

Otolith Chemistry Discriminates among Hatchery-Reared and Tributary-Spawned Salmonines in a Tailwater System

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Abstract.—The Little Red River tailwater (LRRT) system in Arkansas supports a naturally reproducing population of brown trout *Salmo trutta* and an economically valuable fishery. Natural reproduction by stocked brook trout *Salvelinus fontinalis* and rainbow trout *Oncorhynchus mykiss* is unknown but assumed to be negligible. An artificial tributary to the LRRT was constructed to provide a spawning and nursery refuge for stocked trouts, and shortly thereafter we observed spawning adults and emergent fry in the tributary. As a first step in determining the relative contributions of hatchery- and tributary-produced brook and rainbow trouts recruiting to the LRRT fishery, we distinguished hatchery-reared fry from tributary-spawned fry via otolith chemistry. We used laser ablation inductively coupled plasma mass spectrometry to measure the abundances of cations in otoliths. Significant differences between hatchery and wild stocks were found for six element : Ca ratios. A five-variable (Mg, Mn, Zn, Sr, and Ba) discriminant model was sufficient to distinguish the two brook trout stocks with 100% accuracy, and a two-variable (Sr and Ba) model discriminated between the two rainbow trout stocks with 94% accuracy. Differences in ambient water chemistry between the two rearing habitats most likely accounted for these distinct otolith signatures. Otolith analysis of brook trout parr of unknown origin captured in the LRRT system suggests that natural reproduction is occurring there and that this stock may be supplemented with tributary-spawned immigrants. After interannual variation in habitat-specific otolith chemical signatures is quantified, such an analytical tool would be useful to managers in the LRRT system and elsewhere in determining the relative contributions of various stocks to reproductive and harvested populations.

Many salmonine populations are supplemented or sustained by hatchery stocking (Elliott 1994; Youngson and Verspoor 1998; Quinn 2005). The resultant influence on the ecology and evolution of wild salmonines is the subject of much interest as managers attempt to balance the often conflicting objectives of ensuring fish for harvest while maintaining the natural genotypic diversity found in wild populations (Skaala et al. 1996; Gross 1998; Weber and Fausch 2003). Hatchery salmonines may negatively influence the

survival, growth, behavior, and reproductive efforts of wild conspecifics (e.g., Petersson et al. 1996; Skaala et al. 1996; Unwin and Glova 1997; Einum and Fleming 2001). Furthermore, coexisting salmonines of hatchery and wild origin often exhibit significant differences in harvest susceptibility (Flick and Webster 1962), survival, behavior, and reproductive success (e.g., Dickson and MacCrimmon 1982; Swain and Riddell 1990; Mesa 1991; Rhodes and Quinn 1998). Methods that reliably distinguish hatchery-produced from naturally produced salmonines, and thus allow estimation of the relative contributions of each stock to the reproductive or harvestable population, would be of great importance (Seelbach and Whelan 1988).

Within the last several decades, studies of stock

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identification and life history reconstruction have taken advantage of the biochemical, structural, and chronological properties of otoliths (Campana and Thorrold 2001). Because otoliths are acellular and metabolically inert, they serve as quasi-permanent recorders of abiotic and biotic conditions encountered by individual fish throughout entire lifetimes (Campana 1999). Otoliths accrete daily or subdaily growth layers incrementally according to temperature, food availability, somatic growth rate, and endogenous rhythm (Pannella 1971; Neilson and Geen 1982; Casselman 1990), and in many cases, resulting patterns in microstructure reflect life history events or habitat use (Rybock et al. 1975; Brothers 1990; Paragamian et al. 1992; Hendricks and Torsello 1994; Limburg 1995; Quinn et al. 1999). Thus, predictable variation in otolith microstructure unique to individual fish or groups of fish can serve as natural tags useful in stock discrimination and life history reconstruction.

Similarly, the chemical composition of otoliths also may be useful in studies of life history events or stock structure. Biomineralization of otoliths depends in part on the chemistry of ambient water, blood, and endolymph (the inner-ear fluid that surrounds the otoliths), which in turn may vary according to food supply and habitat (Kalish 1989; Campana 1999; Bath et al. 2000; Elsdon and Gillanders 2004). Divalent cations of similar ionic radii (e.g., Ba^{2+} , Sr^{2+}) can replace Ca^{2+} in the aragonitic crystal lattice or can coprecipitate in carbonate form (Campana 1999). These and other metal ions (e.g., Na^+ , Mn^{4+} , and Zn^{2+}) also may be included in the protein matrix or within the crystalline lattice interstices (Campana 1999). Many authors have found strong and predictable associations between ambient water chemistry and otolith chemistry in laboratory and field studies (e.g., Bath et al. 2000; Thorrold and Shuttleworth 2000; Wells et al. 2003; Elsdon and Gillanders 2004; Zimmerman 2005), such that stock, habitat of origin, or migratory history of individuals can be inferred (e.g., Thorrold et al. 1998; Zimmerman and Reeves 2000; Limburg et al. 2001; Cairns et al. 2003; Zimmerman et al. 2003; Brazner et al. 2004).

