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Warner Sucker Investigations (2009)

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Shannon E. Richardson, Paul D. Scheerer, Stephanie A. Miller, Steven E. Jacobs, Glenn
Swearingen, Brooke Berger, John Deibner-Hanson, and John Winkowski
Oregon Department of Fish and Wildlife
28655 Highway 34
Corvallis, Oregon 97333

Mark Terwilliger and Patrick Hayden
Department of Fisheries and Wildlife
Oregon State University
104 Nash Hall
Corvallis OR 97331

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INTRODUCTION

The Warner sucker (*Catostomus warnerensis*) is endemic to the Warner Valley, an endorheic subbasin of the Great Basin in southeastern Oregon and northwestern Nevada. Historically, this species was abundant and its range included three permanent lakes (Hart, Crump, and Pelican), several ephemeral lakes, a network of sloughs and diversion canals, and three major tributary drainages (Honey, Deep, and Twentymile Creeks) (U.S. Fish and Wildlife Service 1985). Warner sucker abundance and distribution has declined over the past century and it was federally listed as threatened in 1985 due to habitat fragmentation and threats posed by the proliferation of piscivorous non-native game fishes (U.S. Fish and Wildlife Service 1985).

The Warner sucker inhabits the lakes and low gradient stream reaches of the Warner Valley. The Warner sucker metapopulation is comprised of both lake and stream life history morphs. The lake suckers are lacustrine adfluvial or potamodromous fish that normally spawn in the streams. However, upstream migration may be blocked by low stream flows during low water years or by irrigation diversion dams. When this happens, spawning may occur in nearshore areas of the lakes (White et al. 1990). Large lake-dwelling populations of introduced fishes likely reduce recruitment by preying on young suckers (U.S. Fish and Wildlife Service 1998). The stream suckers inhabit and spawn in Honey, Deep, and Twentymile Creeks.

The Recovery Plan for the Threatened and Rare Native Fishes of the Warner Basin and Alkali Subbasin (U.S. Fish and Wildlife Service 1998) sets recovery criteria for delisting the species. These criteria require that: 1) a self-sustaining metapopulation is distributed throughout the Twentymile, Honey, and Deep Creek (below the falls) drainages, and in Pelican, Crump, and Hart Lakes, 2) passage is restored within and among the Twentymile, Honey, and Deep Creek (below the falls) drainages so that the individual populations of Warner suckers can function as a metapopulation, and 3) no threats exist that would likely threaten the survival of the species over a significant portion of its range.

Objectives of our 2009 investigations included: 1) obtain a mark-recapture population estimate for suckers in the Twentymile Creek drainage and describe their current distribution, 2) describe associations between the distribution of suckers and habitat variables in Twentymile Creek, 3) evaluate a non-lethal ageing technique, 4) track radio-tagged lake suckers (tagged in 2008) in Hart and Crump Lakes to assess spring movement patterns, 5) track spring spawning movements of lake suckers across a PIT-tag antenna installed at the mouth of Honey Creek, 6) test the feasibility of trapping larval suckers near the mouth of Honey Creek using larval drift nets and light traps to describe the relative abundance and timing of larval sucker movements, and 7) obtain a mark-recapture population estimate of suckers at the Summer Lake Wildlife Management Area (WMA), where a self-sustaining population became established after a fish salvage from Hart Lake during the 1991 drought.

METHODS

Distribution and Abundance in the Twentymile Creek Subbasin

In the summer of 2009, we surveyed approximately 21 km of the mainstem of Twentymile Creek and its primary tributary, Twelvemile Creek (Figure 1). We used backpack electrofishers to obtain a two-pass mark-recapture abundance estimate. The sample frame included the suspected geographical extent of suckers in the subbasin. We divided the sample frame into forty-three 500 m reaches, which we measured using a hip chain. Sample reaches were further divided into 100 m stream sections for fish sampling. We electrofished each 100 m stream section in a single upstream pass and placed all captured fish in buckets. We enumerated and measured all Warner Suckers and recorded the approximate abundance and distribution of all other fish species observed (**APPENDIX A**). At the upstream end of each 100 m section, we processed the fish and released them back to the approximate location from which they were captured. We anesthetized all suckers that we captured using methyl sulfonate (MS222), measured for fork length (FL), and marked with fin clips those suckers ≥ 60 mm FL. We alternated between upper and lower caudal fin clips every 1,000 m (every two sample reaches) throughout the extent of the sample frame to examine small scale movements of fish between pass 1 and pass 2. We reserved a subsample of fin clips and preserved them in 95% ethanol for future genetic analysis. We examined anal fin morphology according to Coombs et al. (1979) to determine the sex of each sucker. During the initial electrofishing pass, we scanned each sucker ≥ 100 mm FL for Passive Integrated Transponder (PIT) tags and recorded detections of previously-installed PIT tags. If no tag was detected, we surgically installed a 23 mm half-duplex PIT tag in the anterior ventral side of the body cavity of all suckers ≥ 100 mm FL. During the second electrofishing pass we recorded the number of marked and unmarked suckers and scanned each fish for existing PIT tags. If no PIT tag was detected, we installed a PIT tag in all suckers ≥ 100 mm FL. An average of 35 days (range 17-71) elapsed between the first and second passes.

We estimated population abundance for the entire Twentymile Creek subbasin using single-sample mark-recapture procedures (Ricker 1975). We divided the Twentymile Creek basin into four strata based on changes in geomorphology, gradient and physical habitat characteristics and obtained separate estimates for each of these strata (Figure 2). We also estimated the abundance of adult suckers and calculated the ratios of adult to juvenile fish. Suckers larger than 160 mm FL were considered mature (Scheerer et al. 2008). We calculated 95% confidence intervals for our estimates using a Poisson approximation (Ricker 1975). Block nets were not deployed during the survey; however, we installed a PIT-tag antenna in stratum two, above the upstream-most diversion, to estimate the magnitude of downstream movement of fish out of the sample reach during our study (Figure 2).

It should be noted that although the subbasin is called the Twentymile Creek subbasin, the majority of the flow at its confluence with Twelvemile Creek comes from Twelvemile Creek.

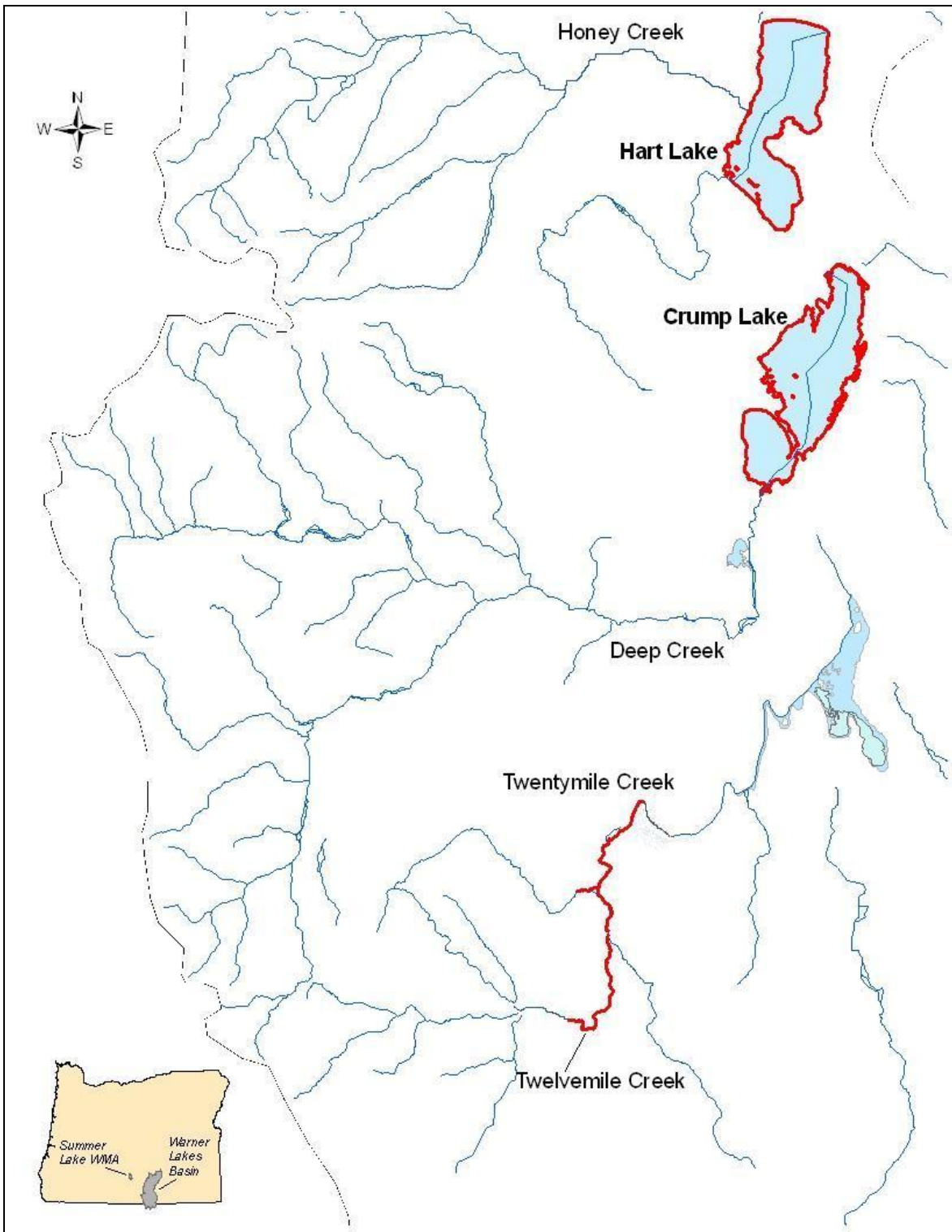


Figure 1. Study area for 2009 Warner Sucker Investigations. Areas surveyed are highlighted by red. The location of the Summer Lake Wildlife Management Area is shown on the Oregon inset map.

