Examining Black Bullhead *Ameiurus melas* Age and Growth in Shallow Minnesota Lakes

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Ageing methods, population age structures, and growth trajectories for Black Bullhead *Ameiurus melas* were evaluated for six populations from shallow lakes divided between two ecoregions in Minnesota, USA. A total of 693 fish were collected in July and August 2018 and measured for total lengths (TL) and weights (g). We collected ageing structures from 320 of these fish. Cross-sections of lapillar otoliths (n=191) and pectoral spine structures (n=75) were used to estimate age by two readers in a blind reading procedure. Fish caught ranged in total lengths from ~40 to 350 mm and weighed between 8 and 380 g. Black Bullheads ranged in age from 0-6 years within both regions of Minnesota and exhibit relatively fast growth until age 3. After age 4, growth slowed down as they approach an asymptote of ~300 mm total length by age 6. Lapillar otolith age estimates had relatively high precision between readers (CV=11.02%), and a reader agreement of 83%. Pectoral spine age estimates had greater precision (CV=5.82%) with a reader agreement of 87%. Comparing the relationship between lapillar otolith and pectoral spine ageing methods yielded low precision and relatively high variability (CV=37.27%). Only 47% of age estimates between methods were in agreement. A linear regression model revealed variability in the relationship between spine lengths (mm) and estimated age (years) for both otolith and spine structures (R^2=0.49, R^2=0.39), respectively. Mean length-at-age shows variable growth patterns between each region of Minnesota.

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Introduction

The Black Bullhead *Amereius melas* is an ecologically important Ictalurid fish species throughout its native range in North America; centrally located within the Mississippi River drainages from as far north as southern Saskatchewan and south to the gulf drainage and northern Mexico (Page and Burr 1991). Black Bullhead are widespread across the midwestern United States, found in a variety of different waterbodies and have a reputation for having high tolerances to environmental conditions (Hanchin et al. 2002, Mork et al. 2009). This species is an opportunistic feeder that orients towards lake bottoms, feeding on a wide range of invertebrates and plant material. Often, Black Bullheads are highly abundant in turbid systems with poor water clarity and high nutrient levels (Mork et al. 2009). This benthivorous species contributes to increased turbidity, declines in macrophyte production and wildlife use, and can alter fish assemblages in shallow aquatic systems (Braig and Johnson 2003, Mork et al. 2009). The most recent, comprehensive and complete literature investigating Black Bullhead populations comes from studies on non-native European populations where there are management concerns for the invasive impacts (Copp et al. 2016, Pedicillo et al. 2008). There is limited literature depicting population characteristics, age structures, and life-history traits of Black Bullhead within their native range (Mork et al. 2009). Determining age and growth is important for understanding the dynamics of any fish population. Growth determinations are valuable in identifying the ecology of a species, which can help guide management decisions (Quist and Isermann 2017).

The purpose of our study was (a) to determine which Black Bullhead ageing method yields the lowest coefficient of variation, using pectoral spine and lapilli otolith structures, (b) determine if spine lengths measured on live fish has a close correlation to age, providing a non-lethal method for future ageing estimations, and (c) compare growth trajectories and age structures among lakes. Literature regarding Ictalurid fishes is variable as to what structures are most feasible for ageing methods amongst species. Similar research conducted on the Yellow Bullhead, *Ameiurus natalis* from South Florida report pectoral spines as being more reliable than otolith structures. Previous studies on other catfish species indicate higher ageing accuracy with the use of otoliths as spines can be problematic due to degradation of the central lumen resulting in the absence of the first annulus.

A comparison of ageing methods for this species is non-existent for Minnesota and is critical to evaluate age and growth within the northern extent of its native range. We hypothesized that pectoral spines would provide more ageing accuracy than otoliths. Additionally, we predicted variable growth between two regions within Minnesota given the differences in ecological attributes.