In a hatchery environment, it is relatively easy, although often unintentional, to impart unique chemical marks upon the otoliths of captive fish that later permit reliable discrimination from wild fish after stocking. Results from many laboratory and field studies (e.g., Schroder et al. 1995; Secor et al. 1995; Gaudie 1996; Bath et al. 2000; Veinott and Porter 2005; Zimmerman 2005) seem to suggest that, if differences in certain aqueous metal concentrations exist between two water bodies and all else is equal, fish reared in those two environments should retain

distinct otolith chemical signatures. Furthermore, differences in food consumed by hatchery-reared and wild fish might also induce differences in otolith chemistry (see Behrens-Yamada and Mulligan 1990; Schroder et al. 1995). Bickford and Hannigan (2005) were able to distinguish among two hatchery stocks and one wild stock of walleye *Sander vitreus* based on differences in otolith chemical fingerprints, which presumably arose in part as a result of differences in ambient water and food chemistry between natural and artificial rearing habitats. Therefore, relying on natural otolith markers imparted by differences in ambient water or food to discriminate between stocked and wild fish is a passive alternative to mass-marking the otoliths of hatchery-reared fish with fluorescent calciphilic compounds or metal supplements (e.g., Behrens-Yamada and Mulligan 1990; Mohler 1997).

The Little Red River tailwater (LRRT) system in central Arkansas provides an economically valuable sport fishery for naturalized brown trout *Salmo trutta*. This fishery is augmented by a put-grow-and-take fishery for stocked rainbow trout *Oncorhynchus mykiss*, brook trout *Salvelinus fontinalis*, and cutthroat trout *O. clarkii* (Hicks 2000). Construction of the Greers Ferry Dam and Reservoir on the main-stem Little Red River in 1964 resulted in a hypolimnetic release into the river valley below. Many of the original warmwater fish and invertebrate fauna were displaced after dam construction (McGary and Harp 1973; Johnson and Harp 2005), and currently salmonine and asellid isopod assemblages dominate the upper reaches of the LRRT. Brown trout became naturalized soon after 1977 plantings, and hatchery plantings ceased after 1983. In contrast, rainbow trout have been stocked since 1966 (~370,000 fish were stocked in 2005) and brook trout since 1994 (~10,000 in 2005) (Sherri Shoults, U.S. Fish and Wildlife Service, personal communication). Cutthroat trout were introduced in the early 1990s but are no longer stocked and very rarely caught by anglers (Arkansas Game and Fish Commission [AGFC], unpublished data).

It is generally assumed that these hatchery-reared salmonines do not spawn successfully in the LRRT (AGFC, unpublished data), and thus that harvestable stocks are sustained solely by hatchery propagation. In 2001, the AGFC and U.S. Army Corps of Engineers constructed a small tributary to the LRRT to provide spawning and nursery refugia for brook, rainbow, and cutthroat trouts. Collins Creek was originally a dry gully but now is watered perennially by hypolimnetic water diverted from Greers Ferry Reservoir (Figure 1). Shortly after irrigation commenced, adult rainbow trout and brook trout (undoubtedly immigrants from the

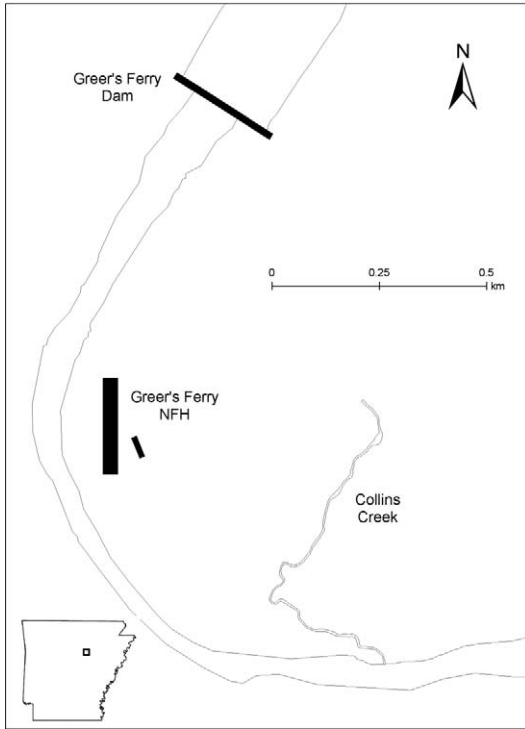


FIGURE 1.—Map of study sites, including Greers Ferry Dam, Greers Ferry National Fish Hatchery, Little Red River tailwater (John F. Kennedy Park site), and Collins Creek.

LRRT) were observed spawning in Collins Creek (T. Bly, personal observation). The AGFC began a biannual electrofishing survey program in Collins Creek and has collected salmonines of various size classes since 2002 (Figure 2), most of which are subyearlings and much smaller than subadults stocked in the LRRT system. Because of the potential for Collins Creek to produce wild fish and thereby supplement hatchery-raised rainbow trout and brook trout stocked in the LRRT, we saw the need for a tool to distinguish between hatchery-reared and tributary-produced individuals. Specifically, we determined the utility of otolith chemical signatures in identifying the natal origin of trout fry in the LRRT system and identified the following three objectives: to identify a suite of elements (i.e., a chemical “fingerprint”) suitable for discriminating between hatchery-reared and tributary-produced fry of rainbow and brook trouts; to make inferences as to the natal origin of subadult brook trout collected in the main-stem tailwater; and to propose a strategy for estimating the relative contributions of all potential sources to rainbow and brook trout stocks in the main-stem tailwater.