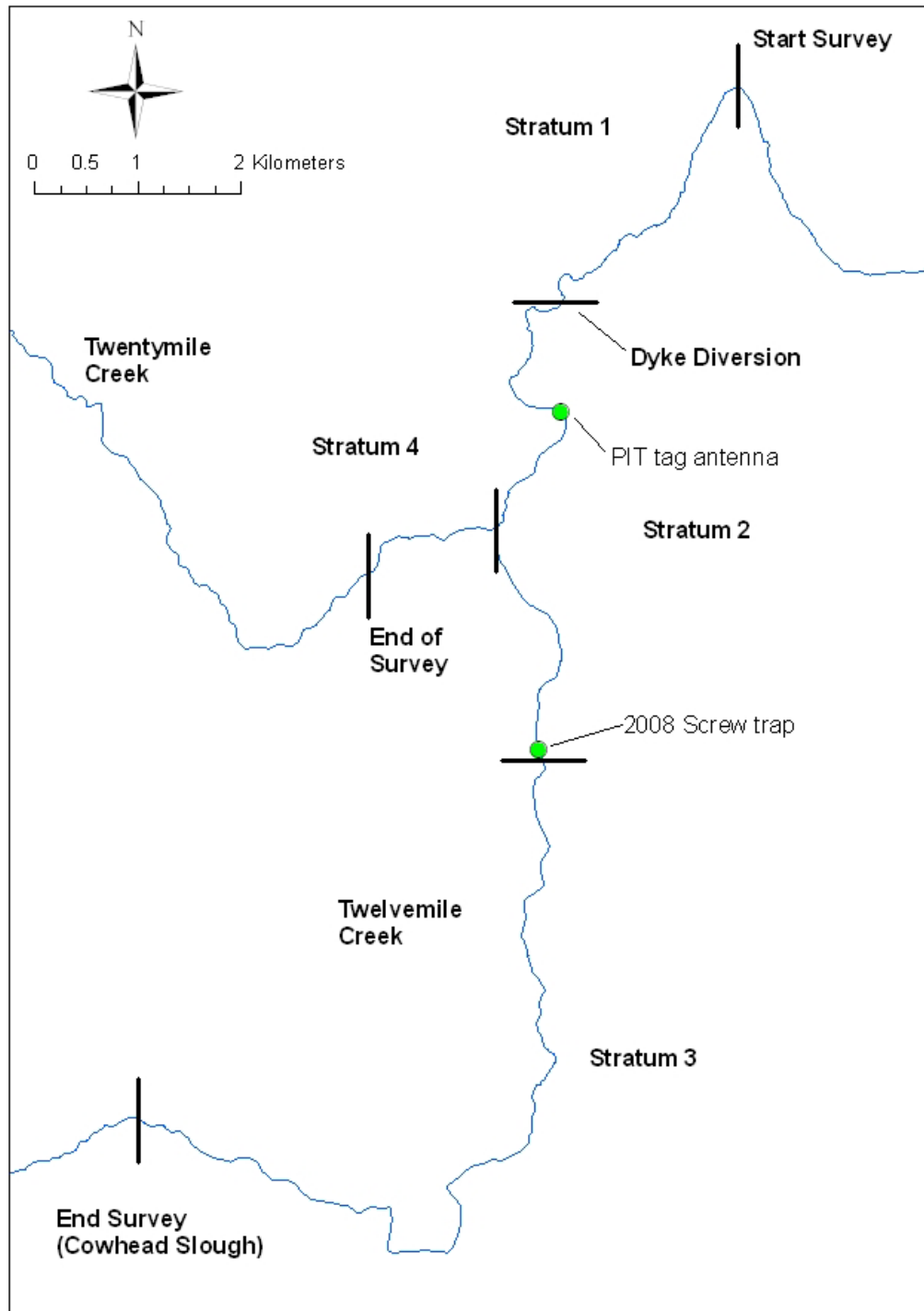


Figure 2. Map of the Twentymile Creek subbasin showing the extent of the four discrete stream strata. Stratum one includes lower Twentymile Creek from the start of the survey to the Dyke diversion. Stratum 2 starts at the Dyke diversion on Twentymile Creek and extends to the beginning of the canyon on Twelvemile Creek. Stratum three starts at the beginning of the canyon on upper Twelvemile Creek and extends to the confluence of Cowhead Slough. Stratum four includes upper Twentymile Creek above the confluence with Twelvemile Creek.

Habitat Variables Collected in the Twentymile Creek Subbasin

Following the second pass of the survey, we collected habitat parameters for each 500 m sample reach. Habitat parameters included: wetted width (m), average depth (m), length and maximum depth of backwater pools (m²), maximum depth (m), water temperature (°C), aquatic vegetation (as a percentage of total surface area), dominant substrate type, dominant riparian cover type, and number of pools. Width, depth, substrate and aquatic vegetation measurements were taken at transects located every 100 m, starting approximately 50 m from the downstream boundary of each reach. We calculated average depth by summing depth measurements collected at 25%, 50% and 75% of the wetted width and dividing by four, to account for zero depth at the stream margins. Maximum depth was the single deepest water depth in each reach. We measured the length, width and maximum depth of backwater habitats when they occurred at a transect location. We determined the dominant substrate from seven equally-spaced points along each transect. At each point (4-inch circle), we recorded whether the majority of the substrate was fines (<1/16th mm), sand (1/16-2 mm), gravel (3-64 mm), cobble (65-256 mm), boulder (>256 mm), bedrock (native consolidated rock), or embedded. We identified dominant riparian cover categorically (conifer, deciduous, grasses and shrubs, and limited vegetation). We recorded stream temperature at the beginning and end of each 500 m reach and recorded Universal Transverse Mercator (UTM) coordinates, stream elevation, and took photographs at the beginning of each reach.

Warner Sucker - Habitat Associations in the Twentymile Creek Subbasin

Relationships between habitat variables and total fish captured (sum of both electrofishing passes) were explored using linear regression (SAS[®] Institute Inc., Cary, NC). The habitat variables described above had high degrees of co-linearity and as such, those with the highest degrees (>0.50) were removed from the analysis. Reaches where habitat data were not collected (a few upstream reaches in both Twentymile and Twelvemile Creeks where no suckers were collected) were also removed from the analysis. The remaining variables that we included in the model were: maximum temperature recorded per reach, reach area, area of backwater habitat, number of pools, maximum depth, minimum depth, and percent embedded substrate, percent bedrock substrate, and mean percentage of aquatic vegetative cover per reach. We used a log transformation to normalize the data and tested assumptions of normality and homogeneity of variance. The most parsimonious final model was selected based on both adjusted R² and mallow Cp model selection criteria (Burnham and Anderson 2002). Our model did not exceed one independent variable for every ten sample sites.

Validating a Non-lethal Ageing Technique

To evaluate a non-lethal technique for ageing Warner suckers, we examined five hard structures commonly used to age fish: (1) lapillar otoliths, (2) scales, (3) opercles, (4) pectoral fin rays, and (5) pelvic fin rays. We collected 16 Warner suckers (mortalities from the stream investigations) for analysis. To increase sample size, we also examined hard structures from several non-listed species of western suckers, including largescale suckers (*C. macrocheilus*, n = 25), bridgelip suckers (*C. columbianus*, n=5), and mountain suckers (*C. platyrhynchus*, n=2), and assumed that growth marks observed on hard structures would be comparable among species. Hard structures were removed from the right-hand side of all fish, except in a few cases when a structure was lost or damaged during dissection; in

those cases, the left compliment was used. Preparation procedures varied for each hard structure as described below:

Lapillar otoliths

We embedded lapilli in EpoThin® epoxy resin and made a 1.0 mm-thick oblique section, running anterodistal-posteromedial, which included the core using a Buehler Isomet® low-speed saw with a diamond-tipped wafering blade. We decided that an oblique section was necessary because the main growth axis of the lapillus projects ventrally, the core is not centrally located, and the longest axis of the lapillus sits at an oblique angle relative to the head of the fish. We mounted sections on glass slides using Crystal Bond® adhesive, sanded with 600-1200 grit wet/dry sandpaper to remove saw marks and gain proximity to the core, and polished on a felt pad with 0.5 µm alumina powder. We flipped the otolith several times during grinding and polishing to create a thin section showing visible increments from core to edge (see Secor et al. 1992). We examined lapilli using a Leitz Biomed® compound microscope with transmitted light. We assigned ages from counts of growth increments that were comprised of a wide translucent and narrow opaque band. Note that ageing otoliths requires us to first sacrifice the fish.

Pectoral and pelvic fin rays

We removed the two anterior-most fin rays from each fish by cutting the rays at the articulation point with a scalpel. We allowed the fin rays to air dry before embedding them in EpoThin® epoxy resin, and then made cross-sections using a Buehler Isomet® low-speed saw with a diamond-tipped wafering blade. We made five serial sections (0.5 mm thick) from the base of each ray towards the distal tip, in order to assess annuli loss with distance away from the body. The first section was made at the point where the proximal end of the fin ray curved away from the body toward the distal tip. We mounted sections on glass slides using Crystal Bond® adhesive and examined growth increments using a Leitz Biomed compound microscope with transmitted light.

Opercles

We clipped the entire opercle from each fish, trimmed off excess tissue, and allowed the structure to air dry. To prepare them for reading, we placed opercles in boiling water for several minutes, after which we removed the integument and other tissue using forceps. We again allowed the structure to air dry. Larger opercles exhibited fenestrated reinforcement bone immediately ventral to the hyomandibular socket that prevented observation of the earliest growth marks; in those cases, we used a Dremel® tool to remove the reinforcement bone so that all growth marks were viewable. We aged opercles using a light table for background illumination. For large opercles, we used a swing-arm stereoscopic dissecting microscope to enumerate growth marks at the edge of the structure. Note that ageing opercles requires us to first sacrifice the fish.

Scales

We used a scalpel to remove scales from an area dorsal of the lateral line and anterior to the dorsal fin. We removed mucus and epidermis from each scale by immersing them in tap water and gently scraping with a scalpel. We sandwiched five scales from each fish between two microscope slides and examined growth increments using a dissecting scope and reflected light.

We estimated within-reader precision in terms of indices of absolute percent error (APE) as outlined in Beamish and Fournier (1981). Growth increments on all structures were counted three times by both an experienced and inexperienced ager, without

information regarding fish size, species, or capture date. Due to the wide difference in between-reader precision, we used the median age obtained from the experienced reader to compare ages among structures. We also evaluated the ease of reading each structure by examining the percentage of time when all three age determinations for a structure agreed.

To determine the relationship between otolith age and ages obtained from other hard parts, we assigned the experienced reader's median age to each sample and constructed age-bias plots using lapilli as our standard for true age. We decided to use otoliths as a standard based on our level of experience ageing these structures and the nature of somatic growth in these species. As a group, western suckers display determinate growth (Terwilliger et al. in review); therefore, as somatic growth slows with age, so does growth of skeletal structures, which then become increasingly difficult to age as growth marks become narrow and pack on the edge of the structure. Otoliths continue to grow even as somatic growth slows or stops, resulting in relatively easy to read and evenly spaced growth marks.

Radio Tracking in the Warner Lakes Basin

In the spring of 2008, we surgically implanted radio transmitter tags (Lotek[®]) into 32 Warner suckers (25 from Hart Lake and 7 from Crump Lake) (Scheerer et al. 2008). We tracked these radio-tagged individuals along transects spaced approximately 250 ft apart in Hart and Crump Lakes from early-April through mid-June 2009. Transects were established using GPS endpoint coordinates acquired by plotting transects on topographic maps to ensure that all areas of the lakes and shoreline were sampled equally. Individuals were tracked on a weekly basis using a mobile radio tracking receiver from a boat, an all-terrain vehicle, or from an Oregon State Police airplane. Each time a fish was located, we recorded the date, the power reading and code of the radio tag, and the geographic coordinates acquired from a hand held Global Positioning System (GPS) receiver. Shallow lake levels during the spring of 2009 limited our ability to track effectively. To increase our access to shallow-water areas of the lake, we used a boat with a Go-Devil[®] motor (Figure 3). This motor has a propeller that is positioned just below the water surface and permits access to water as shallow as 0.2 m deep.



