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## USING OTOLITH MICROCHEMISTRY TO CLASSIFY YELLOW PERCH AS STOCKED OR NATURALLY PRODUCED

—Fisheries managers routinely use stocking to supplement fish populations (Schramm and Piper 1995, Fisher 1996). Stocking eyed-eggs offers substantial cost savings compared to stocking fry and fingerlings (PFBC 2011); however, traditional stocking evaluation using oxytetracycline (OTC) marking of otoliths is ineffective for eyed-eggs of some species (e.g., yellow perch, [*Perca flavescens*]). Thus, there is a need for additional approaches to be able to classify fish as stocked or naturally produced. Fish otoliths are paired calcified structures in the inner ear that permanently deposit trace elements in proportion to water column concentrations (Campana 1999, Campana et al. 2000). Coupled with otolith growth increments (i.e., annuli), elemental accumulation permits retrospective evaluation of environmental history (e.g., natal origins, movement) if water chemistry is spatially heterogeneous and temporally constant (Elsdon et al. 2008). Otolith microchemistry can be used to evaluate stocking contributions (Pracheil et al. 2014) and in the context of eyed-egg stockings, may be useful for classifying fish as stocked or naturally produced.

Yellow perch is a popular sport fish species in South Dakota (Gigliotti 2007) and is routinely stocked by fisheries managers to supplement weak year classes (Schoenebeck et al. 2010). The South Dakota Department of Game, Fish and Parks (SDGFP) propagates yellow perch for stocking (e.g., eyed-eggs, fry, fingerlings) and also stocks adult perch through trap and transfer operations (Lott 1991, Fisher 1996). However, the contributions of yellow perch stockings in South Dakota are largely unknown because it is difficult to differentiate stocked fish from resident individuals (Brown and St. Sauver 2002). Our objective was to assess the utility of otolith microchemistry to distinguish hatchery-reared yellow perch stocked at the eyed-egg stage from naturally produced individuals.

We conducted our study at Blue Dog State Fish Hatchery (Hatchery), a SDGFP fish propagation facility in Day County, South Dakota. The Hatchery receives well water from a shallow aquifer (13.4 m deep) and surface water from Blue Dog Lake (2.6 m deep, 1.8 m mean depth). Well water is used to incubate eggs of coolwater fish species, whereas lake water is used for fingerling production in drainable ponds (0.2–0.8 ha). South Brush Pond, located in Brookings County, South Dakota, contains both stocked Hatchery-reared and naturally produced yellow perch.

We used two groups of yellow perch to evaluate the effectiveness of otolith microchemistry for classifying yellow perch collected from South Brush Pond as stocked or naturally produced. The first group (Well,  $n = 25$ ) included age-0 individuals that were incubated in Heath trays (Heath Teena Aerospace [now Hexcel Structures], Kent, Washington) on Hatchery well water (constant 10 °C) for 15 days to 162 temperature units (TU = 1° C above 0° C for 24 hrs) or

16 days to 173 TUs in April 2013. Otolith formation within the embryo occurred at 119 TUs (i.e., day 12; M. Ward, unpublished data), thus there was a sufficient time period (i.e., 3 or 4 days) for otoliths to absorb well water chemistry before eyed-eggs were stocked into Hatchery ponds filled with Blue Dog Lake water (Kalish 1990, Wright et al. 1992). The second group (Pond,  $n = 17$ ) included age-1 individuals with unknown natal origins but a known capture location (i.e., South Brush Pond). They were either incubated on Hatchery well water (constant 10° C) in April 2012 (same water and incubation protocol as 2013) and stocked as eyed-eggs into South Brush Pond in the same month or naturally produced in South Brush Pond. Thus, we identified natal origins of Pond yellow perch stocked at the eyed-egg stage by comparing otolith core chemistry to site-specific signatures of the Hatchery and South Brush Pond. We collected Well yellow perch from Hatchery ponds during pond draining in August 2013. We collected Pond individuals from South Brush Pond in September 2013 using modified-fyke nets with 9.5 mm bar mesh, 0.9 × 1.5 m frames, and 15.2 m leads.