Methods

Study area.—Tributaries of the Little Red River originate in the Ozark highlands in central Arkansas and flow south-southeast until converging in what is now Greers Ferry Reservoir (GFR), Heber Springs, Arkansas. Below the dam, the tailwater is fed by cold, hypolimnetic water discharged from the reservoir; flows range from dam leakage to approximately 8,000 ft³/s (228 m³/s), depending on hydropower production (John Kielczewski, U.S. Army Corps of Engineers, personal communication). Summer temperatures suitable for salmonine survival in the LRRT occur from the base of the dam downstream approximately 48 km to Pangburn, Arkansas. Greers Ferry National Fish Hatchery (GFNFH) is located on the LRRT approximately 0.5 km below the dam and is supplied by water from the GFR hypolimnion via underground pipes. Rainbow and brook trouts are raised from eyed embryos (obtained from broodstock spawned in other hatcheries) to age-1 or age-2 subadults for stocking in the LRRT and elsewhere. The same piping system supplies water to both Collins Creek and GFNFH; the water originates from a standpipe located approximately 500 m east of GFNFH and flows into the LRRT approximately 500 m south of GFNFH (Figure 1). From source to debouchement, Collins Creek is approximately 1,000 m long; its width ranges from 1 to 3 m, and the depth in most reaches ranges from 1 to 50 cm, although it exceeds 1 m in two pools. The lower 75 m of Collins Creek is subject to inundation by LRRT during periods of peak hydropower generation, and the habitat consists of short riffles alternating with sand- and silt-bottom pools containing dense mats of waterweed *Elodea canadensis*. Upstream of the flood-prone reach to the source standpipe, the gradient is relatively steep, the thalweg water velocities range from approximately 40 to 80 m³/s, and the substrate consists of a gravel-cobble mix interspersed with boulders.

Field sampling.—The AGFC maintains three 100-m-long reference sites on Collins Creek where standardized electrofishing surveys of salmonines have been conducted twice yearly (May–July and October) since 2002. On 24 May 2005, we retained a subsample of brook trout and rainbow trout fry (total length [TL], 30–50 mm) collected from the uppermost site for otolith analysis. We chose this site because downstream channel constrictions and resultant high water velocities would prevent upstream migration of salmonine fry from the LRRT (see Heggenes and Traaen 1988); therefore, fry collected here were undoubtedly Collins Creek progeny. After electrofishing, we collected stream-water samples for trace metal