Figure 3. Boat with the Go-Devil motor, which enabled sampling in shallow water.

Investigations in the Mouth of Honey Creek

We installed a flat plate PIT tag antenna at the mouth of Honey Creek to track potential spawning movements of previously PIT-tagged Warner suckers. The antenna was operated from 6 April 2009 through 8 June 2009. Because the Hart Lake elevation was low (**APPENDIX B**), the Honey Creek channel extended ≈ 100 m across the dry shoreline of Hart Lake before entering the lake. In addition, we set two larval drift nets, measuring 28 cm by 47 cm with 500 μ mesh, and two larval light traps at the mouth of Honey Creek on 20 May, 28 May and 8 June 2009. We fished these traps to determine the timing and extent of larval sucker out-migration from Honey Creek.

Abundance in the Summer Lake Wildlife Management Area

On 11 July 2009, we obtained a mark-recapture abundance estimate of the refuge population of Warner suckers located in the artesian well-fed ditch on the Summer Lake Wildlife Management Area. We used the methodology described above for backpack electrofisher surveys, with the following exceptions: the recapture survey was conducted two days after the marking phase, and fork length (FL) was measured on a sub-sample of 50 fish. No PIT tags were installed. Fin clips were saved from all marked fish for future genetic analysis.

RESULTS

Abundance and Distribution in the Twentymile Creek Subbasin

We estimated 4,612 (95% CI: 3,820-5,567) Warner suckers larger than 59 mm FL (presumptive age 1+ fish) in the Twentymile Creek drainage. Of this total, 1,169 (95% CL: 969-1,412) were adults (>159 mm FL).

Suckers were captured and marked from each of the four strata, although the majority (86%) of the population was found in stratum two (3,786; 95% CI: 3,112-4,603) (Tables 1 and 2). Most suckers were captured in the middle portion of the sample frame (Figure 4). We also noted the distribution of other native fish species, including redband trout (*Oncorhynchus mykiss* ssp.), speckled dace (*Rhinichthys osculus* ssp.), tui chub (*Gila bicolor*), and roach (*Hesperoleucus symmetricus*). Non-native species included largemouth bass (*Micropterus salmoides*) and brown bullhead (*Pomoxis nigromaculatus*). Speckled dace, redband trout, and Warner suckers were found throughout the drainage, whereas the other species were limited to stratum one and the first 100 m of stratum two (**APPENDIX A**).

Table 1. Mark-recapture data for Warner suckers in the Twentymile Creek drainage, listed by stream stratum. Data in “marked” column were collected in the first pass; data in the “captured” and “recaptured” columns were collected in the second pass.

Stratum	Length (km)	Estimate	95% CI	Marked	Captured	Recaptured	Total handled
1	2.5	677	(299 - 1,334)	46	71	4	113
2	7.6	3,779	(3,112 - 4,603)	520	717	98	1,139
3	9.2	155	(63 - 311)	22	26	3	45
4	2.0	49	(15 - 85)	6	13	1	18
All	21.3	4,612	(3,820 - 5,567)	594	827	106	1,315

Table 2. Density estimates for Warner suckers in the Twentymile Creek drainage, listed by stream stratum. Adult fish were >159 mm FL and juvenile fish were ≤159 mm FL.

Stratum	Length (km)	Suckers per kilometer		
		All	Adults	Juveniles
1	2.5	271	66	204
2	7.6	498	121	377
3	9.2	17	15	2
4	2.0	25	1	24
All	21.3	217	55	162

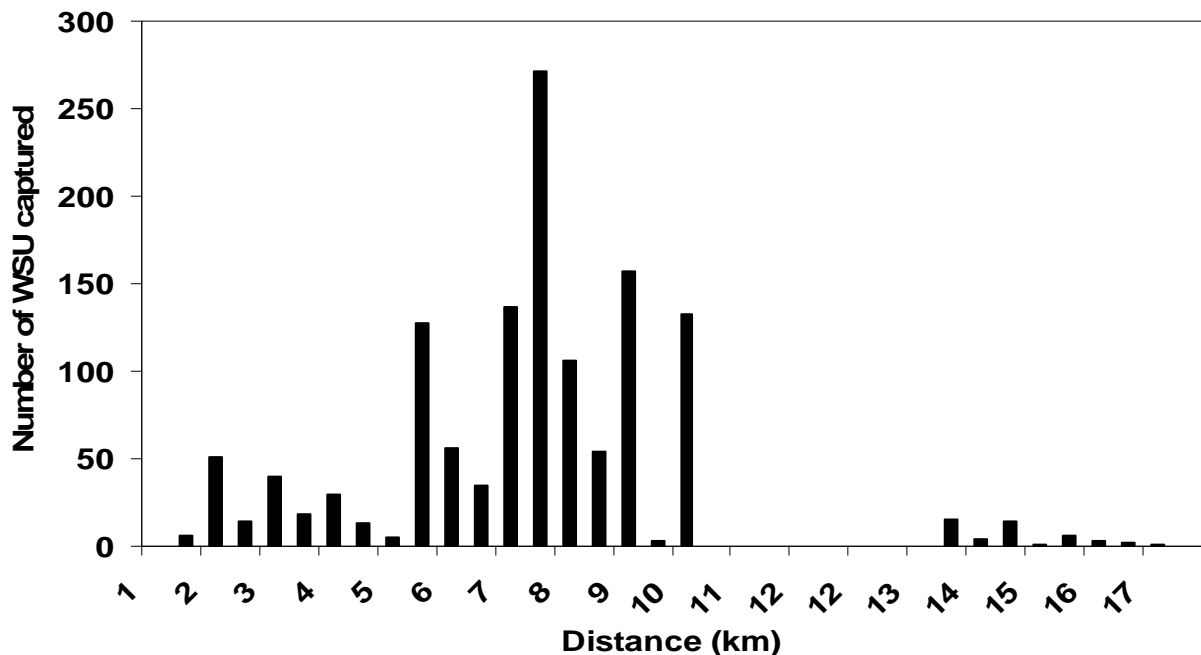


Figure 4. Distribution of Warner suckers in the Twentymile Creek drainage of the Warner basin based on electrofishing captures in 500 m sample reaches. Distance is from the downstream boundary of Stratum 1.

Length Frequency and Growth

The length frequency distribution for Warner suckers captured in the Twentymile Creek drainage in 2009 was broad with one obvious peak at approximately 80 mm and another possible peak around 190 mm (Figure 5). Captured suckers ranged from 50 mm to 383 mm FL with a mean of 114 mm FL (S.D. = 55.8). The ratio of adult to juvenile fish captured was approximately 1:3 (n=337 fish \geq 160 mm and n=992 fish \leq 159 mm).

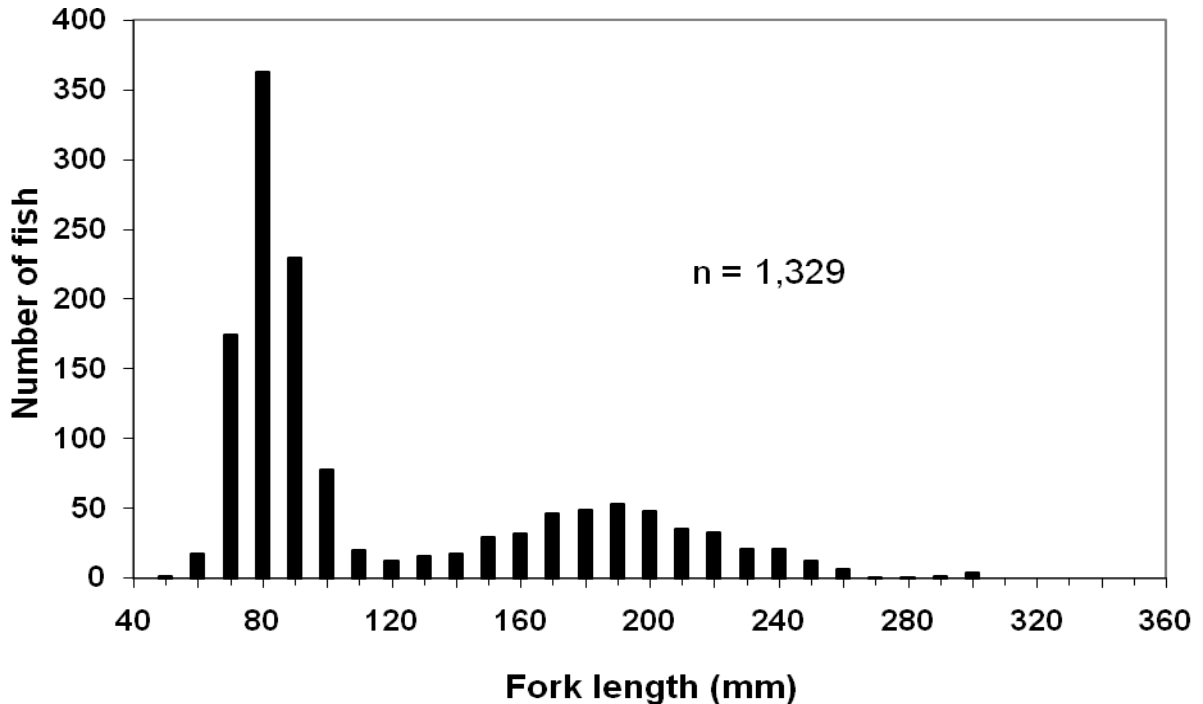


Figure 5. Length frequency histogram for Warner suckers in the Twentymile Creek drainage, summer 2009.

During the 2009 field season, we detected two fish with PIT-tags implanted in 2008, offering critical insight into sucker growth in streams. Both fish were tagged in 2008 at the screw trap fished near the mouth of the canyon (upper end of stratum 2). The first fish was 121 mm when tagged and was 215 mm when recaptured (+94 mm) and the second fish was 120 mm when tagged and 210 mm when recaptured (+90 mm). Days between tagging and detection were 509 and 433, respectively, resulting in an average annual growth of 67 mm for the first fish and 76 mm for the second. Growth of fish tagged and subsequently recaptured during the 2009 season ranged from 0 mm to 18 mm (17-71 days extant).

Movements of Fin-Clipped and PIT-Tagged Suckers in the Twentymile Creek Drainage

During the first sampling pass, we alternated upper and lower caudal fin clips every two sample reaches (1000 m) to assess small scale movements of fin-clipped fish. We recaptured 106 fin-clipped fish on the second sampling pass and, by examining fin clips, detected apparent movement in 11 (10%) of the marked fish. Although some errors occurred during the marking (seven fish had wrong fin lobe clipped), the low rate of movement by marked fish indicates that we generally met the assumption of closed populations for the sample strata and that this source of bias did not substantially affect the accuracy of population estimates.