Methods

Fish Collection

We collected Black Bullheads from six lakes located within two different ecoregions as defined by the state of Minnesota's Pollution Control Agency (Table 1). Three lakes in southern Minnesota are within the southeastern extent of the western corn belt plains. These waters are characterized as hypereutrophic, which were highly turbid water, having high nutrient levels, low light attenuation, and low dissolved oxygen levels. The other three lakes are located within the northern lakes and forests region within the Chippewa National forest. These lakes reside in managed woodlands and exist in a more natural state classified as being more eutrophic on the Carlson's trophic state index by Carlson (1977). Sample sites were chosen based on regional similarities in water chemistry, and classification as a shallow lake.

Fish were sampled using a variety of gears including: Fyke nets, gill nets, a seine net, and boat electrofishing. All captured fish were measured for total length (TL) to the nearest millimeter. For ageing purposes, we kept five fish for each 10 mm length group from each of the six lakes. Pectoral spine lengths were measured posterior to the articulating process to the distal tip of the spine (± 1 mm). Spines were removed anterior to the articulating process, wiped clean of any tissue and skin, and stored in scale envelopes to dry for further analysis. Lapillar otoliths were also removed in the field, wiped clean, and placed into scale envelopes to dry for laboratory analysis.

Ageing Procedures

Pectoral spines and otoliths were analyzed for age following the methods outlined by Koch and Quist (2007) and by Quist and Isermann (2017). Procedures for pectoral spines and otoliths were similar consisting of removal, setting, cutting, and reading. However, otoliths consisted of additional procedures such as "half-pours" and sanding of cross sections. This was to ensure that the otolith was suspended in the resin and completely covered as so not to break upon the sawing procedure.

Pectoral spine processing

Spine processing consisted of removing any excess material from the spine, cutting the tapered ends of 1.5 mL flat top microcentrifuge tubes (Fisher Scientific™), numbering each tube with unique information, filling the inner portion of the tube caps with molding clay, and setting the spine upright within the tube. Tubes were then filled with low-viscosity two- part epoxy resin (EpoThin™) to encase the entire spine. Spines were embedded in epoxy to reduce damage and to easily facilitate manipulation during the cutting process. Spines cured for 48 hours and were then removed from the centrifuge tube and placed back into original envelopes. Spines were secured in a low-speed saw (Isomet™ 1000, Buehler®) and cut with a 101.6 mm diamond wafering blade (Pace®, Technologies™).

Table 1. Types of data collected for ageing and growth analysis including: the region (S = Southern, N = Northern), site name, sampling date, approximate surface area (acres), sample size (*n*), and fish capture method (EF = electrofishing, SN = seine netting, GN = gill netting, and FN = Fyke netting).

Region	Site Name	County	Sampling Dates	Area	Sample Size	Method
S	Pickerel	Freeborn	July and August 2018	588	120	EF,SN,GN,FN
S	Geneva	Freeborn	July and August 2018	2,214	202	EF,SN,GN,FN
S	Rice	Freeborn	July and August 2018	697	183	EF,SN,GN,FN
N	L. Pigeon	Itasca	August 2018	285	43	EF,FN
N	Damon	Itasca	August 2018	68	74	FN
N	Anderson	Beltrami	September 2018	87	71	EF,FN

Three cross sections approximately 0.3 to 0.5 mm thick were taken at the distal end of the basal process from each spine to compensate for any annuli that may be obscured by complete formation the central lumen and placed back into envelopes. Reading of spines consisted of each reader individually viewing all cross sections independently with a Type-B immersion oil (Cargille Labs, Inc.) to enhance annuli rings under a stereomicroscope (Laxco™ MZS4-MZ33) at 30x magnification. Translucent zones between annuli in spines were most distinguishable utilizing reflected light on a black background. All cross sections were observed to be sure we captured the best display of the most discernable annuli. Determined age was then transferred onto a data sheet and agreed age was later determined if any discrepancies in age were found between readers.