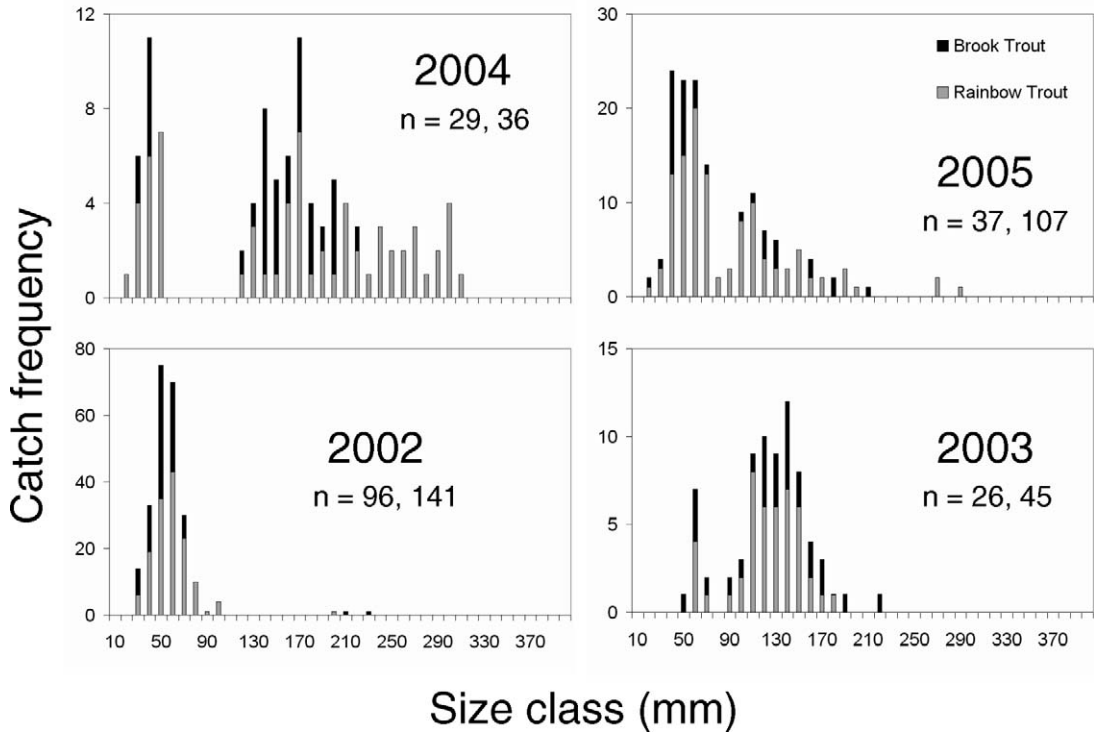


FIGURE 2.—Total catch frequency distributions of brook trout (dark bars) and rainbow trout (light bars) collected during the Arkansas Game and Fish Commission's annual standardized surveys of Collins Creek from 2003 and 2004, by size-class. The numbers shown indicate the sample sizes of brook trout and rainbow trout, respectively, for body sizes smaller than the sizes stocked into the Little Red River tailwater. Bar height represents the total number of juvenile trout collected.

analysis according to EPA method 200.8. We also collected samples of brook trout and rainbow trout fry (TL, 30–35 mm) and water from GFNFH raceways. All fish and water samples were transported on ice to the Water-Rock-Life Laboratory at Arkansas State University, where fish were frozen whole at -20°C and streamwater samples were refrigerated at 4°C . Given the logistical constraints associated with transferring live, field-caught fish to a clean laboratory for otolith extraction, transport on ice and subsequent freezing is a common protocol (e.g., Thorrold and Shuttleworth 2000). Although freezing may have a small but detectable effect on the measured concentrations of a few elements in otoliths, this effect can be ignored if all otoliths are handled consistently (Milton and Cheney 1998).

During the AGFC's annual salmonine electrofishing survey of LRRT in October 2005, we retained approximately 20 brook trout (TL, 200–250 mm) collected from John F. Kennedy (JFK) park, a reference site located 300 m below the dam and in close proximity to GFNFH and the mouth of Collins Creek (Figure 1). Based on the lack of fin erosion and

body scarring typically observed on hatchery-raised trout even several months after stocking in the LRRT (T. Bly, personal observation; AGFC, unpublished data), these fish appeared, at least superficially, to be wild in origin. However, because body size was slightly larger than the typical size-at-stocking (TL, ~ 160 mm), we considered these fish to be of unknown origin and assumed they could have been spawned in Collins Creek, GFNFH, or even the uppermost reach of the LRRT. Previous collection efforts yielded no postemergent brook trout fry from LRRT–JFK park, so we were unable to obtain a sample of fish known to have been spawned at this site. Fish samples were handled as described above.

Otolith preparation and analysis.—Within 2 weeks of collection, fish were thawed slightly and dissected, and the sagittal otoliths (hereafter referred to as “otoliths”) were removed with acid-washed nonmetallic instruments. The otoliths were cleaned of endolymph and tissue by rinsing in Millipore-filtered, reverse-osmosis-processed ($18.3\text{ M}\Omega$) water; sonicating in 2% ultrapure hydrogen peroxide; and rinsing again with ultrapure water. The otoliths were trans-

ferred to acid-washed Teflon tubes and placed under a laminar-flow hood in a class-100, EPA-certified clean laboratory to dry for 24 h. Then the otoliths were mounted with the sulcus side up on petrographic slides with thermoplastic glue and lightly polished in the sagittal plane to expose the core and early growth increments. After polishing, the otoliths were rinsed with 1% ultrapure nitric acid, briefly cleaned with a soft-bristled nylon brush, and immediately triple-rinsed with acid and ultrapure water. After drying again in a laminar-flow hood, the otoliths were transported to Arkansas State University's Mass Spectrometry Laboratory.

We used laser ablation (LA) with inductively coupled plasma mass spectrometry (ICPMS) to quantify various metals in the otolith samples. We used the CETAC LSX-500 (266 nm) system to ablate otolith material under consistent operating parameters (10 Hz, 500 shots, 100% energy level) coupled to a Perkin-Elmer SCIEX DRC II Dynamic Reaction Cell ICPMS system for detection and quantification. Exploratory sampling indicated that a 50- μm spot offered the best compromise between the spatial resolution desired and the quantity of ablated material needed for reliable detection. Before each day's sampling run, we adjusted nebulizer gas (argon) flow and lens voltage to optimize instrument sensitivity and performance (i.e., maximizing ^{86}Sr detection while minimizing variability in counts). Using DigiLaz software, we programmed 5–7 shots per fry otolith, located in an arc just distal to the hatch-check, such that the otolith region sampled corresponded with postemergent growth. Otoliths from 30 rainbow trout (16 hatchery and 14 tributary) and 63 brook trout (11 hatchery, 32 tributary, and 20 tailwater) were sampled.

U.S. Geological Survey microanalytical carbonate standard 1 (MACS-1), a laser ablation standard, was used to calibrate the ICPMS and control for instrument drift every 5–9 otolith samples. Water samples were analyzed using solution-based ICPMS according to EPA 200.8. An internal standard of indium (10 $\mu\text{g/L}$) was used to correct for instrument drift, and a blank and five stock-standard solutions were used for calibration after every 10 water samples.

