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Contributions of Stocked and Naturally Reproduced Rainbow Trout in the Deerfield Reservoir System

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ABSTRACT Deerfield Reservoir in the Black Hills of South Dakota and its tributary system are managed as hatchery supplemented rainbow trout (*Oncorhynchus mykiss*) fisheries. Three genetically unique strains of rainbow trout (Shasta, Erwin and McConaughy) are stocked into the system. Recently, juvenile rainbow trout of unknown origin were collected in the tributary system above Deerfield Reservoir, indicating potential natural reproduction. Understanding the genetic origins and ability of these rainbow trout to contribute to the fishery is essential in determining the proper management strategy for these waters. Our objectives were to 1) evaluate the genetic origins of potentially naturally reproduced rainbow trout in Castle Creek and South Fork Castle Creek, 2) evaluate the contribution of potentially naturally reproduced rainbow trout to Deerfield Reservoir, and 3) evaluate the movements of stocked rainbow trout between Deerfield Reservoir and its tributary system. Microsatellite DNA analysis of naturally reproduced fish indicated that genetic material was primarily contributed by Erwin and McConaughy strain rainbow trout. Logistic regression analysis was used to develop a predictive model for known wild and known hatchery fish based on scale circuli growth characteristics. Logistic regression indicated that approximately 50% of the unknown origin fish were of wild origin. Finally, adfluvial movements by the three strains of rainbow trout from Deerfield Reservoir were evaluated using passive integrated transponder (PIT) technology. McConaughy strain fish exhibited the highest proportion of tagged individuals moving upstream followed by Erwin and Shasta strains, respectively. Knowledge of the origins of the genetic background for naturally reproduced rainbow trout as well as their ability to contribute to the sport fishery is essential to determine the appropriate fisheries management strategy for the Deerfield Reservoir system.

KEY WORDS Black Hills, natural recruitment, *Oncorhynchus mykiss*, rainbow trout, scale analysis, South Dakota

Stocking of various salmonids began in the late 1800s in the Black Hills of South Dakota to provide novel recreational fisheries (Cordes 2007). Put-and-take fisheries, consisting of stocked (i.e., 200–380 mm) rainbow trout (*Oncorhynchus mykiss*), are a standard management practice for reservoirs in the Black Hills. Rainbow trout natural recruitment was thought to be non-existent in most locations as little evidence existed in annual surveys conducted by South Dakota Game, Fish and Parks (SDGFP). Deerfield Reservoir, a popular trout fishery in the Black Hills, annually receives approximately 12,000 rainbow trout from three different genetic strains. Post-stocking survival of hatchery origin rainbow trout in Deerfield Reservoir has not been evaluated, but a review of the literature indicated that in many cases hatchery-reared rainbow trout tend to exhibit low long-term post-stocking survival (Vincent 1975, 1987, Marchetti and Nevitt 2003, Rikardsen and Sandring 2006).

Recruitment by hatchery reared rainbow trout has been observed in natural systems (Marcogliese and Casselman 1998). However, factors such as genetic strain (Brauhn and Kincaid 1982, Tymchuck and Devlin 2005), life-history strategies (Behnke 2002, Meka et al. 2003), habitat (Bjornn and Reiser 1991, Grost et al. 1991, Sternecker and Geist 2010), predation (Mueller and Rockett 1962), and competition (Fraser 1972, 1978, Marrin and Erman 1982) must be appropriate for such recruitment to occur. Observations of age-0 trout in recent stream surveys have indicated potential natural reproduction by rainbow trout within the Castle Creek drainage above Deerfield Reservoir (Bucholz and Wilhite 2010), but survival to adulthood by these age-0 rainbow trout was unknown.

Black Hills lakes, reservoirs and streams are classified and managed by the SDGFP based on biological and physical characteristics, including naturalized salmonid populations and water use (Erickson et al. 1993). Deerfield Reservoir is managed as a put-and-take rainbow trout fishery, while both Castle and South Fork Castle Creeks are managed as wild brook trout (*Salvelinus fontinalis*) fisheries, supplemented with hatchery-reared rainbow trout. The presence of potentially naturally reproduced rainbow trout in the main tributary system of Deerfield Reservoir suggested a need to reevaluate the classification and management of the Deerfield Reservoir,

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Castle Creek and South Fork Castle Creek fisheries. While evidence indicated that natural reproduction was occurring, neither the genetic origins of the age-0 trout nor the extent of recruitment to the sport fishery by these naturally reproduced individuals is known. Thus, our objectives of this study were to 1) evaluate the genetic origins of potentially naturally reproduced rainbow trout in Castle Creek, South Fork Castle Creek, and Deerfield Reservoir, 2) evaluate the contribution of potentially naturally reproduced rainbow trout to the sport fishery in Castle Creek, South Fork Castle Creek, and Deerfield Reservoir using scale analysis, and 3) evaluate the movements of wild and each stocked strain of rainbow trout between Deerfield Reservoir and Castle and South Fork Castle creeks.

STUDY AREA

Deerfield Reservoir is located 35 km west of Rapid City, South Dakota on Castle Creek (Fig. 1), and has a full-pool elevation of 1,792 m above sea level. Storage of Deerfield Reservoir is 1,781 ha-m when at full pool, with a regulated mean outflow of about 0.25 cubic meters per second. Deerfield Reservoir has a surface area of 168 ha, maximum depth of 27.4 m, and drains a watershed area of 24,605 ha. The reservoir is operated by the U.S. Bureau of Reclamation in tandem with Pactola Reservoir located downstream on Rapid Creek.