Otolith processing

Otolith setting consisted of preparing PELCO® flat embedding molds (TED PELLA, Inc.) by filling each bullet mold half full of low-viscosity epoxy resin (EpoThin™) and allowing the half-poured molds to cure for 48 hours. Otoliths were then oriented with the macular hump facing downward to avoid the formation of air bubbles. The sulcus edge was aligned perpendicular to the edge of the mold to ensure a cross section cut would run perpendicular through the otolith. Another "half-pour" of epoxy resin was added to the bullet molds containing otoliths and left to cure for at least 48 hours to ensure hardening. Otoliths were removed from bullet molds once fully cured and placed back into original envelopes. Epoxied otoliths were secured in a low-speed saw (Isomet™, 1000, Buehler®) and cut with a 101.6mm diamond wafering blade (Pace®, Technologies™). Otoliths were cut into single cross-sections from 0.5 to 0.7 mm through the center of the nucleus and stored in original envelopes. When reading of otoliths took place two readers independently viewed cross sections under a stereomicroscope (Laxco™ MZS4-MZ33) at 30x magnification with the Type-B immersion oil (Cargille Labs, Inc.) to enhance annuli rings. Transmitted light was used for viewing otoliths, but if cross sections were too thick for transmitted light to penetrate thoroughly sections were sanded down with 400 grit sandpaper (3M™) to allow for better light penetration. We recorded the determined age on a corresponding data sheet and any non-agreements were resolved by both readers agreeing on a certain age with concert reading.

Data Analysis

Percent agreement between readers, and mean coefficient of variation (CV) were used to describe precision and variability for each ageing method, and to compare the relationship each method had to one another (Kimura and Lyons 1991). Percent agreement and mean CV were calculated for representative samples from each population. Linear models were developed to effectively illustrate the age comparisons between two readers for both otolith and pectoral spines, and in comparing agreed age estimates of each other. Total length measurements (mm) of Black Bullheads were used in a non-linear model to illustrate annual growth rates by region using the von Bertalanffy growth equation: $L_t = L_\infty(1\text{-}e - k\,[\,t - t_0\,])$ as described by Isely and Grabowski (2007). A length-frequency distribution was created to assign fish caught into 10 mm length groups. Agreed age estimates from both structures, and spine length measurements (mm) were used in a linear regression model to analyze if the spine length showed correlation to age. Mean total length and standard deviations (SD) were utilized in comparing estimated age classes.

Results

In total, 693 Black Bullhead were collected across the two different ecological regions within Minnesota between the months of July and September 2018. Fish from the three southern sites totaled 507, and a total of 186 were collected from our three northern sites (Fig. 1). Total length of fish caught ranged from 40 to 350 mm. Spine lengths measured on live fish ranged from 8 to 31 mm. Overall, agreed age estimates using both otolith and pectoral spine structures ranged from 0-6 (both readers combined), with 89% of fish aged 0-3.

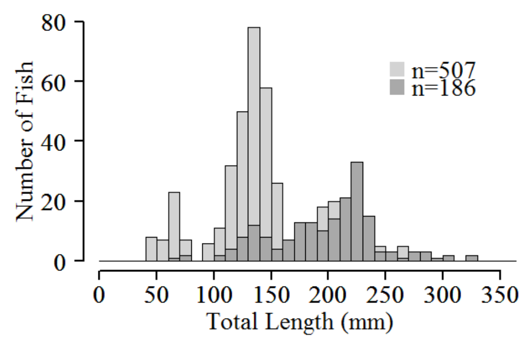

Fig. 1. Length-frequency distribution for Black Bullheads *Ameiurus melas* collected from northern Minnesota lakes (■) and from southern Minnesota lakes (□) organized into 10 mm categories.

Pectoral spine length was related to fish age estimates for otoliths and pectoral spines. Growth of Black Bullhead was highly variable between age classes (Fig. 2). Variability of spine length compared to ageing estimates is shown in a linear regression model (Fig. 3) illustrating the relationship between pectoral spine length and estimated ages amongst otoliths and pectoral spines (R^2=0.49, R^2=0.39), respectively, which indicated that fish age cannot be reliably predicted from spine length. Similar results are reported in Murie (2009) in which the relationship between pectoral spine age and spine length provided an R^2 value of 0.48 for Yellow Bullhead in southern Florida.

