trout populations.

Habitat fragmentation is a problem for many populations of genetically pure redband trout (Muhlfeld et al. 2001a; Simmons et al. 2010). Waterfalls, road culverts, water diversions, and dams act as barriers by impeding fish movement (Northcote 1997; May et al. 2012). Within the current redband trout distribution, May et al. (2012) identified 561 barriers, including 221 culverts and 209 man-made dams. Population fragmentation by barriers can greatly reduce population size,

fecundity, and long-term genetic viability (Northcote 1997; May et al. 2012). Barriers can be identified by examining site specific redband trout movement (Young 1997; Nielsen et al. 1999; Muhlfeld et al. 2001b; Narum et al. 2010). Overall, very little is known about stream-dwelling resident redband trout movement. In montane environments, redband trout move downstream in fall to overwinter in deep pools (Muhlfeld et al. 2001a).

The purpose of this investigation is to describe the genetic structure and summer (May 30<sup>th</sup>—September 30<sup>th</sup>, 2013) movement patterns of redband trout in Dry Creek, a 3<sup>rd</sup> order tributary of the Boise River drainage in southwestern Idaho (Fig. 1). Many of the lower elevation (<1130m) desert reaches experience complete annual summer drying. Redband movement will be described in relation to summer drying patterns and potential barriers on Dry Creek (road culvert and artificial waterfall). Only one study (Muhlfeld et al. 2001a) investigates the movement of stream-dwelling resident redband trout, and this study focuses only on fall and winter movement. Our investigation is the first to describe redband trout movement during summer in an intermittent stream and is one of only a few studies describing redband trout ecology in the Boise River drainage (but see Meyer et al. 2010, Neville & Dunham 2011). We placed PIT tag array above and below potential barriers to examine fish movement patterns and to determine if the road culvert and artificial waterfall were barriers to fish dispersal. The genetic structure of Dry Creek redband trout above and below the potential barriers was used to assess fish movement across these barriers. Genetic data was also used to determine if Dry Creek redband trout have interbred with hatchery rainbow trout and if the Dry Creek population differs from other resident populations of redband trout in southwestern Idaho. Refuges of pure resident redband trout populations could one day be important for rehabilitation of all life history forms of the subspecies (Thurow et al. 2007). The Land Trust for the Treasure Valley has recently expressed interest in securing a conservation

easement for portions of the Dry Creek watershed (personal communication, Tim Breuer, Land Trust for the Treasure Valley). Knowledge about the movement and genetic integrity will help managers decide if a redband trout conservation plan for Dry Creek is needed.

## **Study Area**

Dry Creek is a 3<sup>rd</sup> order tributary of the Boise River drainage approximately 10-km north of Boise, Idaho (Fig. 1). It flows through montane forest, sagebrush-steppe, rangeland, and urban areas before meeting the Lower Boise River in Eagle, Idaho (Fig. 1). For the purposes of this study, Dry Creek was divided into upper and lower reaches. Upper reaches (>1130-m in elevation) occur above the Shingle Creek and Dry Creek confluence, which is approximately 24.5-stream-km from the mainstem Boise River. Areas downstream of the Shingle and Dry Creek confluence were classified as lower reaches. The Dry Creek watershed encompasses approximately 169-km<sup>2</sup> and ranges in elevation from 775-m to 2100-m (Gould et al. 2013). The watershed consists of private and public lands, with the USFS (United States Forest Service) and BLM (Bureau of Land Management) holding the majority of land in the upper reaches of Dry Creek. Lower reaches are held primarily by private land owners. Below the tree line (1310-m) the watershed is dominated by bunch grasses (Poaceae) and sagebrush (Artemisia sp.) with a riparian community of mountain alder (Alnus sp.), black cottonwood (Populus trichocarpa), willow (Salix sp.) and wild rose (Rosa acicularis). Douglas fir (Pseudotsuga menziesii) and ponderosa pine (Pinus ponderosa) comprise much of the upland and riparian communities above 1310-m, although alder, willow, and wild rose are also present. Soils are typically shallow (< 2-m) and consist of gravelly sands and gravelly loams; bedrock is granitic, derived from the Idaho Batholith (Geroy et al. 2011; Smith et al. 2011). Annual air temperatures range from -14° C to 33° C (Smith et al. 2011). Approximately 57-cm of precipitation falls in the upper reaches of the drainage while the lower reaches receive an average