Chemical quantification procedures.—We used GeoPro software (CETAC Technologies) to integrate ICPMS data from each otolith subsample and estimate corresponding concentrations of each of 35 analytes. Here, ^{42}Ca was used as an internal standard (Ca is 38% by weight in MACS-1 and otoliths, and we monitored ^{43}Ca to estimate total Ca. We then calculated limits of quantification (LOQs) for each of the 35 analytes as three times the standard deviation of concentrations measured from seven blank sample replicates. Any

analyte whose concentration in otolith subsamples from all fish cohorts consistently fell below its respective LOQ was omitted from subsequent analyses. Because solution-based ICPMS is a more sensitive analytical procedure than is LA-ICPMS (Campana 1999; Harden 2004; but see Ludsin et al. 2006), we ignored analytes quantifiable in water that were below quantifiable limits in otoliths (e.g., rare earth elements). Screening of data in this manner left us with 17 analytes suitable for statistical analysis (i.e., one or more isotopes each of Na, Mg, Ca, Mn, Fe, Cu, Zn, Sr, and Ba).

To control for unavoidable variation in the amount of otolith material ablated with each laser shot (Bickford and Hannigan 2005) and to extract the most biologically meaningful data (Campana 1999), we compared the concentrations ($\mu\text{g/g}$) of these analytes to corresponding concentrations of Ca and calculated the analyte : calcium ratios for each subsample. For each otolith, we calculated means and coefficients of variation ($\text{CV} = 100 \times \text{SD}/\text{mean}$) of 17 analyte : calcium ratios using data from five to seven subsamples each. Because estimated Ca concentrations were extremely consistent throughout all subsamples (overall range, 368,000–391,000 $\mu\text{g/g}$ using ^{43}Ca ; $\text{CV} < 1\%$), a high variability in analyte : calcium ratios sampled from a small region within an individual otolith most likely would indicate external contamination and/or the “nugget effect” (i.e., sampling of an atypically concentrated and localized aggregation of metal ions). Therefore, we rejected any otolith with a CV greater than 20 for major analyte : calcium ratios or a CV greater than 40 for minor and trace analyte : calcium ratios; ultimately, we retained approximately 85% of otoliths for further analysis.

Statistical analysis.—To correct for nonnormal distributions and heteroscedasticity (Zar 1999), we $\log_{10}(x + 1)$ transformed all mean analyte : calcium ratios (e.g., Gillanders 2002) from individual otoliths before the analyses. To test the hypothesis that otolith chemistry differed predictably between salmonines originating in Collins Creek and GFNFH, we performed multivariate analysis of variance (MANOVA) and associated analysis of variance (ANOVA) on mean element ratios separately for each species. We identified the analyte ratios that differed significantly between stocks, and if ratio differences estimated from two or more isotopes of a given element were significant (e.g., ^{86}Sr and ^{88}Sr), we chose the isotope exhibiting the lowest CV within each otolith. Generally, concentrations calculated from multi-isotopic elements were very similar (within 5%). We then performed linear discriminant function analysis (DFA) on mean elemental ratios using stepwise selection ($\alpha = 0.05$ to enter and leave the model) to better visualize

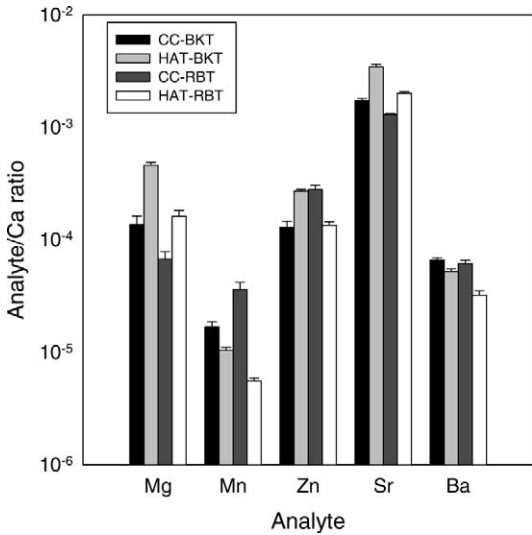


FIGURE 3.—Mean + SE values of Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca, and Ba:Ca ratios from trout fry otoliths. Abbreviations are as follows: BKT = brook trout, RBT = rainbow trout; CC = Collins Creek progeny, HAT = hatchery progeny.

the multivariate distinctness of each stock’s chemical fingerprint and possibly reduce the number of elements necessary for future studies (e.g., Brazner et al. 2004). A “leave-one-out” jack-knifing algorithm (Der and Everitt 2002) allowed us to estimate the reclassification accuracy of the DFA. After performing the above DFA separately for rainbow trout and brook trout, we reran the analysis for brook trout but included data from age-1 LRRT fish coded as a unique stock (i.e., included in the training data set and not as an unknown). These brook trout could have originated from one of three natal habitats (Collins Creek, GFNFH, or LRRT), but as mentioned previously, we had no a priori otolith signature of known LRRT-spawned fish for comparison and classification purposes. Therefore, rather than testing explicit hypotheses, this final analysis was limited to comparing patterns of LRRT otolith signatures in multivariate ordination space to those of known Collins Creek and GFH otoliths visually and then inferring natal origins of individual fish.