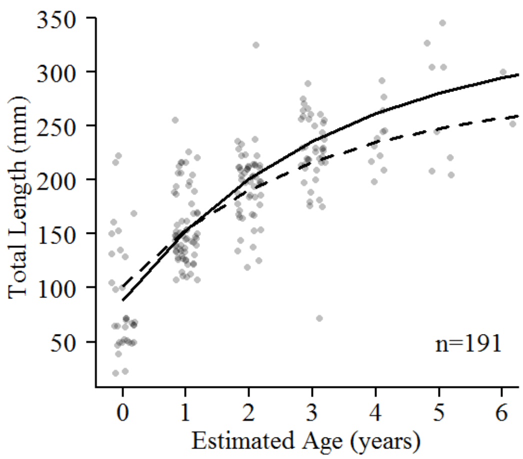

Fig. 2. Total length (TL) at estimated age at time of capture fit with von Bertalanffy growth curves illustrating annual growth rates for Black Bullheads sampled on southern lakes (solid line) and northern lakes (dashed line). Age estimates derived from agreed otolith ageing.

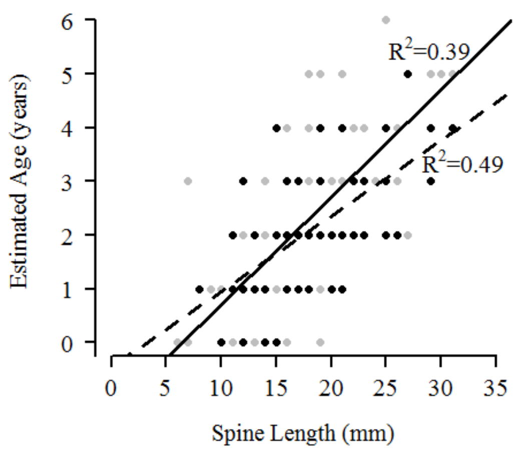

Fig. 3. Linear regression model relating agreed age estimates obtained from; pectoral spine (dashed line) and lapillar otolith (solid line) structures, to spine length measurements (mm). Black points represent agreed otolith age estimates and gray points represent agreed spine age estimates.

Pectoral spines provided the most precise age estimates in comparison to otoliths. Pectoral spine (n=75) ageing estimates between two readers was determined to have a CV of 5.82% whereas with otoliths (n=191), the calculated CV value was 11.02% (Fig. 4). When comparing the agreed age estimates from each method (n=75) to one another, the CV= 37.27%, which indicates discrepancy between the two procedures (Fig. 4).

The von Bertalanffy growth model (Fig. 2) depicts accelerated growth from estimated age-0 fish to estimated age-4 fish, from a mean TL of 89.5 (SD=53.1) to 237.9 mm (SD=29.1), respectively. In general, Black Bullhead increased in length quickly within their first 3 years after which growth slows and approaches asymptote by estimated age-5 with a mean TL of 265.3 mm (SD=59.4). Estimated age-3 black bullheads (n= 19) collected in northern Minnesota had a mean TL=210 mm (SD=42.5) as compared to fish collected in southern Minnesota (n=22) which had a mean TL=239 mm (SD=24.2). Estimated age-4 black bullheads sampled in northern Minnesota (n=3) had a mean TL=208 mm (SD=9.0), as compared to a mean TL=252 mm (SD=23.6) from fish collected in southern Minnesota (n=8). Growth modeling indicates faster growth in the southern lakes compared to the northern lakes expressed as; (L_∞=333.40, K=0.31, t_0=-1.00) and (L_∞=279.17, K=0.35, t_0=-1.30), respectively.

Discussion

Pectoral spines are the preferred ageing structure for Black Bullhead because preparation is less time consuming than otoliths, are a non-lethal ageing method, and have the ability to yield precise results. Precision of age estimates from Black Bullhead pectoral spines were lower than precision estimates derived from otoliths. Although structure related bias was detected between the two procedures with pectoral spines underestimating age of older fish in comparison to otolith ages. Consideration must be taken when ageing with pectoral spines due to failure to identify the first annulus occurring to the expansion of the central lumen and obliteration of the first annulus (Buckmeier et al. 2002).