of 37-cm (Kelleners et al. 2009; Williams et al. 2009; Gould et al. 2013). Most precipitation falls during winter; with snowfall accounting for 77% of annual precipitation (Smith et al. 2011). Flows in the upper reaches of Dry Creek rise in early February and peak in April at 0.6-m<sup>3</sup>/s to 1.8-m<sup>3</sup>/s (Fig. 2; Dry Creek Experimental Watershed, Department of Geosciences, Boise State University). Springs near the headwaters of Dry Creek provide perennial flow, but reaches of Dry Creek below the Bogus Basin Rd. crossing are often dry by mid-summer. Flows to these areas typically return in late September as air temperatures cool and evapotranspiration by riparian vegetation decreases (Fig. 2) (personal communication, James McNamara, Boise State University). Much of the Dry Creek watershed is disturbed by cattle grazing and the area above Bogus Basin Rd. supports a network of mountain biking and hiking trails. A culvert (first constructed in 1941) and artificial waterfall (rock-debris from construction of Bogus Basin Rd) are present at the intersection of Dry Creek and Bogus Basin Rd. (Fig. 1). The artificial waterfall is located approximately 10-m below the road culvert. The two structures together are hereafter referred to as the Dry Creek barrier.

## **Materials and Methods**

## Genetic Structure

One hundred thirty-six redband trout were collected during April and October, 2012. One hundred-eight were collected above and 28 were collected below the Bogus Basin Rd. barrier. Fish were captured using a Smith Root 15-D electrofisher set at 200-volts and 20-Hz. Fish were anesthetized with 50-ppm MS-222, weighed (g) with an O-Haus portable balance and measured (total length, mm). I cut 5-mm fin clips from a pelvic fin using scissors sterilized in ethanol. Fin clips were stored in vials of ethanol or on sheets of absorbent paper. Genetic analysis of fin clips from 136 redband trout were carried out in November 2012 at the Idaho Department of Fish and Game Fish Genetics Laboratory (Eagle, Idaho). DNA was extracted using the Nexttec Genomic

DNA Isolation Kit from XpressBio (Thurmont, MD) or OIAGEN DNeasy Tissue Kits (Valencia, CA). Prior to DNA amplification of SNP loci using primer-probe sets (fluorescent tags), an initial polymerase chain reaction (PCR) "pre-amp" was implemented using whole genomic DNA. The PCR conditions for the pre-amp step were as follows: an initial mixing step of 95° C for 15-min, followed by 14 cycles of 95° C for 15-sec and 60° C for four minutes, ending with a final 4° C dissociation step. Each fish was genotyped at 187 SNPs. Genotyping was performed using Fluidigm 96.96 Dynamic Array IFCs (chips). For each genotyping run, 96 samples (including an extraction negative control, a PCR negative control, and a PCR positive control) and 96 TaqMan SNP assays were hand-pipetted onto the 96.96 chips. Sample cocktail and SNP assay cocktail recipes are available by request from Mike Ackerman, Idaho Department of Fish and Game. Each 96.96 chip was pressurized to load the DNA and SNP assays into the array using a Fluidigm IFC Controller HX. SNP amplification on the 96.96 chips were performed using either an Eppendorf Stand-Alone Thermal Cycler (protocol: thermal mixing step of 50° C for 2-min, 70° C for 30-min, and 25° C for 10-min, a hot-start step of 50° C for 2-min and 95° C for 10-min, followed by 50 cycles of 95° C for 15-sec and 60° C for 60-sec, and a final cool down step of 25°C for 10-min) or a Fluidigm FC1 Fast-cycler (protocol: thermal mixing step of 70° C for 30-min and 25° C for 10min, a hot-start step of 95° C for 60-sec, followed by 50 cycles of 95° C for 5-sec and 25° C for 25-sec, and a final cool down step of 25° C for 10-min). Chips were imaged on a Fluidigm EP1 system and analyzed and scored using the Fluidigm SNP Genotyping Analysis Software version 3.1.1.