We used a syringe filtration method (Shiller 2003) to measure water chemistry as in previous otolith microchemistry studies (e.g., Zeigler and Whitledge 2010, Phelps et al. 2012). While wearing nitrile gloves, we collected duplicate samples of Hatchery well water and South Brush Pond surface water in 250 mL acid-washed polyethylene bottles and stored them in sealed coolers (i.e., no light penetration) before filtration. We used high-resolution inductively coupled plasma mass spectrometry (ICPMS) to quantify trace elements (i.e., strontium [Sr], barium [Ba]) and calcium (Ca) concentrations; all water analyses were completed in the Department of Marine Sciences at the University of Southern Mississippi (Shiller 2003). We converted water elemental concentrations to micromolar ratios against Ca ( $\mu\text{mol}\cdot\text{mol}^{-1}$ ) as this element is a pseudointernal standard (Bickford and Hannigan 2005, Whitledge et al. 2007). We expected Sr:Ca and Ba:Ca ratios (i.e., signatures) of Hatchery well water to be temporally stable because the water source did not change during this study. Thus, we sampled Hatchery well water on one occasion in April 2013. Due to its size and uncontrolled water source, we sampled South Brush Pond water on two occasions in May 2013 and September 2013 to assess temporal patterns in Sr:Ca and Ba:Ca.

We sacrificed yellow perch immediately after collection and placed them on ice to prevent potential stress-induced changes in otolith chemistry (Kalish 1992). We removed sagittal otoliths from each individual in a laboratory using plastic forceps triple-washed in nitric acid (Campana et al. 2000). We used the otolith with the most well-defined annuli for age estimation by two independent readers (agreement > 95%); when readers did not agree ( $n = 2$ ) the age estimate of the more experienced reader was used. For trace element analysis, otoliths were triple-rinsed in distilled, ultrapure water; air-dried for a minimum of 24 hrs; and stored in acid-washed

2 mL polypropylene microcentrifuge tubes (Zeigler and Whitledge 2010). After initial cleaning, we sectioned age-1 otoliths in the transverse plane using a low-speed Isomet diamond blade saw (Buehler, Lake Bluff, Illinois). We cleaned the saw blade after each section with aluminum oxide lapping film (3  $\mu\text{m}$  grit) to prevent otolith contamination. We did not section age-0 otoliths due to their small size and fragility. Otoliths and otolith sections were sanded and polished with 400 grit sandpaper and aluminum oxide lapping film, mounted on petrographic slides (Donohoe and Zimmerman 2010), and sonicated in ultrapure water before trace element analysis.

Although otolith core chemistry can reflect maternal signatures, particularly for marine species such as Atlantic herring (*Clupea harengus*) and Caribbean reef fish (*Stegastes partitus*) as revealed by common marine elements (e.g., manganese; Brophy et al. 2004, Chittaro et al. 2006), multiple studies (e.g., Brazner et al. 2004a, b, Collingsworth et al. 2010) have concluded that yellow perch otolith chemistry (e.g., Sr:Ca, Ba:Ca) is most strongly influenced by ambient water signatures without maternal effects. Thus, we identified natal origins of Pond yellow perch by quantifying otolith core signatures of Well individuals and edge signatures of Pond individuals, which were synchronized with water sampling in the Hatchery (April 2013) and South Brush Pond (September 2013). We measured otolith trace element (i.e.,  $^{88}\text{Sr}$ ,  $^{86}\text{Sr}$ ,  $^{138}\text{Ba}$ ,  $^{137}\text{Ba}$ ) concentrations using laser ablation ICPMS at the University of California–Davis Interdisciplinary Center for Inductively Coupled Plasma Mass Spectrometry. We used an Agilent Technologies 7500a quadrupole ICP-MS coupled to a New Wave Research UP-213 nm laser with He as the carrier gas for spot ablation analysis. Laser parameters were 70 % energy, 10 Hz, 40  $\mu\text{m}$  spot size, 25 s dwell time, 50 s acquisition, and 25 s background. For each spot, a 15 s laser warm-up time was followed by a 20 s dwell time during which we ablated the sample. We used USGS synthetic glass standard GSE-1G as the calibration standard and two additional reference standards (GSD-1G and MACS-3) as quality controls for verification of instrument accuracy and precision. We ablated each standard in three to five locations after every four samples to adjust for possible instrument drift. We converted isotopic counts to elemental concentrations ( $\mu\text{g}\cdot\text{g}^{-1}$ ) after correction for gas blank, matrix, and drift effects using Glitter 4.4 (GEMOC CSIRO, Macquarie Research Ltd., Macquarie University, Sydney, Australia). Data processing with Glitter 4.4 required a 95 s washout time after each ablation. Otolith elemental concentrations were well above mean detection limits (i.e., 0.01 for  $^{88}\text{Sr}$ , 0.07 for  $^{137}\text{Ba}$ ), which we calculated as mean blank values plus three standard deviations (Wells et al. 2003). We reported all water and otolith data as element:Ca ratios ( $\text{mmol}\cdot\text{mol}^{-1}$ ).