Castle Creek is a tributary of Rapid Creek. The headwaters of Castle Creek are located approximately 17 km southwest of Hanna, South Dakota and flow approximately 22 km southeast into Deerfield Reservoir, the only reservoir on Castle Creek. South Fork Castle Creek is a tributary of Castle Creek entering Castle Creek from the south approximately 400 m upstream of Deerfield Reservoir. Castle Creek upstream of Deerfield Reservoir drains approximately 23,600 ha.

METHODS

Genetic origins of naturally reproduced rainbow trout

We collected potentially naturally reproduced rainbow trout (n = 45, <150 mm total length [TL]) from the Castle Creek tributary system during spring and summer of 2009 using backpack electrofishing (Smith Root LR-24, Vancouver, Washington, USA). Collected fish were frozen whole and transported on ice to South Dakota State University (SDSU). Fish remained frozen at SDSU and were subsequently transported on ice to the University of Wisconsin-Stevens Point (UWSP) where they were thawed and caudal fin tissue was collected for analysis. We also removed pelvic fins (n = 25) from hatchery-reared rainbow trout raised at McNenny State Fish Hatchery, Spearfish, South Dakota. These fish represented known genetic strains (Erwin, McConaughy, and Shasta) historically stocked into Deerfield Reservoir. We preserved all fins from hatchery-reared fish in 95% ethanol.

We isolated total genomic deoxyribonucleic acid (DNA) from pelvic fin tissue using the Promega, 96 Well Format

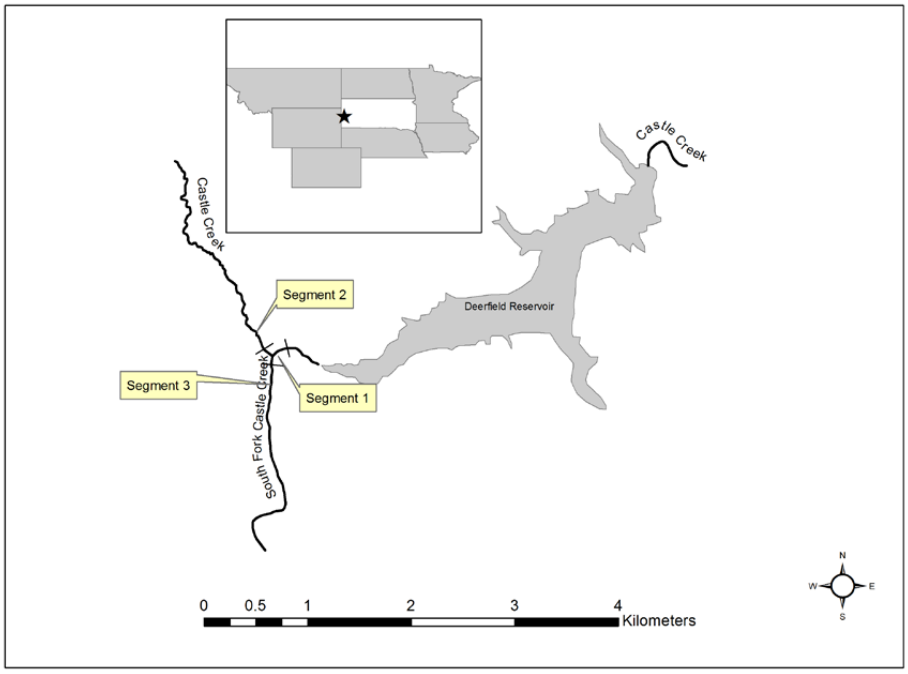

Figure 1. Map of Deerfield Reservoir, South Dakota, USA, the Castle Creek tributary system and associated stream segments. Dashed lines indicate locations of the passive PIT readers in the Castle Creek tributary system.

DNA Kit (Promega Corp., Madison, Wisconsin, USA), following the recommended protocol developed at the Molecular Conservation Genetics Laboratory at UWSP (B. L. Sloss, UWSP, personal communication). We quantified (ng/μL) the DNA using a Nanodrop® ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA).

We selected eight microsatellite loci for genetic analysis based on a literature review: (OMM5233, OMM5177; Coulibaly et al. 2005), (OMM1008, OMM1051; Rexroad et al. 2002*a*), (OMM1097, OMM1088; Rexroad et al. 2002*b*), (OMM1325; Palti et al. 2002) and (OMM5047; Rexroad et al. 2005). We used polymerase chain reactions (PCR) to amplify microsatellites for buffer detection. Loci names, primer sequences, dye labels, multiplex number, primer concentration PCR processes, reaction buffers, and conditions for each individual PCR are summarized in Davis (2012). We genotyped successful PCR reactions with an ABI 3730 Genetic Analyzer (Applied Biosystems, Inc. [ABI], Foster, California, USA), and determined sizes with reference to GeneScan 600 LIZ size standards. The resulting genotype data were analyzed using GeneMapperID v3.2 (Applied Biosystems, Inc.). In cases where the PCR reaction failed or poor results (e.g., poor amplification) were obtained during genotyping, we attempted samples a second time using either the same sample of DNA, re-purified DNA, or using an additional DNA template. All PCR reactions and genotyping were conducted by ACGT, Inc. (Wheeling, Illinois, USA).

After optimizing previously described conditions, we genotyped all samples from the three known hatchery strains (reference populations) and samples of wild fish of unknown origin using the eight markers. We included samples for further analysis when two alleles were amplified at each individual locus. In cases where three alleles were determined for a single locus, we noted weak peaks for the second allele. In cases where data were unclear with stuttering or too poor of amplification to determine a second allele, we used Peak Scanner v1.0 (Applied Biosystems, Inc.) to try and determine the two amplified alleles. In cases where two alleles were not distinct, we removed those samples from further analysis. Additionally, we removed samples from analysis when two alleles were amplified at fewer than five loci to remove bias associated with missing data. We used microsatellite Toolkit 3.1.1 (Park 2001) to calculate number of individuals genotyped, allele counts by population, allele frequencies for all populations by locus, observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content (PIC). To calculate the number of alleles and mean number of alleles at each locus, we used GenAlex (Peakall and Smouse 2006).