During the 2009 field season, we implanted PIT tags into 236 Warner suckers during pass one and into another 185 suckers during pass two, for a total of 421 fish. We recaptured 63 tagged suckers (27%) on the second pass. Of the 63 recaptured PIT-tagged fish, 55 (87%) were captured from the same reach where they were tagged. Of the 8 fish (13%) that showed movement beyond the reach where they were tagged, six were recaptured upstream of the location where they were marked and two were recaptured downstream. The average distance moved by all recaptured PIT-tagged fish was ≈ 100 m. Among fish that were recaptured in reaches other than those they were tagged, average movement was 0.65 km from the tagging location with a range of ≈ 0.4 -1.2 km (Table 3). The fish traveling the greatest distance was marked below the upper diversion (Dyke Diversion) and was recaptured above the diversion, indicating that the fish successfully navigated the fish ladder at the diversion.

Table 3. Movements of Warner suckers PIT-tagged and recaptured during the 2009 survey of Twentymile Creek. Downstream movements are shown in italics.

PIT tag number	Date tagged	Date recaptured	Days to detection	Distance from tagging location (m)	Average distance traveled per day (m)
152365660	05-Aug-09	26-Aug-09	21	858	41
152365754	27-Jul-09	18-Aug-09	22	400	18
152365773	13-Jul-09	01-Sep-09	44	958	22
152365777	13-Jul-09	01-Sep-09	44	1,179	27
152365779	14-Jul-09	01-Sep-09	43	664	15
152365784	14-Jul-09	01-Sep-09	43	664	15
<i>152365383</i>	<i>22-July-09</i>	<i>11-Aug-09</i>	20	450	94
<i>152365396</i>	<i>27-July-09</i>	<i>17-Aug-09</i>	21	400	19

To estimate the number of suckers that emigrated from the sample frame during the study, thereby testing whether the assumption of a closed system for our mark-recapture estimate was violated, we monitored PIT tag detections at the antenna (Figure 6) installed in stratum two (note: we did not install this antenna lower in the system because there were several unscreened diversions in stratum 1). We tested the ability of the antenna to detect PIT tags weekly and verified that it functioned continuously during the period when the mark-recapture surveys were conducted.

Nine fish were detected at the antenna (Table 4); eight were tagged in 2009 and one was tagged during the 2007 stream surveys. All nine fish were tagged in stratum two. Two

of the nine fish that were detected at the antenna were tagged below the antenna and seven fish were tagged upstream of the antenna. Because these seven fish represent only a small proportion (2%) of the total number of fish PIT-tagged upstream of the antenna, we feel that emigration from the study area had a negligible effect on our overall abundance estimate. Due to both the location of our antenna and potential entrainment into irrigation diversions, we were unable to assess the potential effect on our estimate of the loss of suckers from stratum 1. The average downstream movement of suckers tagged in 2009 and detected at the antenna was approximately 2.4 km.

Table 4. Detections of PIT-tagged suckers at the antenna located on Twentymile Creek. The distance from antenna represents the distance between the tagging location and the antenna. Fish that were tagged downstream of the antenna location are shown in italics.

PIT tag number	Date tagged	Total detections	Distance from antenna (m)	Days to first detection	Average distance traveled per day (m)
132590831	9/5/2007	15	2,559	651	4
<i>152365389</i>	<i>7/27/2009</i>	7	528	3	176
152365399	8/4/2009	16	2,369	1	2,369
152365426	8/6/2009	7	3,204	22	146
152365454	8/4/2009	1	2,369	3	790
152365475	8/6/2009	3	3,204	12	267
152365509	8/4/2009	1	2,369	29	82
<i>152365750</i>	<i>7/27/2009</i>	6	528	4	132
<i>152365756</i>	<i>7/27/2009</i>	1	819	4	205

Figure 6. PIT tag antenna installed in Twentymile Creek. Photo shows the box containing the multiplexer and batteries and the solar panel. The flat plate antenna wires are in the stream channel.



Habitat Associations

Warner suckers were most abundant in strata one and two in the Twentymile Creek drainage. These strata were characterized by low gradients, with a wide channel and deep pools. The stream channel in these strata had low water velocities, abundant macrophytes, and was dominated by fine substrates. We also captured high densities of suckers in backwater habitats. Redband trout overlap with Warner suckers throughout the basin, but appear to utilize different habitat types. Redband trout were more abundant in stratum three and four, which included the canyon and upper reaches of Twelvemile Creek. These areas had higher gradient, high velocities, and coarse substrates.

Stream temperatures collected at the start and end of each 500 m reach offer a snapshot of conditions present during sampling. We found temperatures were lowest in the canyon (stratum three) and highest in the most downstream stratum. Maximum temperatures that we recorded ranged from 15.5°C to 29.0°C. In Figure 7, we plotted maximum recorded temperatures in three categories: $\leq 22^\circ\text{C}$, 22.1-28.0°C, and $>28^\circ\text{C}$. These categories represent approximate critical thermal thresholds. Specifically, at 22°C, reduced levels of dissolved oxygen negatively affect juvenile Klamath sucker growth (Terwilliger et al. 2003) and 28°C is the upper potentially harmful temperature for adult Klamath suckers (Wood, et al. 2006). Although suckers were most abundant in the warmer, downstream areas, it is possible that high temperatures in the downstream portion of their distribution affect their survival. Tait and Mulkey (1993a) found a negative relationship between sucker occurrence and temperatures exceeding 28°C.

The number of pools per reach ranged from 5 to 15. The mean depth per reach ranged from 0.11 m to 0.52 m (Figure 8) and the maximum depth per reach ranged from 0.85 m to 2.0 m. Several pools were too deep for field crews to measure, so actual maximum depths may be greater than recorded. The highest captures of suckers were associated with the deepest sample reaches in stratum 2 (Figure 8).

Aquatic vegetation was present in most reaches and ranged from 0% to 100% of the wetted surface area (**APPENDIX C**). Presence of aquatic vegetation was higher in stream channels with low gradient and low velocity (both relative to basin means); these channels typically had fine substrates. The presence of aquatic vegetation may have been over-reported, however, as the lower reaches had an abundance of algal growth which the crews included in their estimates of percent aquatic vegetation. When electrofishing, we found that suckers were commonly associated with aquatic macrophytes along the margins of deep pools.

The substrate in strata one and two was dominated by gravel and cobble. The third stratum, which includes the canyon, was dominated by cobble and boulder. Substrate in upper Twentymile Creek, (data was limited to the first 500 m of the reach) was dominated by sand and fines (Table 5). Suckers were most common in areas dominated by gravel-sized and smaller substrate.

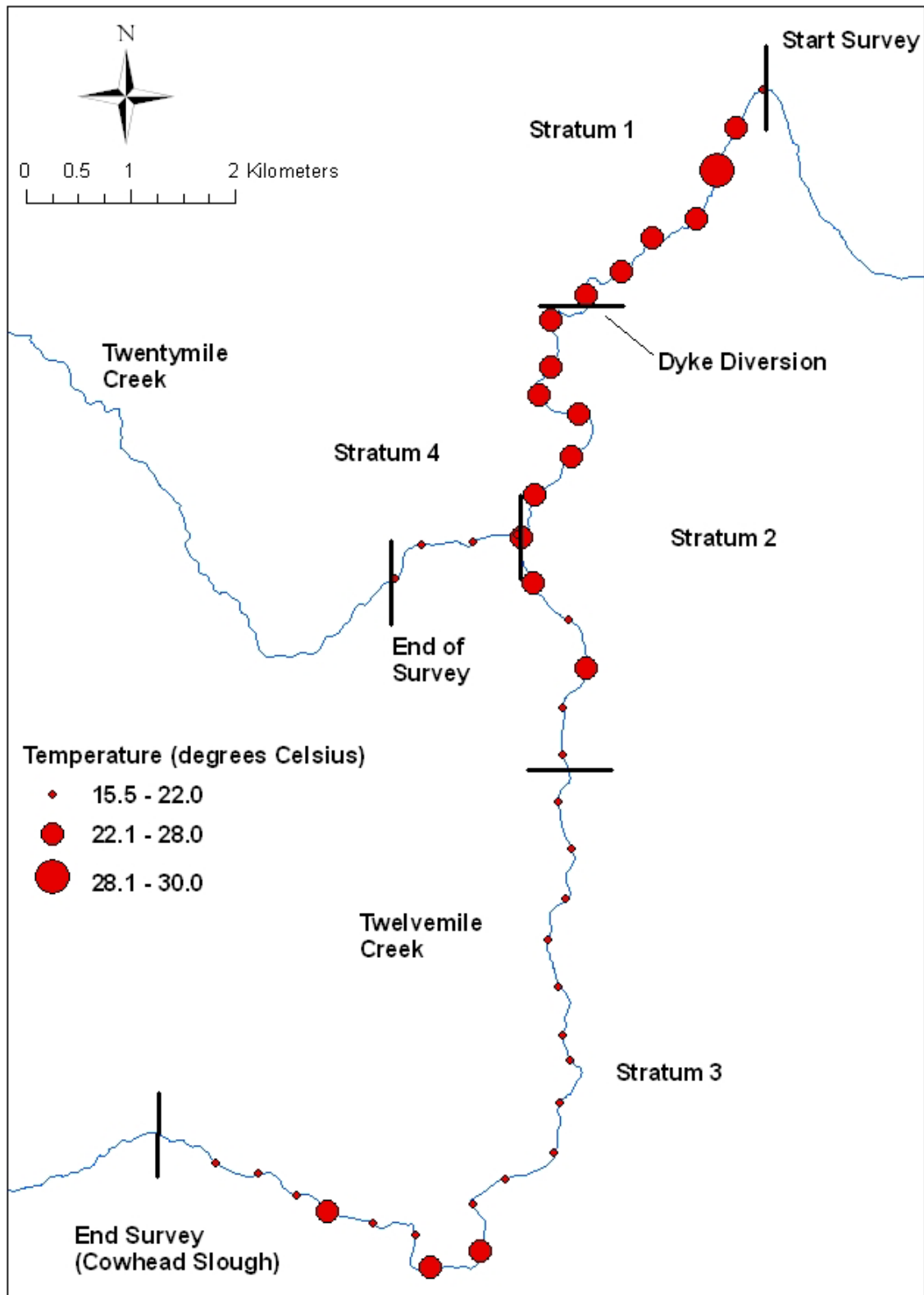


Figure 7. Maximum daily stream temperatures recorded in the Twentymile Creek drainage, Summer 2009. Temperature categories represent approximate critical thresholds for juvenile (22°C) and adult Klamath suckers (28°C).