Total and subpopulation Hardy-Weinberg heterozygosity was used to calculate pairwise  $F_{ST}$  between Dry Creek redband trout upstream and downstream of the culvert at Bogus Basin Rd. Pairwise  $F_{ST}$  is a value of differentiation between two groups. Two populations with large genetic

differences between them have pairwise  $F_{ST}$  values closer to 1. Differences between Dry Creek redband trout and reference populations of pure and introgressed redband trout (Table 1) were investigated using individual clustering analysis in STRUCTURE, as well as Principle Components Analysis (PCA) in GENALEX (Peakall & Smouse 2006, 2012; Pritchard et al. 2000). Reference samples from known populations of hatchery rainbow trout, inland redband trout, inland introgressed redband trout, and steelhead (Table 1) were obtained from the Idaho Department of Fish and Game Genetics Lab. The Bayesian clustering algorithm in STRUCTURE uses individual genotypes to create groups with maximum adherence to patterns expected under Hardy-Weingberg equilibrium in order to determine population structure (Neville & Dunham 2011). The method of Neville and Dunham (2011) was used to force STRUCTURE to characterize two clusters (k = 2; 2011). STRUCTURE estimates the proportional ancestry (Q) for each individual ranging from 0 (pure coastal rainbow trout type) to 1 (pure redband trout type). Simulations were repeated 4 times using a burn-in length of 100,000 iterations and 100,000 Markov chain Monte Carlo replicates.

Dry Creek redband trout genotypes were also compared to reference populations (see Table 1) using PCA. PCA is a statistical technique used to reduce the number of variables in a multivariate dataset to those that explain the most amount of information in the original set of variables (Dunteman 1989). The goal is to reduce the dimensionality of the original data set. I used the PCA function in GENALEX (Peakall & Smouse 2006, 2012) to graph Dry Creek redband trout and reference population genotypes using the principal components which accounted for most of the variation across the 187 SNPS examined.

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# Redband Trout Movement Patterns

Thirty-five reaches 30-m to 120-m in length within the watershed were sampled for redband trout from May to July, 2013 using methods described earlier (see Genetic Structure section) (Figure 3). Fish were scanned with handheld PIT tag readers to determine if they had been previously tagged. Mass (g) and total length (mm) were measured and recorded. Fish were tagged with half duplex Passive Integrated Transponder (PIT) tags underneath the skin posterior to the pectoral fin and then scanned to ensure tag placement. PIT numbers were recorded in HEX format. Fish were then allowed to recover in a 5-gallon bucket without MS-222 before being released back into the reach in which they were captured.

To assess fish movement across the Bogus Basin Road barrier, we installed two 10' BIO Lite in-stream PIT tag antenna systems and Biomark HPR Plus readers on BLM land May 21, 2013 (Fig. 1). The first array is located 925 in-stream-m above the culvert at N 43.68977, W 116.17063 and the second array is located 587 in-stream-m below the culvert at N 43.58988, W 116.30595 (Fig. 1). The arrays are powered with 120W portable solar panels attached to two external batteries. The antennae rest on the bottom of the streambed and continuously scan for PIT tagged fish that pass over them. The read range extends more than 20-cm above the scanner, which is greater than the depth of the stream in that area during base flow. Time, date, and PIT tag number from each PIT tag array were downloaded weekly from May 30<sup>th</sup> to September 30<sup>th</sup>, 2013.

Movement of PIT tagged fish from original tagging location to PIT tag arrays was used to determine the direction and length of redband trout movement. Recaptures of previously tagged redband trout during the fish survey were also used to determine individual fish movement. Net movement during the study period was calculated by measuring the distance between the maximum upstream and downstream locations for each individual fish detected. Net movement was classified as upstream or downstream by the overall direction the fish moved. PIT detections that occurred while the creek bed was completely dry around the arrays were attributed to predators eating tagged fish and walking across arrays. These data were not included in analysis of redband trout movement.