We compared water Sr:Ca and Ba:Ca between the Hatchery and South Brush Pond using analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD)

test for multiple comparisons on  $\log_{10}$ -transformed water data to meet assumptions of normality and homoscedasticity (Brazner et al. 2004a, Zeigler and Whitledge 2010). We evaluated the relationship between water signatures and otolith signatures of Well and Pond yellow perch using least-squares linear regression. We used nonparametric  $k$ -sample nearest neighbor discriminant analysis with a leave-one-out jackknife procedure (Johnson 1998) to measure the accuracy with which: 1) age-0 Well yellow perch (i.e., known origin) could be reclassified to Hatchery well water based on otolith core signatures and 2) age-1 Pond yellow perch (i.e., known capture location) could be reclassified to South Brush Pond based on otolith edge signatures. After quantifying the accuracy of different models ( $k = 2\text{--}8$ ), we used the model with the lowest error rate ( $k = 2$ ) to identify natal origins of Pond individuals using the known origin/capture location data set (Ruttenberg et al. 2005).

Hatchery well water had a Sr:Ca signature of  $1.85 \pm 0.04 \mu\text{mol}\cdot\text{mol}^{-1}$  (2 SE) and a Ba:Ca signature of  $0.81 \pm 0.01 \mu\text{mol}\cdot\text{mol}^{-1}$ . In South Brush Pond, water signatures overlapped between sampling periods for Sr:Ca (May:  $3.99 \pm 0.04 \mu\text{mol}\cdot\text{mol}^{-1}$ ; September:  $4.02 \pm 0.04 \mu\text{mol}\cdot\text{mol}^{-1}$ ) and Ba:Ca (May:  $0.30 \pm 0.01 \mu\text{mol}\cdot\text{mol}^{-1}$ ; September:  $0.30 \pm 0.01 \mu\text{mol}\cdot\text{mol}^{-1}$ ). Mean water signatures from the Hatchery and South Brush Pond were distinct for Sr:Ca ( $F_{1,2} = 1547.01$ ,  $P < 0.01$ ) and Ba:Ca ( $F_{1,2} = 2700.98$ ,  $P < 0.01$ ). Water chemistry was positively related to otolith chemistry for Sr:Ca ( $R^2 = 0.99$ ,  $P = 0.01$ ) and Ba:Ca ( $R^2 = 0.99$ ,  $P < 0.01$ ). Well and Pond yellow perch were reclassified to known natal and capture environments with 100% accuracy ( $n = 42/42$ ), permitting reliable natal origins assessment for Pond individuals. Approximately two-thirds (65%,  $n = 11$ ) of Pond individuals collected in this study hatched in South Brush Pond, whereas 35% ( $n = 6$ ) were Hatchery-reared.

Our results indicate that otolith microchemistry can be used as a tool for distinguishing hatchery-reared yellow perch stocked at the eyed-egg stage from naturally produced individuals. This finding supports previous research demonstrating the utility of otolith microchemistry for identifying fish natal origins (Brazner et al. 2004b, Murphy et al. 2012). High reclassification accuracies associated with bivariate (e.g., combined Sr:Ca and Ba:Ca) otolith signatures permitted reliable assessment of age-1 natal origins, supporting a previous yellow perch study (Collingsworth et al. 2010) that concluded using Ba:Ca in combination with Sr:Ca can reduce the uncertainty of otolith microchemistry. Although not large, our site-specific sample sizes for Well ( $n = 25$ ) and Pond ( $n = 17$ ) yellow perch were typical for published otolith microchemistry studies (e.g., median = 16 fish/site; Pracheil et al. 2014). Moreover, the accuracy and reliability of otolith microchemistry may offset its limitations (e.g., cost, personnel time), particularly in the context of eyed-egg stockings, for which fisheries managers have been unable to use OTC marking to classify yellow perch as stocked or naturally produced.

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