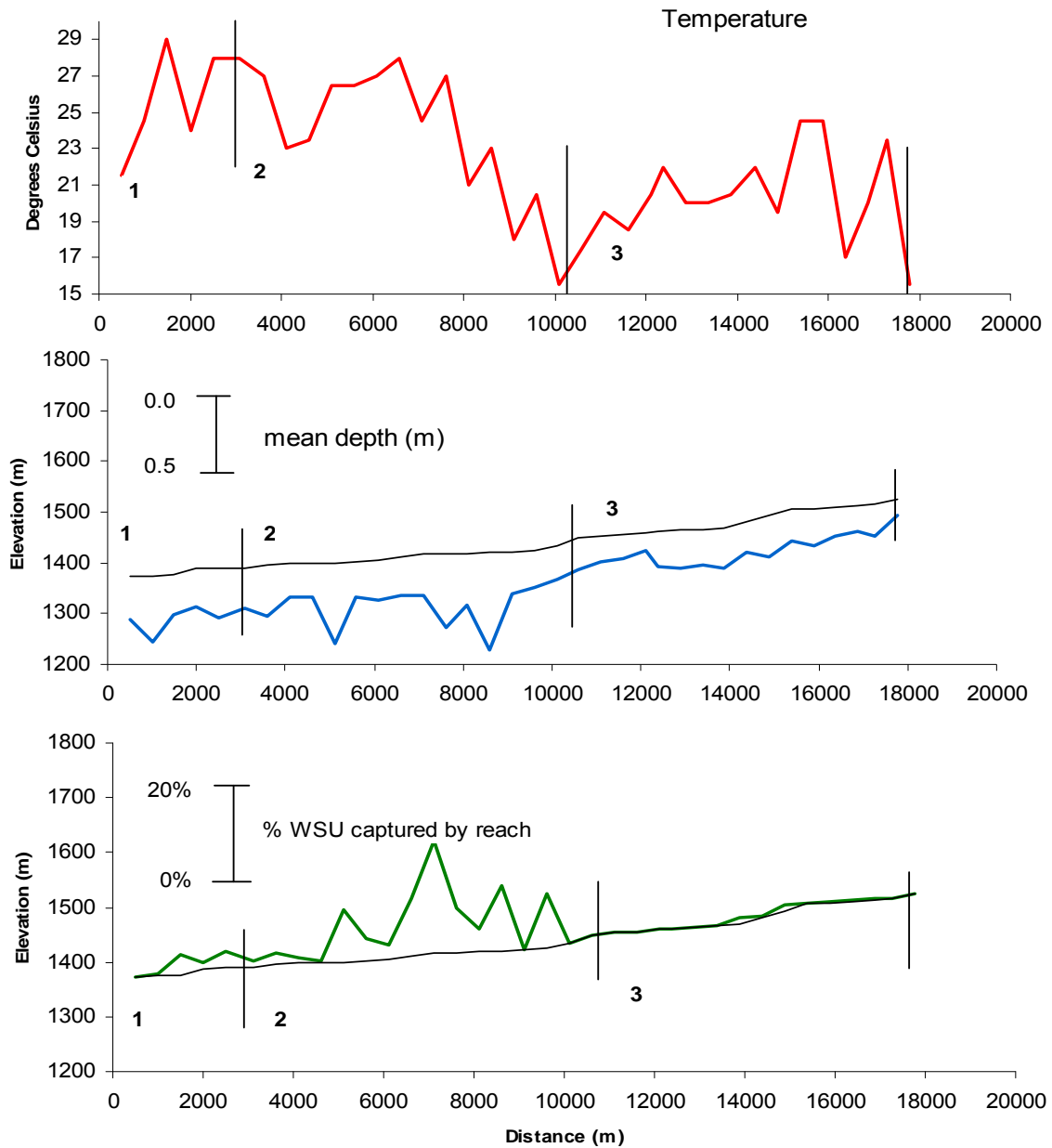


Figure 8. Relationships between the percentage of total suckers captured (bottom), mean depth (middle) and the maximum recorded temperatures (top), recorded for each reach and plotted along the longitudinal profile of the Twentymile Creek drainage. Numbers and vertical bars denote strata.

Table 5. Substrate composition for the Twentymile Creek subbasin by stratum. Numbers represent the percentage of all transects within the stratum dominated by each substrate type.

Substrate type	Stratum 1	Stratum 2	Stratum 3	Stratum 4
Fines	13%	7%	4%	30%
Sand	11%	3%	1%	27%
Gravel	37%	35%	20%	14%
Cobble	25%	35%	40%	23%
Boulder	8%	13%	32%	4%
Bedrock	5%	5%	2%	0%
Embedded	0%	2%	3%	1%

Fish - Habitat Association Model

A two-variable model accounted for 52% of observed variation in total number of fish captured ($P < 0.0001$, $DF=2, 37$). Significant variables included maximum recorded temperature ($P = 0.0037$) and mean depth ($P = 0.0028$); both of which were positively correlated with $\log(\text{fish abundance})$. The final model was $\log(\text{fish abundance}) = -7.86358 + 0.13933 (\text{maximum temperature}) + 5.24991 (\text{mean depth})$.

Sucker Condition

During the 2009 stream surveys, 64% of all of the suckers handled had parasites and/or lesions (Figure 9). We noted infected fish throughout the subbasin, although larger percentages of suckers in the lower two strata were infected. We found a significant positive relationship between elevated water temperature and parasite presence ($P = 0.00002$), although the regression only explains 36% of the total variation in incidence of infection between sites. However, we did not differentiate between levels of infection, i.e. a fish with a single lesion or parasite was recorded the same as a fish with multiple lesions or parasites. The lesions were likely caused by fish trying to rid themselves of the parasite, *Lernaea* sp., by scraping. These lesions commonly had secondary fungal infections (C. Banner, ODFW Pathology, personal communication). Other maladies affecting the suckers included exophthalmos (protruding eye), fleshy tumor-like growths, spinal deformations, and internal parasites (tapeworms).

Sex Ratios and Sexual Maturation

Based on anal fin morphology (Coombs et al. 1979), we determined the sex of a subsample of 314 Warner suckers in the Twentymile Creek drainage (24% of the total fish captured). The male to female ratio was 1:1.2 (males=145 and females=169). We found there was some uncertainty when sexing individuals based solely on anal fin morphology. Crews determined the sex of 58 recaptured tagged fish and noted seven (12%) discrepancies (e.g. a fish was identified as a male when marked and identified as a female when recaptured). No breeding tubercles, a secondary sexual characteristic often used to determine sex and sexual maturity, were present on any fish captured. Spawning colors, which may be displayed by either sex, although more frequently by males (Coombs et al. 1979), were present on several individuals. The average lengths of the female and male

suckers were 180 mm FL (range 76 mm- 219 mm) and 191 mm FL (range 102 mm- 220 mm), respectively.



Figure 9. Photos of suckers with various maladies including (A and B) a bulging eye, (C) a tumor, and (D) an infected lesion. Photos depict notable individuals; not all fish showed such extreme examples.

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Validating a Non-Lethal Ageing Technique

The precision estimates obtained for each structure were much higher for the experienced reader compared to the inexperienced reader (Table 6). The experienced reader exhibited highest precision when reading lapilli and pectoral fin rays, while the inexperienced reader's highest precision came from reading pectoral fin rays. This repeatability of estimating age was directly related to the level of expertise of the reader; the inexperienced reader had approximately four months experience ageing fishes, while the experienced reader had 17 years experience ageing fish, with six years ageing lapilli from adult catostomids and cyprinids. For this reason, hereafter we only present data from the experienced reader.

Table 6. Precision estimates (APE) of the two readers for each hard structure aged in the study.

Structure	APE inexperienced	APE experienced
Lapillus	15.61	3.73
Pectoral fin ray	14.33	5.06
Pelvic fin ray	18.60	5.23
Opercle	28.19	11.72
Scale	29.62	20.69

We found lapilli were the easiest structure to read; 68% of the time we assigned the same age for all three reads (Table 7). Also, 58% and 51% of the time we assigned the same age all three times for pectoral and pelvic fin rays, respectively, indicating that fin rays were relatively easy to read. We found both opercles and scales were relatively difficult to age, due to the presence of false growth marks and tightly packed growth marks on the edge of the structure.

Table 7. Percent agreement among three reads for each structure aged in the study.

Structure	Percent agreement
Lapillus	68%
Pectoral fin ray	58%
Pelvic fin ray	51%
Opercle	38%
Scale	44%

Age-bias plots show the best age correlations among hard structures occurred between otoliths and pectoral fin rays (Figure 10). Pectoral rays tend to underestimate ages compared to otoliths in fish older than 15 years and became difficult to read as growth marks became tightly packed along the leading edge of the ray. At younger ages, any age difference between the two structures was typically only 1 year. False rings were present on