## Results

# Genetics

Genetic analysis revealed that fish from above and below the culvert waterfall were not genetically distinct (Pairwise  $F_{ST}$ =0.015). Genetic diversity was relatively low both above and below the culvert/artificial waterfall (He above=0.239 and He below=0.249).

Introgression of Dry Creek redband trout with hatchery rainbow trout was minimal. Only one fish (of the 135 sampled) possessed hatchery genes and this individual was captured below the culvert. Dry Creek redband trout had lower pairwise  $F_{ST}$  values with non-introgressed reference redband trout populations (0.053—0.160) than with introgressed redband trout and hatchery rainbow trout (0.148—0.230) (Table 2). STRUCTURE analysis supported the findings that Dry Creek redband were non-introgressed with hatchery rainbow trout (Fig. 3).

While Dry Creek redband trout were not introgressed with hatchery rainbow trout, PCA revealed that Dry Creek redband trout formed their own group separate from all reference populations, including non-introgressed S. Idaho redband trout (Fig. 4). The first principle component explained 73.18% of the variation in the data. The second principal component explained 8.15%, and the third explained 5.86% of the variation in the data.

## Movement

A total of 407 redband trout (47—250-mm TL) were collected via electrofishing and PIT tagged during the survey. The upstream PIT array detected 37 different redband trout and the downstream array detected 26 individual fish. During the study period, no individual fish crossed both arrays. Overall movement was 91% (52/57) upstream (median=92-m, range 7—765-m) and 9% downstream (median=53-m, range 28—970-m). The fish with the maximum downstream movement moved 970-m downstream and become trapped in an isolated pool where it persisted until August 23<sup>rd</sup>, 2013 before disappearing. For both arrays, 56% (32/57) of detections occurred in June, prior to drying of mid-elevation reaches in mid-July. 7% (4/57) of fish were detected in May, 33% (19/57) in July, and 4% (2/57) in August. The upstream PIT array detected 37.8% (14/37) of its total detections during July. Only 7.7% (1/13) of the detections at the downstream array occurred in the same month. No movement was detected during August or September at either array.

# Discussion

# Genetics

Redband trout above and below the culvert were not genetically distinct populations (Pairwise  $F_{ST}=0.015$ ). This supports the proposal that the culvert and man-made waterfall do not block fish movement. It is possible that fish movement occurs from upstream to downstream, and that the downstream population is a "sink." Redband trout may disperse to the downstream reaches but never return upstream. Given the lack of water below the culvert during late summer, most fish trapped below it would likely perish. I would not be able to differentiate the two populations genetically, but the downstream population would not contribute to the effective population size if fish do not survive or are unable to travel back upstream and reproduce.

Genetic diversity of Dry Creek redband trout was low (He=0.239 above culvert, 0.249 below). Inability to disperse during disturbances such as drought can bottleneck small populations and endanger their long-term survival (Capone & Kushlan 1991; Fagan 2002; Taylor 1997; Novinger & Rahel 2003). If the culvert is a barrier, alteration to facilitate greater fish movement may help to increase genetic diversity, or prevent it from dropping lower. The isolation of small populations often leads to local extinction (Fagan et al. 2005; Hugueny et al. 2011). Isolated reaches need to be large and complex in order for isolated species to survive long term (Novinger & Rahel 2003).

While there are many drawbacks to isolation, barriers to fish movement may be the only immediate solution to protect native tout from hybridization with non-natives. Genetic purity is associated with limitations to gene flow (Nielsen et al. 1999; Muhfeld et al. 2001b). Many habitats that support redband trout with lower levels of introgression are small, fragmented, and isolated (Muhlfeld et al. 2001a, 2001b; Simmons et al. 2010). Novinger and Rahel (2003) also found that barriers limited invasion of non-native trout into populations of native trout. Proportions of hatchery rainbow alleles decrease at higher elevations (Rasmussen et al. 2010), but it is uncertain how significantly physical barriers contribute to this occurrence (Rasmussen et al. 2010; Neville & Dunham 2011). Hybridization is most pronounced in larger streams and rivers, leaving only small and fragmented populations of non-hybridized trout (Weigel et al. 2003; Rasmussen et al. 2010).