Results

Rainbow Trout

In comparing otolith chemistry between Collins Creek and GFNFH rainbow trout, five element : calcium ratios differed (all Bonferroni adjusted, $P < 0.05$): Mg, Mn, Zn, Sr, and Ba (Figure 3). A stepwise DFA retained two variables in the final model, Sr and Ba (overall MANOVA: Wilk’s $\lambda F_{2,27} = 69.58; P < 0.0001$). The first canonical axis loaded positively on

Sr, negatively on Ba, and explained 99% of the total variance. A jack-knifing algorithm showed that the DFA correctly classified 94% of rainbow trout individuals to their respective category of origin, with one Collins Creek fish misclassified as a GFNFH fish. Biplots of canonical axis scores assigned to individual rainbow trout show distinct separation into two natural groups; the Mahalanobis distance between the two groups was significant ($P < 0.0001$; Figure 4a).

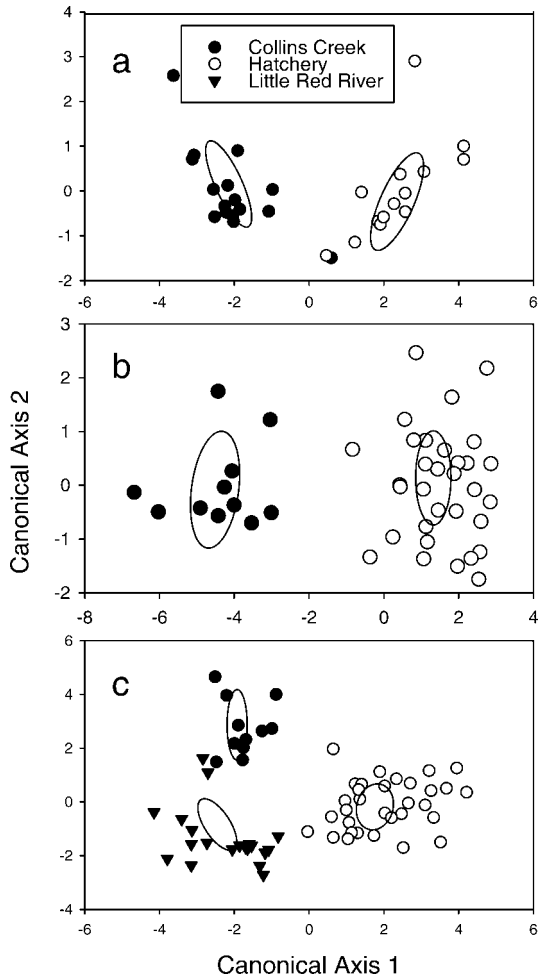


FIGURE 4.—Biplots of the first two canonical axis scores, derived from otolith chemistry signatures, for (a) rainbow trout fry, (b) brook trout fry, and (c) brook trout fry and parr. The solid circles represent Collins Creek progeny, the open circles represent hatchery progeny, and the triangles represent parr collected from the John F. Kennedy Park site on the Little Red River tailwater. The 95% confidence ellipsoids of means are shown. For rainbow trout, n (tributary) = 16 and n (hatchery) = 14; for brook trout, n (tributary) = 11, and n (hatchery) = 32, and n (tailwater) = 20.

TABLE 1.—Mean ratios of select analytes : calcium ratios (SEs in parentheses) in water samples taken twice yearly (May–June and October) from Collins Creek and trout raceways at Greers Ferry National Fish Hatchery, including isotopes used in estimations, from 2002 through 2005. Human error prevented the quantification of Zn concentrations.

Source	²³ Na	²⁴ Mg	⁵⁵ Mn	⁸⁶ Sr	¹³⁵ Ba
Collins Creek	1.360 (0.032)	0.890 (0.073)	0.157 (0.03)	0.0190 (0.0015)	0.0090 (0.0007)
Hatchery	0.313 (0.0027)	0.164 (0.012)	0.0395 (0.018)	0.00287 (0.0009)	0.00116 (0.00034)

Brook Trout

In comparing otolith chemistry between Collins Creek and GFNFH brook trout, five elemental ratios differed (all $P < 0.05$): Mg, Mn, Zn, Sr, and Ba (Figure 3). A stepwise DFA retained all five variables in the final model (MANOVA: Wilk's $\lambda F_{5,37} = 48.92$; $P < 0.0001$), with the first canonical axis explaining 99% of the total variation. Zn and Sr loaded most positively with axis 1, whereas Mn and Ba loaded most negatively. The DFA correctly classified 100% of brook trout individuals into their respective category of origin. Biplots of canonical axis scores assigned to individual brook trout show distinct separation into two natural groups; the Mahalanobis distance between the two groups was significant ($P < 0.0001$; Figure 4b).