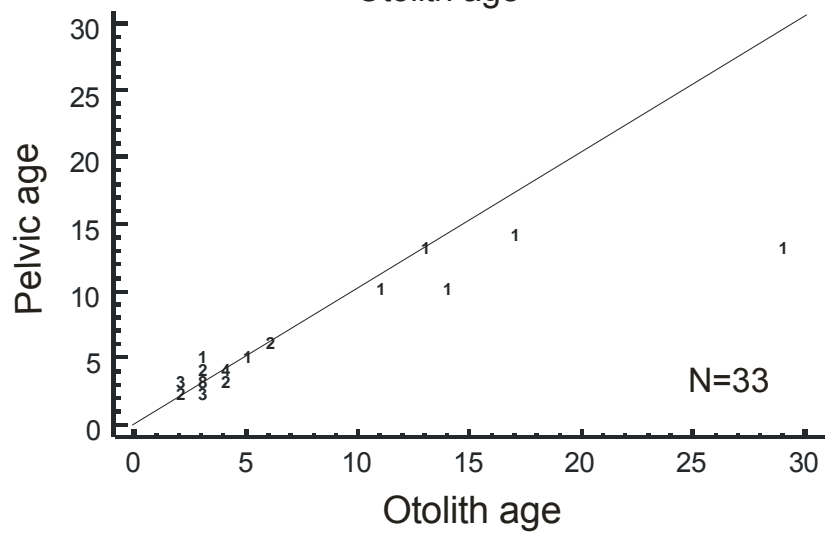
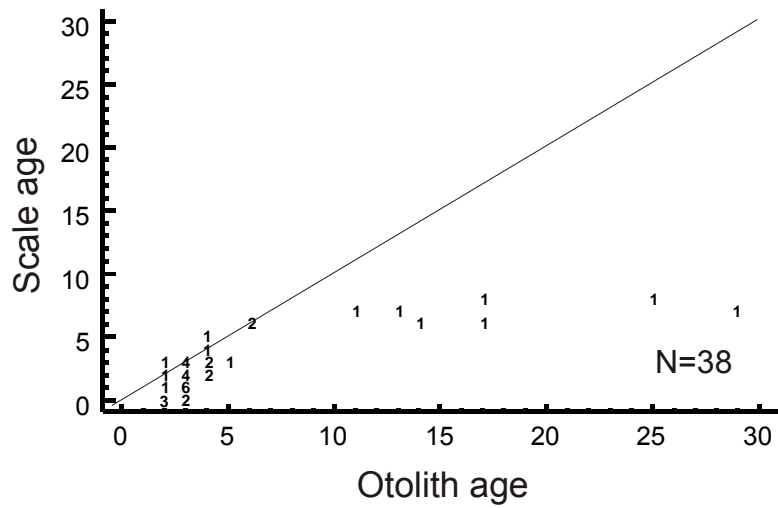
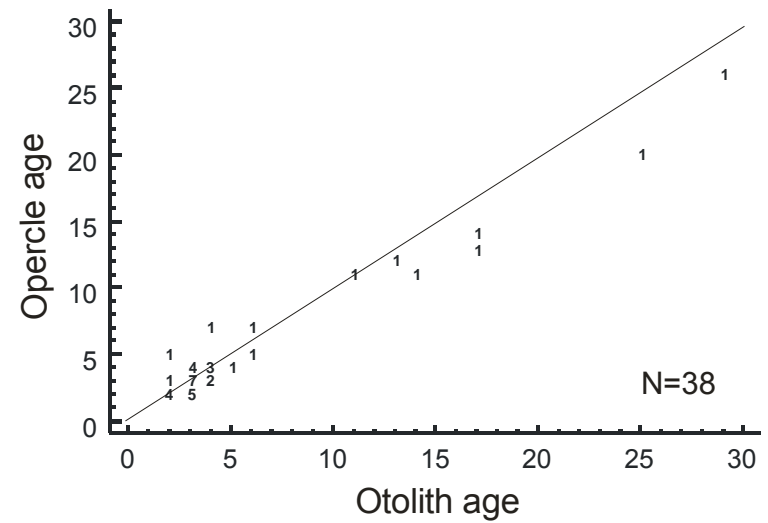
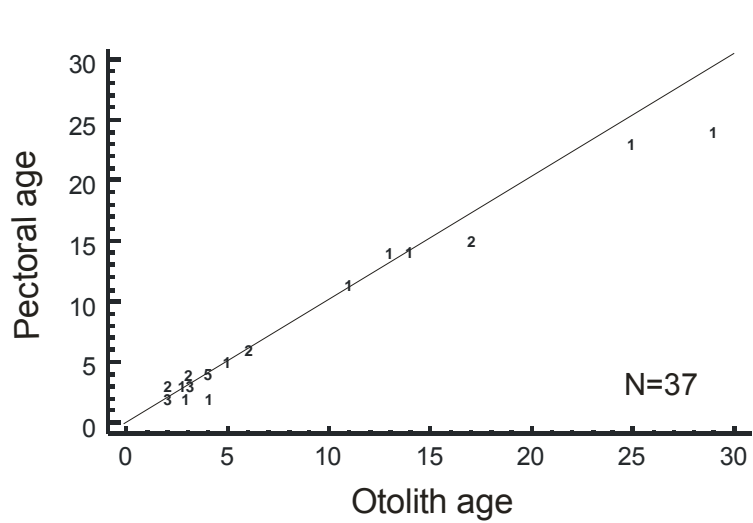


Figure 10. Age bias plots comparing ages from lapilli (otoliths) with other hard structures. Points that fall along the 45° line represent no ageing bias. Sample sizes varied between comparisons due to the inability to read all structures from all fish.

pectoral fin rays but were easily distinguishable from true growth marks, due to their poorer contrast and ephemeral appearance. Pelvic fin rays underestimated ages compared to otoliths for fishes older than ten and the deviation of the assigned age increased as fish age increased. Pelvic rays from older fish contained many false rings, had tightly packed growth marks on the edge, and several were deemed unreadable. Opercles also tended to underestimate otolith age for older fish due to tightly packed growth marks along the edge, and smaller opercles had false growth marks that caused overestimation of age in younger fish. Finally, scales were the poorest choice of an ageing structure, as they highly underestimated otolith age for all age groups. Growth marks on scales were extremely difficult to count, even on relatively young fish.

For pectoral fin rays, an assessment of growth mark loss with distance away from the fin ray base was inconclusive. The three fin ray sections taken closest to the base displayed similar growth marks; however, sections that were 2-2.5 mm away from the base of the ray showed loss of the innermost growth mark. This trend was more pronounced in smaller, younger fish than in larger, older fish. Further, the loss of the innermost growth mark was visually obvious and resulted in an abnormally indistinct translucent area that would have contained the innermost two growth marks.

Spring Movements of Radio Tagged Warner Suckers in Hart and Crump Lakes

During our surveys in the spring of 2009, we located 29 of the 32 Warner suckers that we radio tagged in 2008. Of these 29 fish, only four showed movements and were presumed to be alive; all were located in Hart Lake. Fish movements were limited due to the low water levels in Hart Lake during the spring of 2009. These fish moved around the deeper, northern portion of Hart Lake (**APPENDIX D**). Locations of the radio-tagged fish that did not move (presumed mortalities) are shown in **APPENDIX E**.

Spring Movements of PIT-Tagged Suckers in Honey Creek

Three Warner suckers were detected crossing the PIT tag antenna at the mouth of Honey Creek during the spring of 2009 (Table 8). One male sucker, tagged on 1 June 2006 crossed the antenna on multiple occasions between 5 May and 26 May 2009. Another male sucker, tagged on 18 May 2006 in the screw trap fished at the mouth of Honey Creek, was detected on 8 June 2009. A female, tagged on 14 May 2008, crossed the antenna on multiple occasions between 25 April and 27 April 2009. All of the movements were between 2000 hrs and 0600 hrs (nocturnal).

Trapping Larval Suckers at the Mouth of Honey Creek

No larval suckers were captured in the drift nets and light traps set at the mouth of Honey Creek in late May and early June of 2009. Two traps were fished for a total of 64 hours over 4 nights. A photograph of the trapping effort is shown in Figure 11.

Table 8. PIT-tagged Warner suckers detected by the antenna located at the mouth of Honey Creek during the spring of 2009. Length, weight, and sex were recorded at time of initial tagging.

PIT tag number	Date Detected	Time	Date Tagged	Location Tagged	Length	Weight	Sex
132628638	5/10/2009	2:55	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/18/2009	21:58	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/19/2009	4:10	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/19/2009	20:21	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/20/2009	3:45	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/20/2009	21:09	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/21/2009	1:01	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/21/2009	4:33	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/21/2009	5:32	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/21/2009	20:51	1-Jun-06	Hart Lake (net 5)	336	465	M
132628638	5/22/2009	2:59	1-Jun-06	Hart Lake (net 5)	336	465	M
132628642	6/8/2009	0:23	18-May-06	Honey Creek Screwtrap	215	120	M
152365849	4/24/2009	23:24	14-May-08	Hart Lake (net 3/5)	265	225	F
152365849	4/25/2009	2:55	14-May-08	Hart Lake (net 3/5)	265	225	F
152365849	4/25/2009	4:09	14-May-08	Hart Lake (net 3/5)	265	225	F
152365849	4/27/2009	23:21	14-May-08	Hart Lake (net 3/5)	265	225	F



Figure 11. Larval drift nets and light traps fished near the mouth of Honey Creek.

Warner Sucker Abundance Estimate at Summer Lake WMA

On 11 July 2009, we obtained a population estimate for Warner suckers at the Summer Lake Wildlife Management Area. This population resulted from natural production of adult suckers that were moved to the refuge when the Warner Lakes desiccated during the 1991 drought. The 2009 estimate was 660 fish (95% CI: 421-1,024). This estimate was significantly larger than the 2007 estimate of 142 fish (95% CI: 91-218). These estimates are for suckers in the ditch fed by the artesian well. No suckers were captured in the Lower Sulfur Well Pond. The sinuous channel and the wetland between the ditch and the Lower Sulfur Well Pond were not sampled. The size distribution for suckers collected in 2009 was broader and contained smaller fish than in 2007 (Figure 12), indicating recent successful recruitment at this location. There did not appear to be any difference in the amount or quality of available habitat in 2009 compared to 2007. We installed a HOBO[®] thermograph in the culvert at the lower end of the ditch in 2009.

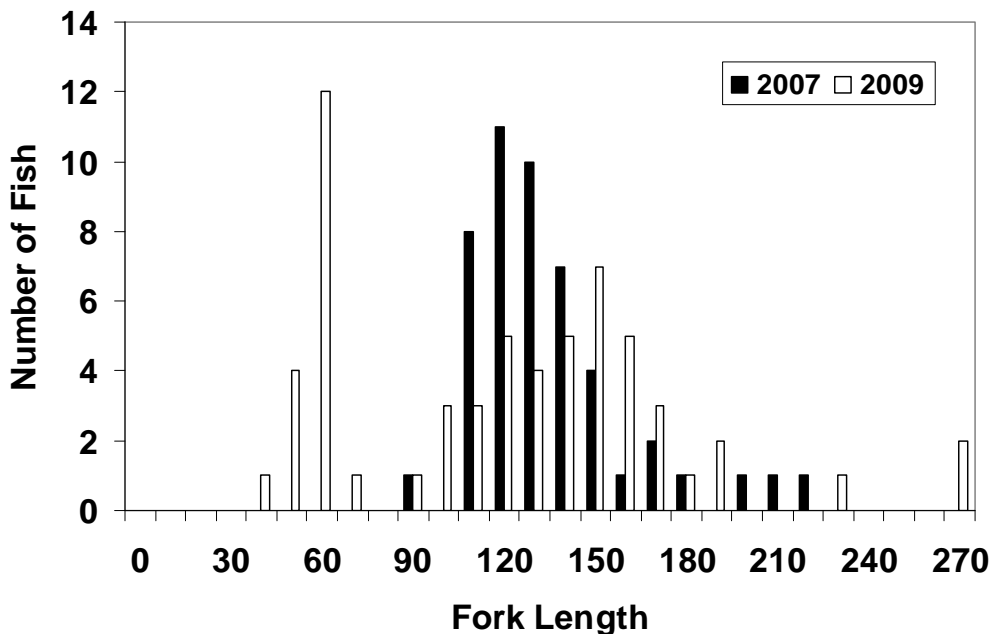


Figure 12. Length frequency histograms for Warner suckers at Summer Lake Wildlife Management Area in 2007 and 2009.

DISCUSSION

The Warner sucker was federally listed as threatened in 1985. Reasons for the listing included watershed degradation, irrigation diversion practices, and predation and competition from introduced fishes (U.S. Fish and Wildlife Service 1998). In most years Hart and Crump Lakes hold water year round, however during droughts the suckers inhabiting the lakes are lost when the lakes desiccate (White et al. 1991; Allen et al. 1994). Stream suckers recolonize the lakes following desiccation (Allen et al. 1994). Irrigation dams and diversions limit movements and genetic exchange between lake and stream suckers (and redband trout) by impeding both the upstream spawning migrations from the lakes into the streams and the downstream migration of young fish into the lakes. To compound these

challenges, when young fish are able to enter the lakes, they face a gauntlet of introduced fishes which both prey upon and compete with them. These conditions have gone relatively unchanged in the 25 years since listing.

Results of previous ODFW studies have found that lake sucker populations are depressed and successful recruitment is limited by the large populations of nonnative fishes in the lakes (Scheerer et al. 2006; 2008). It is our opinion that the stream populations are the strongholds for suckers in the Warner Lakes basin.