To estimate genetic structure and determine the genetic origins of the naturally reproduced (unknown origin) rainbow trout, we used a cluster analysis based on a Bayesian framework implemented in STRUCTURE v2.3.3 (Pritchard et al. 2000). The program infers the proportion of membership of each individual to clusters based on allele frequencies. To infer the number of clusters or populations (K) present in the genomic data set (X), STRUCTURE estimates the proportion of the genotypes of each individual having ancestry to each cluster and applies a prior probability of those data (Pr[X|K]) and then infers the proportion of membership of each individual to clusters inferred from allele frequencies. We did not model a series of independent runs using the Gibbs sampler with varying values of K as has been done in previous research. Instead, a set value (K = 3) was selected *a priori* because three strains were being evaluated as possible contributors to genetic make-up of naturally reproduced rainbow trout. The admixture model assumes that a fraction of an individual's genetic make-up may have been acquired from different strains depending on parental crosses. The results are based on runs of 10^6 Monte Carlo Markov Chain (MCMC) iterations, following a burn-in period of 10^5 iterations.

Contribution of wild rainbow trout based on scale analysis

We collected scales from rainbow trout (n = 26) during the annual fish community survey at Deerfield Reservoir conducted on 18–19 August 2010, prior to stocking of rainbow trout in September. Standard fish sampling survey gear used during the survey consisted of four experimental gill nets (45.7 × 1.8 m, with graded monofilament mesh sizes of 12.7, 19.1, 25.4, 31.8, 38.1, and 50.8 mm bar mesh) and four modified fyke nets (1.3 × 1.5 m frame, 19.1 mm bar mesh, and a 1.2 × 23 m lead) set daily and allowed to fish overnight.

We identified collected fish as being of hatchery origin by an adipose fin clip. With the exception of the first stocking of 2,000 fish in April, we clipped the adipose fin prior to stalking the remaining 10,000 rainbow trout. Complete removal of the adipose fin results in no fin regeneration (Thompson and Blankenship 1997); thus, adipose clipped rainbow trout could be identified as being of hatchery origin regardless of the amount of time they had been in the reservoir. We initially categorized rainbow trout without adipose fin clips as being unknown origin fish. We collected scales from above the lateral line and slightly anterior of the dorsal fin (DeVries and Frie 1996). Once collected, scales were immediately cleaned with water, if necessary, and placed in scale envelopes. We labeled envelopes with length and weight and the adipose condition (e.g., clipped or unclipped) and transferred them to SDSU for analysis.

We collected scales from potentially naturally reproduced rainbow trout (n = 29) in the Castle Creek system above the reservoir during spring and summer of 2009 and 2010 using backpack electrofishing. We identified fish as being potentially naturally reproduced by size (<150 mm) as the system was stocked with 200–290 mm hatchery raised rainbow trout. Scales from hatchery raised rainbow trout (n = 25) were collected at McNenny State Fish Hatchery. We evaluated scales while being viewed under a microfiche reader. We chose a single representative scale from each fish using two criteria: 1) a well-defined focus (i.e., no regeneration) and 2) having

standard shape (i.e., no elongation). When no suitable scale was available, we removed the fish from the study. We placed suitable scales between two microscope slides and viewed them under a compound microscope at maximum magnification (70×). A DP72 microscope digital camera (Olympus America, Lake Success, New York, USA) attached to the microscope captured a digital image of each scale. We made two measurements on each scale using DP2-BSW microscope digital camera software (Olympus America): focus to sixth circulus and fifth to sixth circuli. Differentiation of wild and hatchery origin salmonids had previously been successful using these measurements in previous research (Madden et al. 2010). We assessed data for normality with a Shapiro-Wilks test and for homogeneity of variance using a Folded F-test. Scale measurements between known hatchery and potentially reproduced rainbow trout were compared using a t-test (PROC T-TEST; SAS Institute 2008). We set alpha at 0.10 because this was an exploratory analysis.

We used logistic regression to develop a predictive model from known hatchery and potentially naturally reproduced fish scale measurements to classify the rainbow trout that possess an adipose fin (e.g., unknown origin). We set potentially naturally reproduced fish as the reference group (wild = 0) and hatchery fish as the response group (hatchery = 1). Alpha again was set at 0.10. We used logistic regression analysis on both measurements and we selected the best model based on which model was most significant (i.e. lowest *P* value) and correctly classified the highest percentage of input data. We used the best-fitting model to estimate the probabilities of collected fish being of wild or hatchery origin.

Movements of stocked rainbow trout above Deerfield Reservoir

We stratified the Castle Creek tributary system (e.g., the primary tributary system) into three study (stream) segments. The most downstream segment, segment one, was approximately 100 m above the inlet to Deerfield Reservoir and extended upstream to the confluence of South Fork and Castle Creeks. Segment two began approximately 50 m above the confluence in Castle Creek and extended upstream. Segment three began approximately 50 m above the confluence in South Fork Castle Creek and extended upstream.