After including data from LRRT brook trout into the DFA, all five variables were retained in the model (MANOVA: Wilk's $\lambda F_{12,110} = 27.91$; $P < 0.0001$). The first two canonical axes, respectively, explained 68% and 31% of the variation in the overall model and had loading patterns similar to those of the previous brook trout DFA. Although 100% of both Collins Creek and GFNFH fish were classified correctly into their respective category of origin, 2 of 20 LRRT fish were misclassified into the GFNFH group. Biplots of canonical axis scores show distinct separation among all three groups, except for the misclassified LRRT individuals, which appear to group with Collins Creek fish (Figure 4c). Recall that LRRT fish were of unknown origin but were treated a priori as a distinct stock; therefore, this misclassification of two LRRT individuals merely illustrates a greater degree of similarity with the Collins Creek otolith signature.

Water Chemistry

In comparing within-GFNFH variation in water chemistry (i.e., brook trout versus rainbow trout raceways), no differences were found for all analytes (multiple ANOVAs: $0.10 < \text{all } P < 0.79$). Subsequently, data from both raceways were pooled. Considering the elements detectable in otoliths, all differed between Collins Creek stream water and GFNFH water (multiple ANOVAs and post hoc Tukey tests: $0.0001 < \text{all } P < 0.038$). Considering water and otoliths simultaneously, the differences in chemistry

between sources were not concordant. That is, for all ratios obtained from stream water, the GFNFH values were significantly lower than the Collins Creek values (Table 1). However, in otoliths, the Mg and Sr ratios were higher in GFNFH, whereas the Mn and Ba ratios were lower in GFNFH. The ratios of Zn were greater in GFNFH brook trout than in Collins Creek brook trout, whereas the opposite pattern was found in rainbow trout. Finally, differences in Cu and Fe were detected between GFNFH and Collins Creek water but not between GFNFH and Collins Creek otoliths of either salmonine.

Discussion

Significant differences existed between the chemical signatures of otoliths taken from hatchery-reared and wild-spawned salmonines in the LRRT system. For rainbow trout, a two-variable discriminant model containing Sr and Ba was sufficient to distinguish the two stocks with 94% accuracy. For brook trout, a five-variable model containing Mg, Mn, Zn, Sr, and Ba discriminated between the two stocks with 100% accuracy. When LRRT brook trout of unknown origin were included in the model, most individuals formed a natural group distinct from both Collins Creek and GFNFH fish, suggesting that these fish were spawned in LRRT. Conversely, two individuals exhibited chemical signatures more closely resembling the Collins Creek stock, perhaps a result of previous emigration of tributary-spawned fish into the tailwater. However, this conclusion is tentative until we can ascribe a chemical signature to brook trout known to have originated in LRRT. We assume that, for both species, the unique chemical fingerprints associated with either hatchery rearing or tributary spawning were functions of spatial variation in ambient water chemistry (and possibly food supply; see below) associated with distinct natal habitats. We have no reason to believe that such patterns in chemical fingerprints arose from some innate biochemical difference between hatchery-raised and wild-spawned fish in general but simply resulted from differences in rearing environment.

The lack of complete concordance between patterns in water chemistry and otolith chemistry found in our

study is neither surprising nor troubling. Because the biomineralization of otoliths is regulated by several physiological processes and barriers (many of them poorly understood; Campana 1999), we should not always expect a direct relation between ambient water chemistry and otolith composition. For example, Wells et al. (2003) found direct relations for Sr:Ca ratios between stream water and otoliths but inverse relations for Mg:Ca ratios, and Kalish (1989) documented an overriding effect of growth rate and physiological status on otolith biomineralization. Furthermore, the efficacy of biomineralization of various elements in otoliths may vary with the source of those elements (i.e., food versus ambient water; Behrens-Yamada and Mulligan 1990). Sanchez-Jerez et al. (2002) attributed habitat-specific chemical fingerprints of juvenile eastern striped trumpeter *Pelates sexlineatus* to variation in the tissue chemistry of natural prey items consumed. Although we did not quantify elemental composition of food consumed by the two stocks of experimental fish, significant differences most likely exist. Commercial pellets consumed by hatchery fry contain fish meal of marine origin (Sherri Shoults, personal communication) and thus most likely reflect ambient salinity by containing relatively high Sr:Ca and Mg:Ca ratios. Conversely, tributary-reared fish consumed primarily asellid isopods and chironomid larvae (S.M.C., personal observation), which presumably have relatively low Sr:Ca and Mg:Ca ratios compared with those of marine organisms. Campana (1999) suggested that biomineralization of trace elements such as Mn and Ba is influenced primarily by ambient water chemistry. For these elements, we saw concordance between water and otolith signatures, as both tributary water and otoliths contained greater Mn:Ca and Ba:Ca ratios than did hatchery water and otoliths. The biomineralization of zinc in otoliths may reflect growth history rather than environmental availability (Halden et al. 2000; Miller et al. 2006), especially considering the known binding affinity for zinc to protein (Lobinski et al. 2006). Finally, temperature may play an important role in biomineralization of major or trace elements in some instances (e.g., Elsdon and Gillanders 2004), but Campana (1999) found no overall trend when data were meta-analyzed across species, environments, and temperature ranges. In our study, temperatures in GNFNH and Collins Creek were nearly identical and very stable (due to a common source of hypolimnetic water) and thus probably did not affect our results. Given the potential of many controlling, synergistic, or confounding factors in the biomineralization process, our results still remain valid and useful even though we cannot attribute differences in otolith chemistry between our fish stocks to a specific mechanism with

any certainty. For management purposes (e.g., in stock discrimination), it is sufficient that the otolith fingerprints differ significantly between stocked and wild fish.