Dry Creek redband trout were not introgressed with hatchery rainbow trout (Fig. 3). One fish from below the culvert was found to possess hatchery genes, but the 135 other fish are genetically pure (Fig. 3). Many habitats occupied by redband trout, including Dry Creek, are harsh environments that require adaptations to fluctuating temperature, stream flow (Zoellick 1999,

2004; Gamperl et al. 2002; Schill et al. 2004; Dambacher et al. 2009), and dissolved oxygen. Local adaptations of redband trout may be necessary for continued existence in such harsh natural conditions. Because the habitats redband trout often occupy are at the extremes of trout tolerance, redband trout may operate near their natural thermal limits. Hatchery raised rainbow trout have much narrower acceptable temperature windows than wild redband trout (Behnke 1992). Rainbow trout domestication has also been found to change hormone profiles, foraging behavior, response to predators, (Tymchuk et al. 2009) and pathogen resistance (Currens et al. 1990). Also, some local adaptations of redband trout may only be apparent during periods of harsh environmental conditions like fire, drought, and winter storms (Allendorf et al. 2004). Introgression with rainbow trout could seriously endanger redband trout abilities to survive in their historic habitats (Gamperl et al. 2002). These concerns are increased in the face of oncoming global warming. Dry Creek redband trout appear to be free from introgression, making their population valuable to the preservation of pure redband trout.

The isolation of Dry Creek helps to protect the genetic integrity of Dry Creek redband trout via limiting opportunities for hybridization with mainstem Boise River rainbow trout, which are heavily introgressed with non-native hatchery trout (Neville & Dunham 2011). Redband trout are the only species I have observed in Dry Creek except at the confluence with the Boise River. Redband trout may be the only species able to survive in Dry Creek. Native fishes have been found to be greater adapted to conditions of intermittent streams than introduced species, and therefore have an advantage in such systems (Giddings et al. 2006, Dekar & Magoulick 2007). It is also probable that summer drying downstream or another barrier isolates Dry Creek, pure redband trout from the Boise River. If the Boise River were to be fully connected with Dry Creek, pure redband trout may be lost from the system forever.

Dry Creek redband trout are not only dissimilar to hatchery stocks; they appear to have diverged from other populations of pure southern resident Idaho redband trout (Table 2, Fig. 4). In the PCA, Dry Creek redband trout formed their own separate cluster (Fig. 4). This reinforces the idea that Dry Creek redband trout have been isolated for some time from other populations of redband trout. Dry Creek redband trout actually have lower pairwise F<sub>ST</sub> values with steelhead than other populations of resident redband trout in southern Idaho (Table 2). Anadromous redband trout can give rise to resident forms and vice versa as ocean access changes (Thurow et al. 2007). Steelhead may represent a parent population from which southern Idaho redband trout populations have since drifted. Other studies document that small populations and geographic isolation can lead to genetic drift and local adaptation very quickly (Nielsen et al. 1999; Narum et al. 2010). There are significant single nucleotide polymorphism (SNP) patterns related to local differences in temperature and intermittent stream flows in desert and montane streams (Narum et al. 2010). Narum et al. (2010) found clear patterns of climate adaptation (desert vs. cool) in 5 of 96 SNP markers between populations of redband trout. Isolation by distance was not supported, indicating that environmental conditions were most significant for driving population divergence and local adaptation (Narum et al. 2010). Native populations of redband trout exposed to generations of selective pressures are likely to diverge since local environments drive adaptations (Narum et al. 2010) and small populations exhibit increased genetic drift and loss of variants (Dowling & Childs 1992).

Population disturbances such as bottlenecking events would contribute to the genetic drift of isolated redband trout populations. The severe drought Dry Creek experienced during my study was most likely responsible for a substantial bottleneck of the redband trout population. Genetic data collected in this study are an excellent pre-disturbance baseline that could be utilized in the future to investigate how the Dry Creek redband trout population was affected by drought.