We used passive integrated transponder (PIT) tag technology to assess adfluvial movements of hatchery-reared rainbow trout from Deerfield Reservoir into Castle and South Fork Castle Creeks. We implanted PIT tags into a portion of the hatchery-reared rainbow trout at McNenny State Fish Hatchery from April to July 2010. We stocked Deerfield Reservoir with 2,000 rainbow trout monthly from May to October 2010. Rainbow trout tagged in the hatchery were individually anesthetized with MS-222, Benzoak or Aqui-S E as part of a separate study conducted under Investigational New Animal Drug (INAD) study numbers 11–740 and 11–741. We implanted PIT tags into 600 Shasta strain rainbow trout (mean TL = 286 mm), 595 McConaughy strain fish (mean TL = 281 mm), and 602 Erwin strain fish (mean TL = 203 mm; Table 1). We placed anesthetized fish in a surgical trough and made a 0.5 cm incision on the ventral surface near the pelvic fin. We inserted a PIT tag (HDX PIT tag, 23.1 mm long, 3.9 mm diameter, weighing 0.6 g in air; Texas Instruments, Inc., Dallas, Texas, USA) into the body cavity through the incision; we closed the incision with a single dissolvable surgical suture. We used triadine to sterilize tags, sutures and all surgical instruments (Roussel et al. 2000).

We installed three passive monitoring stations in the Castle Creek tributary system above Deerfield Reservoir during August 2010 and operated through August 2011. We constructed antennae as open-coil inductor loops with 8-gauge multistrand wire (see diagram in Davis [2012]). To encircle the wetted stream channel, wire passed through 2.5 cm diameter PVC pipe secured to the streambed by multiple cinderblocks and was suspended over the water with the support of aircraft cable stretched across the stream channel. We connected each antenna to a radio frequency identification (RFID) half-duplex single antenna reader (HDX RFID, Oregon RFID, Portland, Oregon, USA) powered by two sealed 12 V, deep-cycle marine batteries (Werker Marine Deep Cycle, 100 amp hr battery) connected in parallel. We used a palmtop computer (waterproof PDA, Oregon RFID) to download output data from the readers and displayed individual tag identification, date and time of detections. In addition, the palmtop com-

Table 1. Monthly number of rainbow trout stocked, the strain type stocked and number of trout that were implanted with passive integrated transponder (PIT) tags prior to stocking into Deerfield Reservoir, South Dakota, USA, May–October 2010.

Month	Strain	Number stocked	Number PIT tagged
May	Shasta	2,000	300
Jun	Shasta	2,000	300
Jul	McConaughy	2,000	300
Aug	McConaughy	2,000	295
Sep	Erwin	2,000	300
Oct	Erwin	2,000	301

puter allowed us to adjust the frequency of scans made by the reader and to minimize voltage required for operation to accommodate for variable environmental conditions affecting battery life (e.g., temperature). We placed weather-proof reader boxes and batteries in vertical culverts located outside of the immediate flood zone and locked to prevent tampering. When possible, we retrieved data and changed batteries biweekly. These monitoring stations did not differentiate upstream or downstream movement and direction could only be determined if a tagged individual passed two separate stations. We tested tag detection by each individual reader after initial installation and at randomly spaced intervals throughout the monitoring study. All readers passed detection tests throughout the duration of the study. Collected data were imported to Microsoft Excel and exported to a Microsoft Access database for analysis. We used a chi-square test to test for significant ($\alpha = 0.05$) differences in movement by tagged individuals among the three strains. We conducted pair-wise comparisons to assess differences between strains by study segments. Our research was conducted under approval from the South Dakota State University Institutional Animal Care and Use Committee (Approval Number 10-024A).

RESULTS

Genetic origins of naturally reproduced rainbow trout

A total of 198 fin tissue samples were collected and analyzed. The overall genetic results for these fish can be found in (Davis 2012). For this paper, we only analyzed the 45 samples that were collected from potentially naturally reproduced rainbow trout from the Castle and South Fork Castle Creeks above Deerfield Reservoir. We removed 27 of the 45 samples from analysis because they failed to amplify at five or more loci, resulting in 18 potentially naturally reproduced fish that were genetically identified. Assignment tests conducted with STRUCTURE succeeded in assigning each individual to a cluster, with each cluster relating to an individual's parents source subpopulation. Because three strains are currently stocked, assessment of all samples was determined by detecting three clusters based on multiplexed genotypes. Resolution of the multiplexed markers provided results that were representative of the number of strains present in the system. Potentially naturally reproduced fish consisted of an admixture of primarily two of three strains of rainbow trout stocked into Deerfield Reservoir. The majority of the genetic make-up of these individuals as a whole was assigned to the Erwin cluster, with a smaller portion being assigned to the McConaughy cluster (Fig. 2).

Contribution of wild rainbow trout based on scale analysis

Mean measurements from scale focus to sixth circulus of potentially naturally reproduced, unknown origin, and hatchery raised fish were 2,909 (SD = 273), 2,734 (SD = 333), and 2,651 (SD = 231) µm, respectively (Fig. 3). Mean measurements from fifth to sixth circuli for potentially naturally reproduced, unknown, and hatchery origin fish were 361 (SD = 62), 355 (SD = 63), and 329 (SD = 61) µm, respectively (Fig. 3). Scale measurements from focus to sixth circulus and fifth to sixth circuli were normally distributed for known hatchery origin fish ($P = 0.41$ and $P = 0.75$, respectively) and for potentially naturally reproduced fish ($P = 0.38$ and $P = 0.53$, respectively) and variances were homogenous for both