Several considerations prevented us from drawing more than tentative conclusions regarding age-1 LRRT brook trout. Reliable assignment of individual fish to known stocks based on otolith chemistry requires that all possible temporal and spatial variation in chemical fingerprints be known a priori. If fingerprints associated with certain source (natal) habitats are not known, individuals produced in those habitats may be misclassified or remain as "unknowns." Because we did not collect brook trout fry known to have been produced in LRRT, we do not know a priori the characteristics of the corresponding chemical signature. However, in an example where all potential hatchery fingerprints are known, individual fish whose signatures do not match that of any hatchery are assumed to be wild (e.g., Bickford and Hannigan 2005). The apparent multivariate distinctness of most LRRT brook trout from known hatchery-reared or tributary-spawned stocks provides evidence that another stock exists, which in this case could have originated only in the tailwater itself.

If source-specific fingerprints change over time, then stock identification will necessarily be limited to those age-classes for which researchers have quantified natal fingerprints until a complete library of fingerprints is obtained (Gillanders 2002). Thus, the chemical fingerprints we describe for age-0 trout fry in this study are most useful in monitoring the 2005 year-class but should be extrapolated to other year-classes only with caution. If environmental conditions experienced by fish reared in the hatchery and the tributary were different in 2004 (the year-class of LRRT brook trout analyzed in this study) than in 2005, this temporal variation would be unknown and confounding. As a result, otolith fingerprints from hatchery-raised or tributary-spawned fish from the 2004 year-class might appear as a chemically distinct ordination, and our ability to distinguish between the stocks would be compromised. The significance of this temporal component of uncertainty undoubtedly varies with the habitat studied. Wells et al. (2003) and Bickford (2004) found that water chemistry in their respective study streams did not vary significantly from one year to the next and suggested that habitat-specific otolith chemistry was probably temporally stable as well. Conversely, Gillanders (2002) and Swearer et al. (2003) found interannual and seasonal differences in estuary-specific fingerprints, although they did not discuss temporal variation in water chemistry. Morris et al. (2003) attributed variability in otolith Sr:Ca ratios

among several year-classes of striped bass *Morone saxatilis* spawned in the Roanoke River to annual flooding events. Furthermore, concentrations of certain elements may be more temporally stable than others (Campana et al. 2000, reviewed by Gillanders 2002). Given the characteristic interannual stability in physicochemical characteristics of stream water in this region (Hannigan and Bickford 2003), especially stream water originating from hypolimnetic sources, as well as a consistent food source used in GFNFH (Sherri Shoults, personal communication), it is unlikely that elemental fingerprints imparted on our salmonine fry varied appreciably from 2004 to 2005; still, empirical evidence is lacking.

Visual and electrofishing surveys of adult brown trout suggest that spawning occurs at all suitable riffles throughout the LRRT with the exception of JFK park; conversely, brook trout abundance is highest at JFK park and declines rapidly downstream (AGFC, unpublished data). Furthermore, relatively small (<250 mm) brook trout are caught only at JFK park. Given the tendency of brown trout to suppress natural reproduction of brook trout owing to aggressive interactions for spawning sites and/or foraging microhabitats, and also through redd superimposition (Fausch and White 1981, 1986; Sorensen et al. 1995), we would not be surprised if naturally produced brook trout were confined to JFK park and Collins Creek as a result of brown trout dominance in the rest of the tailwater. Recently, a tiger trout (female brown trout \times male brook trout) was angled from the LRRT (J. Williams, personal observation), suggesting competition for spawning habitat between brown trout and brook trout as well as attempted spawning by brook trout, in the LRRT. If further studies confirm a naturally reproducing population of brook trout in LRRT supplemented by emigrants from Collins Creek, then stocking procedures and harvest regulations may need to be reevaluated.

Standardized and exploratory surveys since 2000 have yielded only a few rainbow trout smaller than average size at stocking (e.g., one 55-mm [TL] individual caught 30 km downstream of the dam in May 2005). However, rainbow trout abundances are high in Collins Creek, with a wide range in sizes, and the possibilities exist for immigration of fry or parr into the LRRT. As with brook trout, additional studies are needed to investigate the natural productive capacity of the tailwater system, such as quantifying the number of outmigrants from Collins Creek and searching more intensively for redds and emergent fry in the LRRT.

The results from this study provide a useful tool for investigating the metapopulation dynamics of salmonines in the LRRT. Based on known chemical fingerprints of

hatchery-reared and tributary-spawned salmonines and the potential to develop a fingerprint library of future year-classes, we should be able to identify the origin of reproductive adults that spawn successfully in Collins Creek or the LRRT and thus be able to evaluate the relative fitness of hatchery and wild individuals.

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