#### Movement

Summer (May 30<sup>th</sup>—September 30<sup>th</sup>, 2013) movement of redband trout in Dry Creek appears to be strongly related to late summer creek drying. Lower sections of Dry Creek dried prior to upstream reaches. Nearly all (91%, (52/57)) of summer redband trout movement in Dry Creek was upstream. Upstream movements also had a greater median distance than downstream movements (92-m v. 53-m). All but one of the redband trout that moved downstream traveled less than 60-m. These fish may have run back upstream after being detected by an array. Davey and Kelly (2007) found brown trout, (*Salmo trutta*) to migrate upstream as downstream reaches dried. The overwhelming upstream trend indicates that Dry Creek redband trout may be behaviorally adapted to the downstream drying pattern of Dry Creek.

Summer movement of redband trout in Dry Creek peaked during June. Most fish moved several weeks before creek drying in mid-July. Dry Creek redband trout could most effectively survive July and August by moving upstream in early summer to avoid reaches that become intermittent. This hypothesis is supported by the timing of PIT tag detections. The upstream array detected movement more frequently at later dates, reflecting the downstream to upstream pattern of drying. Sites around the downstream array became dry earlier than around the upstream array. For a small window in late July, fish in flowing reaches above the downstream array still had the opportunity to move up and cross the upstream array.

Surprisingly, PIT tag detections did not cease after the creek became dry. Tags were still recorded even though no water was present around the array. Many redband trout became trapped in isolated pools in late July and August, 2013. Isolated pools inflict physiochemical stress and

increase vulnerability to predation (Larimore et al. 1959; John 1964; Capone & Kushlan 1991; Magoulick & Kobza 2003; Dekar & Magoulick 2007). High predation by raccoons on trapped or recently deceased fish in Dry Creek is possible given numerous tracks found near isolated pools. Raccoons were also recorded (via motion activated camera) moving over the upstream array on a regular basis while the creek was dry. John (1964) reported that raccoons foiled multiple experiments by catching all fish in a series of shallow, isolated pools in Arizona. I find it highly likely that raccoons were responsible for the continued PIT tag detections in Dry Creek given the tracks, photographs, and reports of raccoons eating fish in other systems.

Dry Creek redband trout appear to move greater distances in summer than other streamdwelling trout. The two largest detected distances moved by any redband trout in Dry Creek during my study was 970-m and 765-m. Seasonal drying likely causes a great increase in summer movement in Dry Creek. No other studies have examined redband trout movement during summer. Hilderbrand and Kershner (2000) found median stream dwelling cutthroat trout (Oncorhynchus *clarki*) home-range size in summer to be 425-m. However, median home range following the first week was only 55-m, indicating that initial movements may have been in response to being captured (Hilderbrand & Kershner 2000). Young (1999) found brown trout in a Rocky Mountain stream to have an average summer home range of 41-m. From October to December in a small stream in Montana, redband trout moved a mean total distance of 64-m, but there was great individual variation (Muhlfeld et al. 2001a). Movement ceased when redband trout found suitable overwintering sites (Muhlfeld et al. 2001a). Dry Creek redband trout movement during summer may be similar—an initial period of movement followed by relatively static positioning when ideal over-summering reaches are found. Preservation of perennial flow in upstream reaches of Dry Creek would help ensure that refuges are available for redband trout during extreme drought.

No redband trout were detected by both PIT tag arrays, suggesting the culvert and waterfall created by rockfall during road construction at Bogus Basin Rd. may have been as a barrier to fish movement during the study period. Low stream flows may make fish passage more difficult. If fish movement across the culvert were to occur, spring would be the most likely time because flows are highest at that time (Fig. 2). Alternatively, the PIT tag arrays may be too far apart (1512-m) for fish to travel during my study. Because my study occurred in late spring and early summer of a severe drought year (Fig. 2), am unable to conclude whether or not the culvert is a barrier to redband trout movement. Genetic data indicated that redband trout in Dry Creek formed a single population, suggesting that at least one way movement through the barrier occurs.