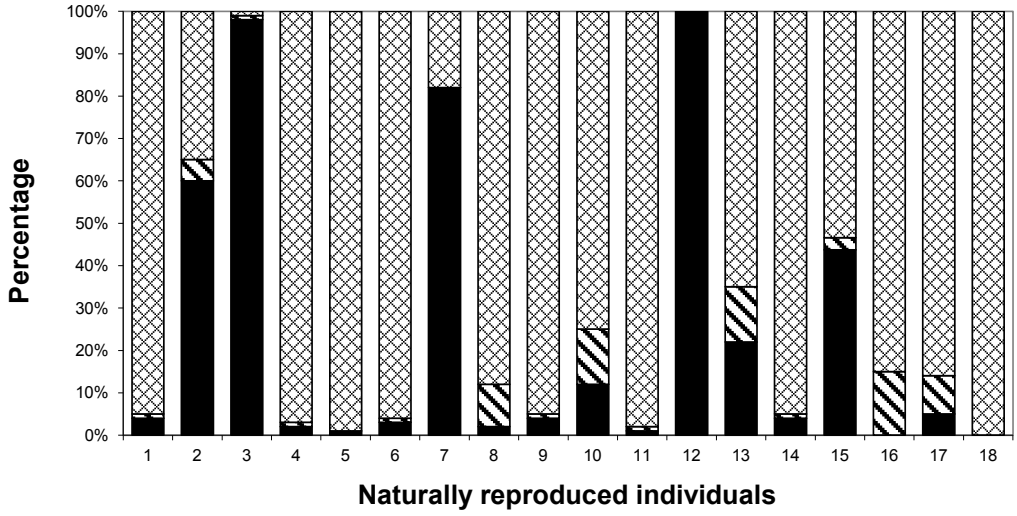

Figure 2. Genetic classification of 18 potentially naturally reproduced rainbow trout (*Oncorhynchus mykiss*) collected from the Castle Creek tributary system above Deerfield Reservoir, South Dakota. Black denotes percent contribution from the McConaughy strain, diagonal lines indicate Shasta strain contribution and cross-hatched sections represent contribution by Erwin strain with individuals of mixed origin showing contribution from more than one strain.

measurements ($F_{1,53}$ = 1.39, P = 0.41 and $F_{1,53}$ = 1.02, P = 0.96, respectively). Significant differences were detected in scale measurements of potentially naturally reproduced fish and hatchery raised fish from the focus to sixth circulus (t_{53} = −3.74, P = 0.0004) and from fifth to sixth circuli (t_{53} = –1.94, P = 0.058).

The predictive model for scale measurements from focus to sixth circulus was

Logit (Probability) = 10.797 – 0.004 (focus to sixth circulus)

Low model fit (model $P < 0.0001$, $r^2 = 0.166$) was expected as substantial overlap existed between the two distributions of known scale measurements, but the model correctly classified 60.4% of the input data.

The predictive model for scale measurements from fifth to sixth circuli was

Logit (Probability) = 2.961 – 0.009 (fifth to sixth circuli)

This model (model P = 0.052, r^2 = 0.05) correctly classified 53.5% of the input data. Subsequent analyses used the predictive model developed for scale measurement from the focus to sixth circulus because classification success was higher for this metric.

The predictive logistic regression equation allowed us to calculate the probability that each of the 26 unknown origin (e.g., no adipose clip) fish in the standardized lake survey belonged to either the potentially naturally reproduced (reference or "0" group) or the hatchery category (response or "1" group). The calculated probability values for our 26 unknown origin samples ranged from 0.05 (relatively certain to be a wild fish) to 0.93 (relatively certain to be a hatchery fish). Of the 26 unknown origin fish, 13 were classified as potentially naturally reproduced fish (probability range 0.05–0.49), while 13 were classified as hatchery origin fish (range 0.50–0.93; Fig. 4).

Movements of stocked rainbow trout above Deerfield Reservoir

We recorded at least one PIT-tagged individual of each strain entering segment one by the week of 3 April (Fig. 5). An increasing trend in detections was evident until the week of 29 May when the maximum combined number of tagged fish entered segment one. During this week the maximum number of Shasta (n = 4), McConaughy (n = 22), and Erwin (n = 24) strain fish were detected in segment one (Fig. 5). Tagged individuals of all three strains continued to be detected in subsequent weeks, but detections became more infrequent.

We detected a total of 103 (17%) of the 595 tagged McConaughy strain rainbow trout in segment one, followed by 33 (5%) of the 601 tagged Erwin strain and 15 (3%) of the 600 tagged Shasta strain (Table 2). Proportions of tagged individuals among the three strains were different for segment one (χ^2_4 = 3,592, $P < 0.001$). Pair-wise comparisons revealed differences in detections between strains for segment one ($\chi^2_1 \geq 1{,}195$, $P < 0.001$ for all three pair-wise comparisons) with