Prior investigations in Twentymile Creek by Tait and Mulkey (1993a; 1993b) and Tait et al. (1995), which consisted of snorkel surveys during summer months within a subset of the entire drainage, resulted in density estimates that were consistently lower than the estimates we obtained from our electrofishing surveys. Tait and Mulkey (1993a) acknowledged that snorkeling generally led to significant underestimation of actual abundance and did not employ a calibration method to adjust their estimates to better reflect actual sucker abundance. They found the highest densities of suckers in the middle section of Twelvemile Creek, which generally coincides with our Stratum 2. Using the EPA's general randomized tessellation stratified (GRTS) sampling design, our sampling in 2007 indicated that Twentymile Creek sucker population was the most abundant stream population in the Warner subbasin (Scheerer et al. 2007).

The 2007 estimate of $4,746 \pm 92\%$ adult suckers in Twentymile Creek drainage had low precision, due to the patchy distribution of suckers and the high level of variability in fish density between sample locations (large number of sites with zero fish and a few locations with very high abundance). This finding prompted us to use a different methodology and led us to conduct a comprehensive mark-recapture survey in 2009. This survey resulted in a similar estimate (4,612) with markedly improved precision (95% CI: $\pm 18\%$). Further, we believe that this estimate is generally unbiased. We examined records of PIT-tagged fish across the antenna and feel that the assumption of a closed population was largely met with little evidence of emigration from the system. In addition, a comparison of the spatial distribution of marked and unmarked fish between passes indicates similar catchability between passes. Assumptions of mark retention and mark detection were addressed by using a combination of PIT-tags in fish ≥ 100 mm and fin clips in fish ≥ 60 mm for the marking method. We did not find any fin-clipped suckers ≥ 100 mm that showed evidence of a scar from PIT-tag surgery, yet did not also have a PIT tag. PIT-tags offer a long-term mark for use into future studies, while fin clips were minimally invasive, remained prominent throughout the survey and were easily observed in all sizes.

An additional benefit of our new methodology was that we were better able to describe the distribution of suckers and habitat complexity within this subbasin, which allowed us to infer associations with habitat conditions. It is notable that our estimate of nearly 5,000 suckers indicates a relatively healthy population size, yet the estimate for redband trout in the Twentymile subbasin in 2007 exceeded 27,000 fish (ODFW 2007). It is uncertain whether the abundance of Warner sucker was greater historically, or meets its potential, in this subbasin.

While the methods used in the 2009 stream surveys resulted in a more rigorous estimate than was previously obtained, the lower reaches remain difficult to sample with high efficiency. We achieved a 9% recapture rate in stratum one compared to 18%, 14%, and 17% recapture rates in strata two through four, respectively. The deep, wide pools found in stratum one limit the utility of backpack electrofishers. There is also the potential

loss of fish from this stratum into irrigation ditches. In 2010, we are studying movements of PIT and radio tagged suckers in the Twentymile Creek subbasin to better describe seasonal fish movements in this drainage. We also plan to test the feasibility of using a barge-style electrofisher in the future to increase sampling efficiency in this lower area.

Length frequency histograms for the Twentymile Creek drainage show bimodal distribution with an initial peak at ≈ 80 mm FL and a second peak at ≈ 190 mm, indicating at least two age groups. Very few suckers smaller than 70 mm FL were captured, although field crews noted the presence of smaller suckers at various locations during the survey. Other fish species less than 70 mm FL were captured, including dace and trout fry. It seems plausible that our sampling gear was not effective at capturing juvenile suckers. Use of passive sampling gears (minnow traps or fyke nets) may improve our sampling efficiency for these smaller life stages.

Most of the suckers we captured ($\sim 75\%$) were juveniles (< 160 mm FL). However, in the canyon portion of Stratum 3, juveniles comprised a much lower proportion of the total suckers captured (11%) and in stratum four, upper Twentymile Creek above the confluence with Twelvemile Creek, juvenile-sized fish dominated the catch (96%). It is possible that suckers are spawning successfully in stratum three and the offspring move, or are flushed downstream, and rear in the lower strata. All suckers captured in Twentymile Creek were captured in the lower 500 m of the creek. The high proportion of juvenile suckers in this area and absence of suckers in the steeper, upstream portion of this creek suggests that suckers may use lower Twentymile Creek as rearing habitat.

Through multiple regression analysis, we found that water temperature and depth may influence sucker distribution and abundance. Capture efficiency was somewhat reduced in stratum one, possibly due to either loss of fish into diversions, or to crews being unable to effectively sample several large pools within the stratum. This may have confounded the analysis, although studies by both Tait and Mulkey (1993a and 1993b) and Tait et al (1995) identified depth and temperature as primary variables explaining sucker abundance. We observed that suckers were typically more abundant in both pool and backwater habitats, and were commonly found in areas of with high levels of aquatic macrophytes and smaller sized substrates; however our survey methods were not sensitive enough to detect the influence of these factors. We plan to refine our future sampling protocols to better evaluate fish-habitat associations for these parameters.

We found a positive correlation between maximum recorded temperature and sucker abundance, i.e. suckers were more common in areas where water temperatures were higher. However, because warmer water temperatures are common in the slower, wider downstream areas, suckers may be keying in primarily to the habitats, rather than the temperature of these habitats. It is also possible that suckers are exploiting cool water refuges that we were unable to detect. Temperature data were collected as single point-in-time readings at lower and upper boundaries of each reach, an approach that provided only a coarse level of resolution in the temperature profile. We plan to install instream temperature loggers at multiple locations in the drainage to collect continuous temperature data in the future. This may provide additional insight into the potential relationship between sucker distribution and stream temperatures.

During both the 2008 lake investigations (Scheerer et al. 2008) and the 2009 stream investigations, we noted high incidences of external parasites, lesions and deformities. The most common parasite observed on both the lake and stream suckers was *Lernaea* sp.

This parasitic copepod, commonly called fish lice, has no intermediate host and can easily spread among fishes. As water levels drop during the summer, available habitat is reduced and water temperatures increase, which may result in increased fish densities in suitable, available habitats. A combination of crowding and potential temperature-induced stress may increase the levels of infection.

During our population estimate we assessed the movements of PIT-tagged stream suckers through electrofishing recaptures and using a flat-plate PIT-tag antenna. Of the 421 stream suckers tagged in 2009, 13% showed detectable movement based on recaptures of marked fish when electrofishing. Only eight fish that were PIT-tagged in 2009 were detected at the PIT tag antenna, which suggests a negligible loss (<2%) of fish from the study area during our survey. Six of the eight fish detected at the antenna moved a minimum of 2.4 km from their tagging location. Most of these fish were tagged near the downstream end of the canyon reach. We had anticipated these relatively low levels of fish movement during the low water portion of the year and in 2010, we are monitoring a multiple in-stream antennas to evaluate seasonal sucker movements of PIT-tagged fish in the Twentymile Creek subbasin. We are also tracking 30 radio tagged fish in the subbasin. We hope to gain understanding of seasonal spawning migration (timing and locations) and stream sucker movements to and from the lakes. In 2010, we will also assess the feasibility of using larval drift nets to assess the timing and magnitude of larval fish drift.

Age-at-maturity and age-class distribution are relatively unexplored aspects of the life history of stream suckers. We detected significant annual growth (67-74 mm) in two recaptured suckers that were tagged in 2008. We anticipate obtaining a considerable amount of additional growth information from the nearly 500 fish that have been recently PIT-tagged in the Twentymile Creek drainage.

Results from our ageing feasibility study indicated that pectoral fin ray sections can be used as a non-lethal means to age threatened Warner suckers. Growth marks on these structures were visually distinct and were relatively easy to count, especially for younger fish. Pectoral fin ray ages matched otolith ages for suckers under 15 years old 79% of the time. Growth marks on pectoral fin rays did become more difficult to read in suckers older than 15 years, but the oldest Warner sucker aged in this study was 5 years. All Warner suckers aged in the study were captured from Summer Lake and were relatively small (range 24-157 mm FL). We recommend taking a fin clip as close to the base as possible, and no farther than 2 mm towards the distal end, to minimize loss of the innermost growth marks. We plan to collect fin rays from suckers captured in 2010 and use this aging technique to describe the population age structure of suckers in Twentymile Creek and the in the lakes. Additionally, we have begun an ageing validation study whereby we inject PIT-tagged Warner suckers with oxytetracycline. This injection will deposit a mark on the otolith at a known time that can be used to validate annual marks on these fish when they are recaptured in the future.

We monitored the refuge population of Warner suckers located at the Summer Lake Wildlife Management Area and found that the population was robust with evidence of recent successful reproduction and recruitment. We propose genetic analysis to determine the level of variability that exists within this population (for example, did a bottleneck occur and/or has substantial drift occurred). This is critical to determine whether this population is suitable for use to repopulate the Warner Basin population, following future drought conditions in the Warner basin.

In the past few years, we have established a close working relationship with landowners in the Twentymile Creek subbasin. In 2010, we are assessing the movements of Warner suckers during the spawning period. We hope to identify timing and location of spawning, timing and magnitude of larval drift, and assess downstream movements from the stream to Crump Lake. We are also monitoring the abundance of Warner suckers and other fishes in the lakes. In addition, we are continuing to collect tissue samples for future genetic analysis. These efforts will provide data critical to recover this species.