The redband trout of Dry Creek have relatively low genetic diversity and appear to have genetically drifted from other populations of southern Idaho resident redband trout. Dry Creek redband trout are a pure population that have not introgressed with hatchery rainbow trout. In the summer of 2013, Dry Creek redband trout movement appeared to be associated with creek drying patterns. Movement was nearly all upstream and peaked several weeks before complete drying. Management to protect upstream reaches with perennial flow will help to preserve Dry Creek redband trout during uncharacteristic drought years. The genetic data suggests mixing of redband trout on either side of the barrier, but during summers with unusually low flows, the Bogus Basin Rd. barrier may prevent fish dispersal. Maintaining isolation between redband trout of Dry Creek and non-native trout of the Boise River is essential to the preservation of pure redband trout in Dry Creek.







Table 1: Descriptions of reference populations of redband trout and hatchery rainbow trout
utilized in this study. Reference genetic data were obtained from Matthew Campbell (Idaho Fish
and Game).

Population Type	River/Creek	Drainage	Ν	Sample Date
Redband trout	Hat Creek	Salmon River	24	9/12/2001
(resident, S. Idaho)	Wolf Creek	Snake River	22	9/13/2001
	Big Jacks Creek	Snake River	25	8/26/2003
	N.F. Owyhee River	Snake River	25	6/11/2003
	Shack Creek	Shack Creek Snake River		9/3/2003
	Bennett Creek Snake River		23	3/22/2001
	Doby George Creek (upper) Snake River		25	5/24/2007
	Doby George Creek (lower)	by George Creek (lower) Snake River		5/29/2007
Steelhead	Snake River	Snake River	1054	2009
(anadromous redband trout)	Salmon River	Salmon River	77	2010
	Pahsimeroi River	Salmon River	34	2009
Introgressed redband trout	Pahsimeroi River	Salmon River	57	2010
(resident)	Lemhi River	Salmon River	60	2010
Population Type	Hatchery	Stock		Sample Date
Hatchery rainbow trout	Mt. Lassen Fish Hatchery	Mt. Lassen X Hildebrand	47	11/1/2006
("coastal")	Ennis Fish Hatchery	Eagle Lake	47	6/28/2006
	Ennis Fish Hatchery	Fish Lake	46	6/28/2006
	Ennis Fish Hatchery	Shasta	46	6/28/2006
	Ennis Fish Hatchery	Erwin	47	6/28/2006
	Ennis Fish Hatchery	Arlee	47	6/28/2006
	Ennis Fish Hatchery	McConaughy	25	6/28/2006
	Ennis Fish Hatchery	Harrison Lake/Desmet		6/28/2006
	Ennis Fish Hatchery	Harrison Lake	28	6/28/2006
	Mt. Lassen Fish Hatchery	Mt. Lassen X Donaldson	45	11/1/2006
	Shepherd of the Hills Hatchery	Shepherd of the Hills	38	6/26/2006
	Shepherd of the Hills Hatchery	Missouri Arlee	43	6/26/2006
	Nampa Fish Hatchery	Troutlodge	46	7/13/2006
	Mt. Whitney Fish Hatchery	Mt. Whitney	39	4/16/2007



**Figure 3:** Two cluster (k=2) STRUCTURE diagram of Dry Creek redband trout and 24 reference populations. Each individual fish is represented by a vertical bar with proportionate coloring to indicate ancestry from each of two possible clusters. Light grey coloring represents redband trout ancestry and dark grey coloring represents coastal rainbow trout ancestry. Reference population genotypes obtained from Matthew Campbell (Idaho Fish and Game).

<b>Table 2:</b> Pairwise FST values between Dry Creek redband trout and populations of genetically
pure steelhead, southern Idaho resident redband trout, introgressed redband trout, and hatchery
rainbow trout.

Population type	Genetic status	Number of populations	Min. pairwise FST	Mean pairwise FST	Max. pairwise FST
Steelhead (anadromous redband trout)	pure	3	0.053	0.060	0.067
S. Idaho resident redband trout	pure	8	0.068	0.095	0.160
Introgressed redband trout	introgressed	2	0.148	0.155	0.211
Hatchery rainbow trout	introgressed	14	0.155	0.193	0.230



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