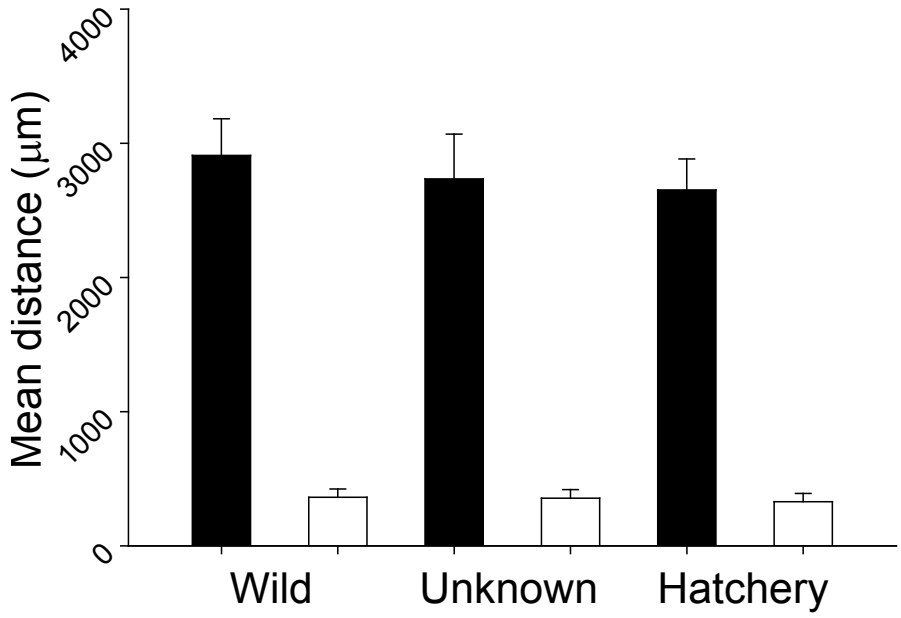

Figure 3. Mean (SD) distance (µm) from focus to sixth circulus and from fifth to sixth circulus respectively, for scales removed from wild, unknown, and hatchery origin rainbow trout (*Oncorhynchus mykiss*) collected from the Deerfield Reservoir system and McNenny State Fish Hatchery, South Dakota. Black bars denote focus to sixth circulus, while white bars denote fifth to sixth cirucli measurements.

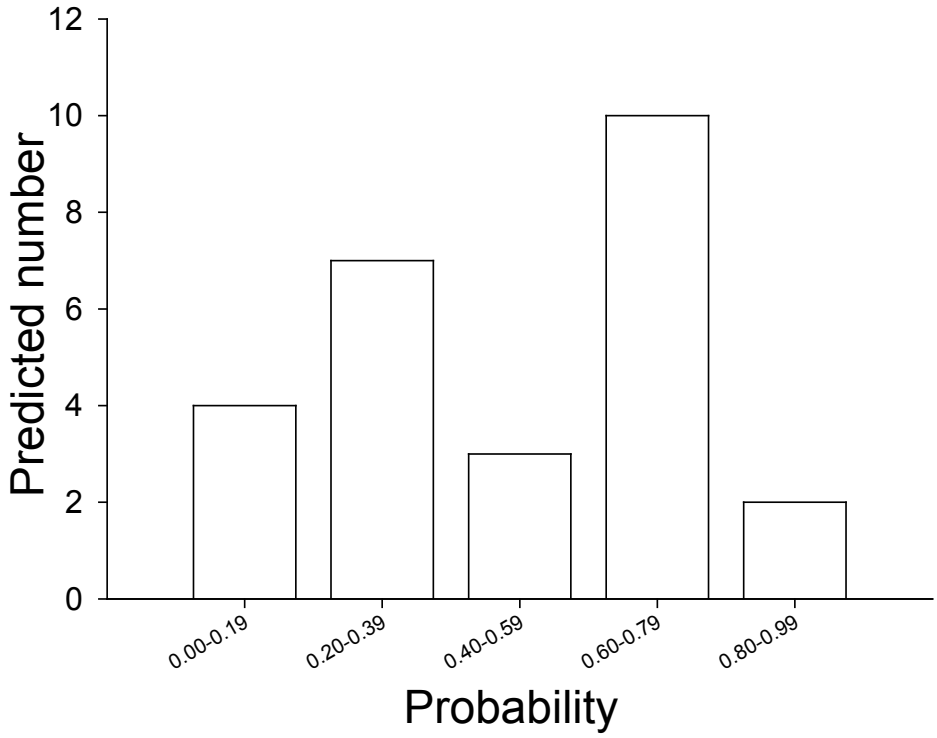

Figure 4. Predicted numbers of unknown origin rainbow trout (*Oncorhynchus mykiss*) collected from Deerfield Reservoir, South Dakota, USA, being of hatchery or wild origin based on probabilities from logistic regression. Values <0.50 were classified as "wild" origin; values ≥0.50 were classified as "hatchery" origin.