ACKNOWLEDGEMENTS

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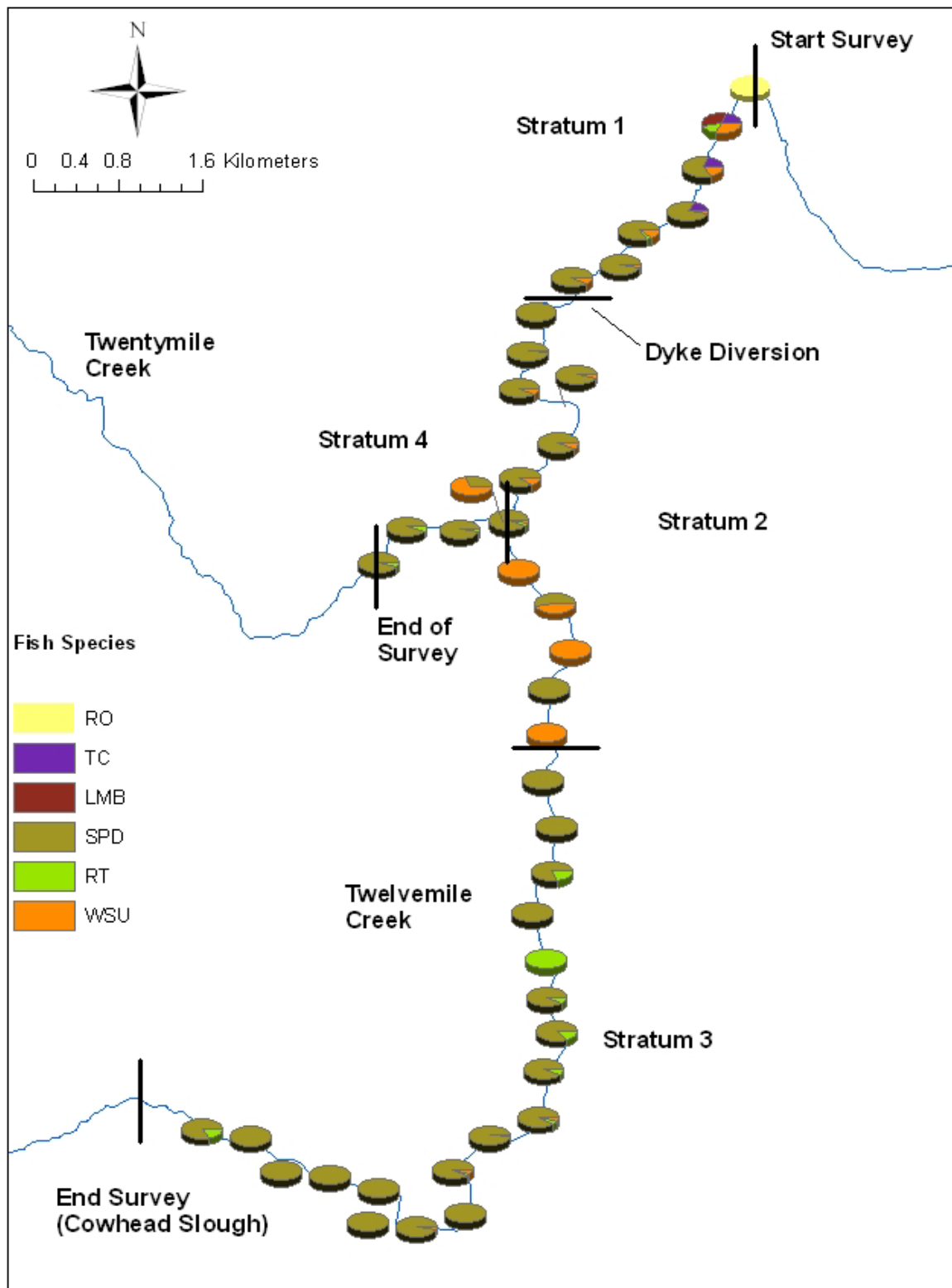
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APPENDIX A. Distribution of fish observed in the Twelvemile Creek subbasin. Pie charts show the approximate proportion of species in each reach. Fish codes: RO- roach, TC- tui chub, LMB- largemouth bass, SPD- speckled dace, RT- redband trout, and WSU- Warner sucker.



APPENDIX B. Photos showing low lake elevations in 2009.

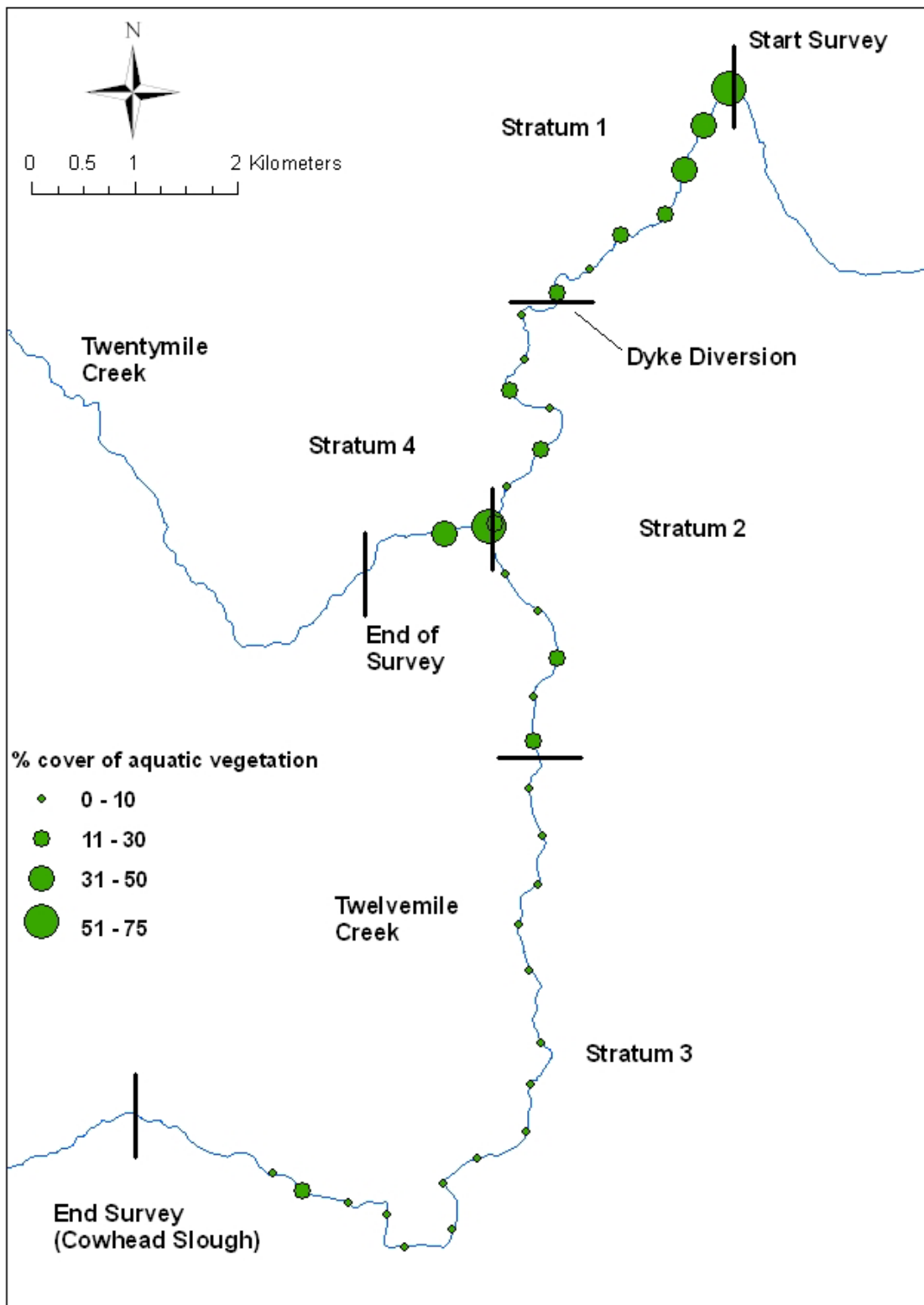


Hart Lake in the spring of 2009

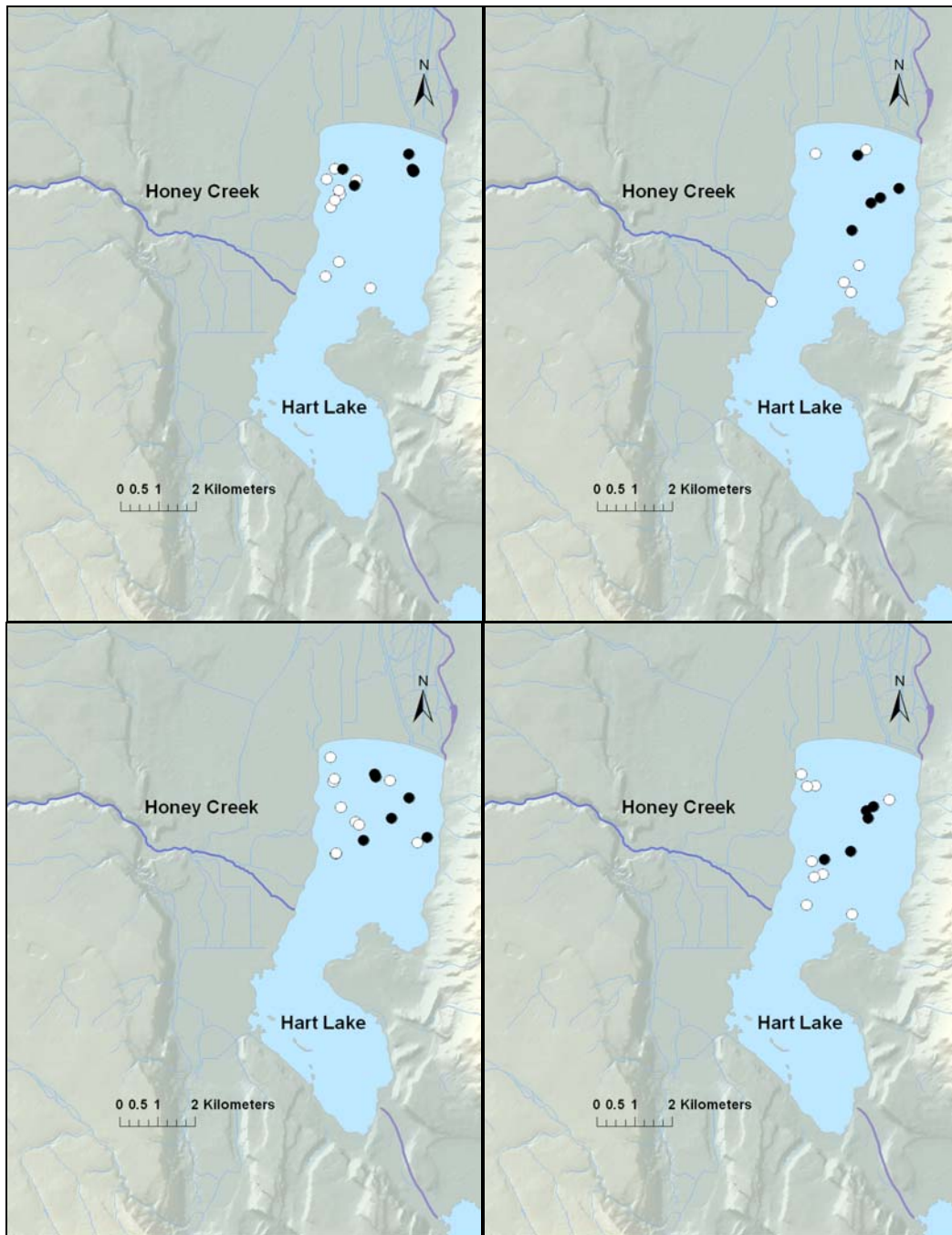


Aerial tracking of radio-tagged suckers. Looking north toward Hart Lake (top of photo).

APPENDIX C. Average percentage of aquatic vegetative cover per reach.



APPENDIX D. Detections of Warner suckers radio tagged in Hart Lake in 2008. Black circles represent 2009 tag detections and white circles represent 2008 tag detections. Starting at the upper left and moving clockwise, these maps represent tracking locations for fish with tag numbers 12, 14, 17, and 15, respectively.



APPENDIX E. Detections of Warner suckers that were radio tagged in Hart and Crump Lakes in 2008 and that were mortalities in 2009. No suckers tagged in Crump Lake in 2008 were found to be alive in 2009. Tag numbers are listed next to white circle. Note that several tags were located on shore, including those on the western shoreline of Hart Lake, which was dry in 2009.