When comparing variable growth by region, it is observed that Black Bullheads collected from the northern lakes have an average slower growth as compared to those collected from southern lakes. Abundance and mean length at age 3 have shown to be positively correlated with increased nutrients in South Dakota glacial lakes (Hanchin et al. 2002). Lakes located in the southern region of Minnesota were found to be hypereutrophic and extremely rich

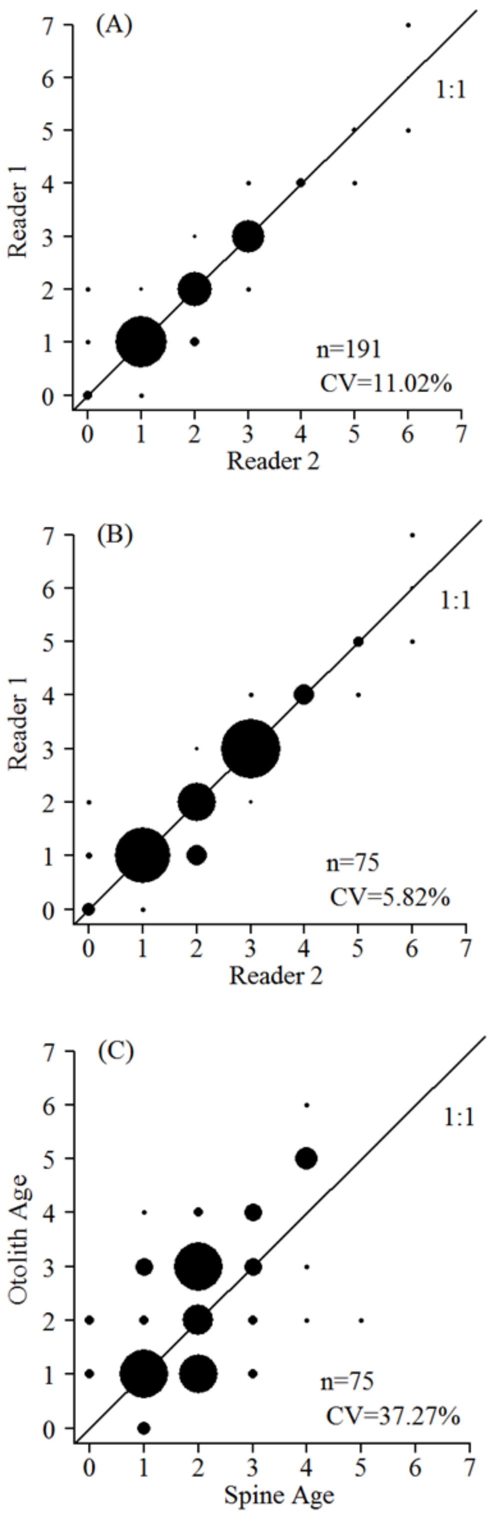

Fig. 4. Scatter plots comparing ageing estimates between reader 1 and reader 2 using; A.) otolith structures, B.) pectoral spines, and C.) agreed age estimates for otoliths versus pectoral spines. Sample sizes are represented by circle size.

in nutrients and minerals due to their location in agricultural areas. Northern lakes were also shallower than southern lakes, which can contribute to overpopulation resulting in slower growth (Hanchin et al. 2002). Variable growth by region can also be attributed to longer growing seasons in the South due to temperatures staying warmer longer then their Northern counterparts.

Although the extent of this study was limited to six populations, our results provide important information on the characteristics of Black Bullhead growth in different ecoregions within Minnesota. Our results contribute to the lack of literature for ageing Black Bullhead and the use of non-lethal methods for ageing purposes. Pectoral spines offer a suitable non-lethal alternative to otoliths for ageing of Black Bullhead and are recommended for use of ageing.

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