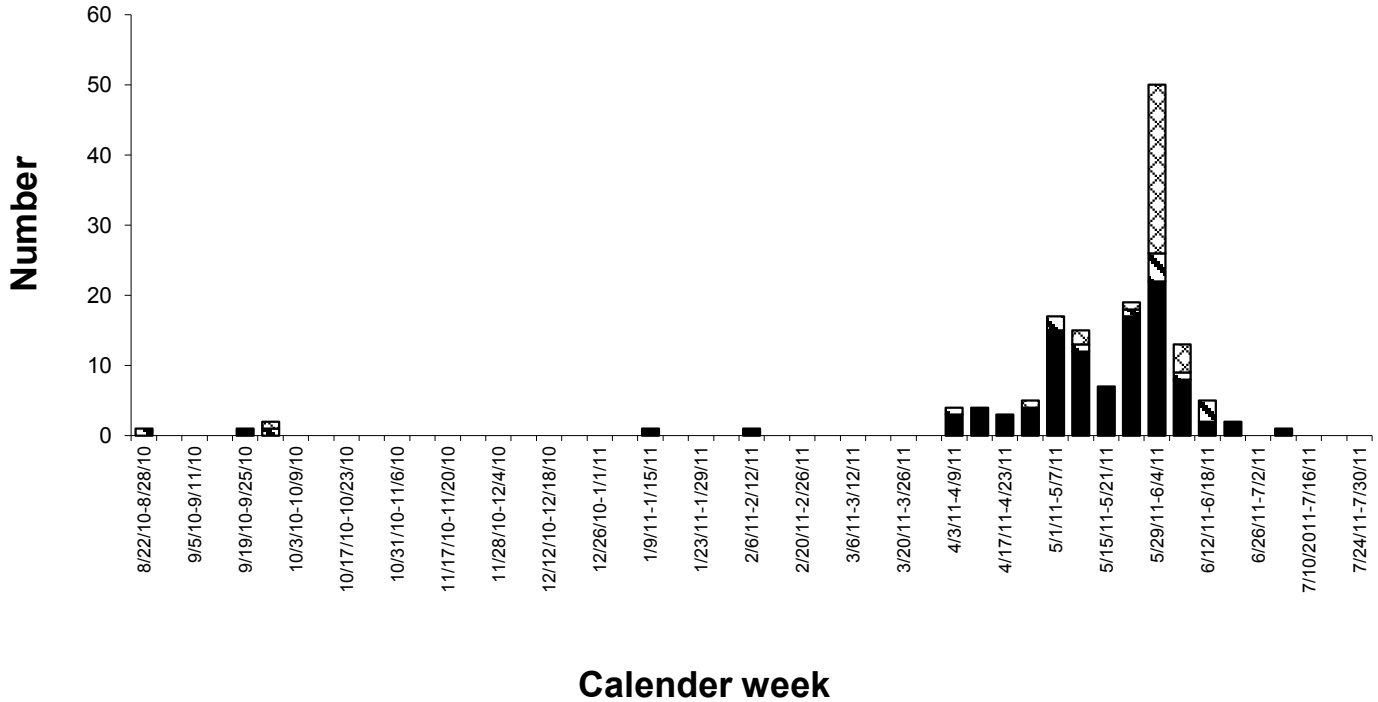

Figure 5. Number of Shasta, McConaughy and Erwin strain rainbow trout (*Oncorhynchus mykiss*) moving into segment one of Castle Creek above Deerfield Reservoir, South Dakota, USA, by week from August 2010–August 2011. Black bars represented the McConaughy strain, diagonal bars indicated the Shasta strain and cross-hatched bars are Erwin strain.

McConaughy strain fish exhibiting the highest movement into segment one, followed by Erwin and Shasta strains.

We observed a similar pattern of movement as PIT tagged individuals moved upstream of segment one. Of the 103 tagged McConaughy strain rainbow trout that were detected in segment one, 36 moved upstream and were detected in segment two, while 22 of the 33 tagged Erwin strain detected in segment one were detected in segment two. Only two of the 15 Shasta strain detected in segment one were detected in segment two. Proportions of tagged individuals among the three strains were different for segment two ($\chi^2_4 = 307$, $P < 0.001$). Similarly, pair-wise comparisons revealed differences in detections between strains for segment two ($\chi^2_1 \geq 48$, $P < 0.001$ for all three pair-wise comparisons) with McConaughy strain fish exhibiting the highest movement rate, followed by Erwin and Shasta strains.

Of the 103 tagged McConaughy strain rainbow trout that were detected in segment one, we detected 18 upstream in segment three. Of the 33 tagged Erwin strain that were detected in segment one, five moved upstream and were detected upstream in segment three. Two of the 15 tagged Shasta strain fish detected in segment one were detected in segment three. Proportions of tagged individuals among the three strains were significantly different for segment three ($\chi^2_4 = 302$, $P < 0.001$). Pair-wise comparisons revealed significant differences in detections between strains for segment three ($\chi^2_1 \geq 48$, $P < 0.001$ for all three pair-wise comparisons) with McConaughy strain fish exhibiting the highest movement rate, followed by Erwin and Shasta strains.

DISCUSSION

The three components of this study contributed to a complementary assessment of the extent of natural reproduction of rainbow trout in the Deerfield Reservoir System. Our genetic analysis of potentially naturally reproduced rainbow trout, <150 mm TL, indicated that the Erwin followed by the McConaughy strain contributed the most genetic material. Very little Shasta strain genetic material was found in the potentially naturally reproduced fish. Poor amplification of alleles during the PCR process did result in the removal of 27 of the 45 fish, which resulted in a smaller sample size. Of the 27 removed samples, 24 were potentially naturally reproduced rainbow trout, in which individuals were frozen whole after collection. These individuals were then thawed prior to collection of fin tissue for genetic analysis. The process of freezing and thawing may have potentially denatured the DNA and resulted in poor amplification, as has been observed in previous research (Fraser and Strzeżek 2005). These samples were then removed prior to program STRUCTURE analysis to mitigate any potential bias. All samples included in the analysis exhibited amplification at >5 loci, which increased the likelihood of correct assignment. Program STRCTURE has shown to perform well with small data sets in previous research (Hauser et al. 2006). The eight-marker set used in this application proved useful in assigning portions of individual rainbow trout genetic make-up to cluster (i.e., strains). This marker set was developed for rainbow trout by other researchers and was modified from an original multiplex that included 12 microsatellite markers. This preliminary study (Johnson et al. 2007) was very similar in design to our study and included Shasta strain rainbow trout.

Our scale analysis, supported by a logistic model, was a useful tool and estimated that approximately 50% of the unclipped (e.g., unknown origin) rainbow trout present in the Deerfield Reservoir annual fish population survey were potentially natural reproduced. Moderate overlap in scale measurements occurred between potentially naturally reproduced

Table 2. Comparisons of tagged individuals of each strain that entered segments one, two and three within the Castle Creek tributary system, South Dakota, USA. Values in parentheses represented the number of tagged individuals detected in each segment. *P*-values are from pairwise Chi-square tests assessing the number of fish tagged and proportions of tagged fish that moved.

Strain	Strain	χ^2	P-value
	Segment 1		
Erwin (33)	McConaughy (103)	1,196.0	<0.001
Shasta (15)	McConaughy (1030	1,195.0	<0.001
Shasta (15)	Erwin (33)	1,201.0	<0.001
	Segment 2		
Erwin (22)	McConaughy (36)	136.0	<0.001
Shasta (2)	McConaughy (36)	118.0	<0.001
Shasta (2)	Erwin (22)	48.0	<0.001
	Segment 3		
Erwin (5)	McConaughy (18)	136.0	<0.001
Shasta (2)	McConaughy (18)	118.0	<0.001
Shasta (2)	Erwin (5)	48.0	<0.001

and hatchery fish, which lowered overall confidence. However, we are confident that rainbow trout with focus to sixth circulus measurements >3,200 µm are most likely of natural reproduction, while rainbow trout whose measurements were <2,600 µm are most likely of hatchery origin. We are certain that the four rainbow trout classified at 0.00–0.19 probabilities were indeed naturally reproduced fish, but become less confident as classifications approach 0.50, with limited confidence that 0.40–0.49 fish were reliably categorized as wild. Along those same lines, we believe that the two rainbow trout at 0.80–0.99 are of hatchery origin, with less certainty as categorizations approach 0.50.

Prior research has used both annulus and circulus spacing to distinguish between wild and hatchery reared fish (Seelbach and Whelan 1988, Stokesbury et al. 2001, Madden et al. 2010). In cases where differences in circulus spacing could not be detected, the first annulus is often used (Marcogliese and Casselman 1998). No annulus could be detected on the potentially naturally reproduced fish. Thus, we utilized measurements of circulus spacing. Interestingly, hatchery origin fish had shorter mean distances in both focus to sixth circulus and fifth to sixth circuli than wild origin fish, which was contrary to previous research with the same species (Madden et al. 2010). This suggests that the potentially naturally reproduced fish exhibited faster growth than the rainbow trout raised in a hatchery.

The PIT tag analysis demonstrated that tagged individuals of all three stocked rainbow trout strains exhibited adfluvial movement between Deerfield Reservoir and the Castle Creek tributary system. The McConaughy strain was more likely to enter Castle Creek above Deerfield reservoir during spawning time, followed by Erwin strain rainbow trout. Additionally, tagged individuals of all strains moved upstream through segment one and into segments two and three. Overall, temporal movement patterns were similar among all three strains and similar to other rainbow trout populations (Mellina et al. 2005, James 2011). Adfluvial movements by the McConaughy strain were noted in McConaughy Reservoir, Nebraska (Van Velson 1974), a geographically unique reservoir that historically supported a self-sustaining population of rainbow trout. Van Velson (1974) also observed fall and spring migrations by McConaughy strain rainbow trout from the reservoir to spawning locations in smaller tributaries upstream. Only spring migrations were observed in this study for the tagged McConaughy strain individuals. A possible reason for this lack of detection of a possible fall migration would be the timing of stocking and detections by remote readers.

The Erwin strain is regarded as a domestic strain of rainbow trout that is used extensively in aquaculture and research (Ryce et al. 2001). To our knowledge, adfluvial movement by Erwin strain rainbow trout has not been observed elsewhere and not reported in the literature. Erwin strain fish were the last strain to be stocked into Deerfield Reservoir (Sep and Oct stockings) and, consequently, were exposed to the shortest period of angler harvest in comparison to the other two strains, which may have attributed to the Erwin strain having the second highest number of tagged individuals entering segment one. The Erwin strain contributed the most genetic material to natural reproduction, despite not having the highest number of PIT-tagged individuals detected within the Castle Creek Tributary system. Fish were only tagged and detected in one year; thus, it is possible that fish may delay spawning until a second year. Gall et al. (1988) found that environmental conditions had marked effects on timing of spawning for two independent stocks of rainbow trout and that the average age of spawning was age-2 for five consecutive generations. The sudden change in environmental conditions associated with the movement from a hatchery to a natural environment could have delayed spawning movements in their first year. Alternatively, spawning could be occurring within the reservoir or in the mouth of creek downstream of the first PIT antenna. While few cases exist, non-migratory rainbow trout have shown the ability to spawn in lacustrine environments (Robertson et al. 1961). Juveniles could then move into the tributary after hatching in search of nursery habitat.

The Shasta strain rainbow trout is typically considered a domesticated strain (Barnes et al. 2009) and is often selected for put-and-take fisheries as they often yield high angler harvest rates (Brauhn and Kincaid 1982) despite low survival in the wild (Vincent 1987). Barnes et al. (2009) reported high angler catch rates for Shasta strain rainbow trout in small natural lakes in South Dakota. Stocking of Shasta strain rainbow trout occurred in May and June in Deerfield Reservoir, exposing these fish to the longest period of harvest for any of the three strains evaluated. Only 15 tagged Shasta strain individuals entered segment one. Time of spawning could also have influenced minimal spring detections as McCarthy et al. (1975) classified the Shasta strain as a fall spawning fish. Similarly, Pavlidis et al. (1992) identified mid-November to late-December as the spawning period for Shasta strain rainbow trout. Our PIT-tagging study included the autumn time period immediately following stocking, but no detections were made.

MANAGEMENT IMPLICATIONS

If a unique fishery is desired within the tributary system, increased stocking of McConaughy strain rainbow trout might be advantageous as they comprised the highest percentage of tagged individuals exhibiting adfluvial movements from Deerfield Reservoir. A self-sustaining rainbow trout population would likely benefit from the stocking of strains that are more likely to naturalize and successfully spawn. As the Erwin and McConaughy strains both contributed to natural reproduction, an emphasis on stocking those strains could increase the probability of wild fish contribution to this fishery. In contrast, a reduction in stocking numbers of Erwin and McConaughy may reduce inter and intra-specific competition for resources, such as spawning habitat and

cover. More research is needed to evaluate the proper stocking strategy to promote a more self-sustaining fishery, along with continued assessment of potential natural reproduction within the system. If the management strategy for Deerfield Reservoir is a put-and-take fishery, the current stocking practices seem suitable.

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