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MANAGEMENT BRIEF

Evaluation of Aging Structures for Silver Carp from Midwestern U.S. Rivers

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Abstract

To combat the potential deleterious effects that Silver Carp Hypophthalmichthys molitrix have on native populations, management of this species is essential. Before developing population-level models, a determination of which aging structure for estimating the age of Silver Carp is needed. To our knowledge, no consensus has been reached on which structure should be used for estimating Silver Carp ages. We collected 120 Silver Carp from the Illinois, Mississippi, Missouri, and Ohio rivers via electrofishing to evaluate aging structures. Removal time, processing time, and discernible annuli were evaluated for scales, opercles, vertebrae, pectoral fin rays, postcleithra, and asterisci and lapilli otoliths. Asteriscus otolith, opercle and scale annuli were difficult to discern and not evaluated further. Total processing times for postcleithra (246.1 s) and lapilli (251.2 s) were the most time-efficient; pectoral fin rays and vertebrae were more time intensive. Between-reader precision and agreement rates resulted in lapilli being the most precise, followed by postcleithra, pectoral fin rays, and vertebrae. Comparisons of structures with lapilli revealed that pectoral fin rays exhibited 78% agreement, 49% agreement with postcleithra, and 53% agreement for vertebrae. In terms of agreement ± 1 year to lapilli, pectoral fin ray, postcleithrum, and vertebra resulted in high agreement (>85%). Age bias plots revealed that these discrepancies consistently underestimated ages compared with lapilli. Discrepancies may be attributed to erosion of the central lumen of fin rays and postcleithra, while locating the first annulus on vertebrae may have led to this disparity. Based on previous studies, evaluation of overall processing times, assessment of between-reader precision. between-reader agreement rates, and bias that may be involved with alternative structures, we recommend lapilli otoliths be used for estimating age of Silver Carp. Future efforts should focus on validating accuracy of lapilli for estimating Silver Carp ages.

To combat the potential deleterious effects that Silver Carp *Hypophthalmichthys molitrix* have on native fishes, management of this species is essential (Kolar et al. 2005; Irons et al. 2007; Conover et al. 2007). Developing population-level models

that enhance our ability to reduce or eradicate stocks can minimize their effects on native species (Sakai 2001). To develop these models, a thorough understanding of their demographics (e.g., age, growth, and mortality) is needed. Accurate ages are required for age-structure analysis, growth analysis, and mortality rate estimation, all of which are key parameters in population modeling (Campana 2001). The effects of inaccurate age estimates cannot be overstated (Bradford 1991; Richards et al. 1992; Morison et al. 1998); models built on erroneous age data result in overly optimistic estimates of population dynamics, particularly when age is underestimated (Campana 2001).

Despite the need for accurately estimated ages, researchers focus on using time-efficient structures while still being able to acquire adequate representations of population demographics (Isermann et al. 2003; Maceina et al. 2007). Few studies have formally evaluated the amount of time it takes to remove and process different structures used to estimate age (Isermann et al. 2003). Many studies have offered anecdotal information on removal and processing times and have requested further evaluation of effort (Boxrucker 1986; Welch et al. 1993; Kocovsky and Carline 2000; Buckmeier et al. 2002; Koch and Quist 2007; Stolarski and Hartman 2008). As such, removal and processing times are undoubtedly important components of estimating ages, especially in cases where accuracy and precision may be sacrificed.

Despite the necessity for an efficient structure that exhibits an accurate and precise age estimate, no consensus has been formed for Silver Carp. Shefler and Reich (1977) used scales to estimate their age for growth within Lake Kinnerect, Israel. Kamilov (1984) established that the first ray of the pectoral fins, the pterygiophore of the first ray of the dorsal fins, and vertebrae were suitable for aging, whereas scales, opercles, and otoliths were not. However, the structures were not compared within that

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study. Other structures such as the postcleithra have also been reported suitable for aging (Johal et al. 2000). More recently, Williamson and Garvey (2005) used pectoral fin rays to estimate Silver Carp ages. The central theme of these demographic studies suggests multiple structures are used for estimating ages and that there is a need for a comparative study.

Because agencies may have concern with the times associated with aging structures, our first objective was to compare removal and processing times associated with several structures. Due to a lack of consensus and because there has been no formal comparative evaluation of Silver Carp structures, we evaluated scales, opercles, vertebrae, pectoral fin rays, postcleithra, and asterisci and lapilli otoliths, all commonly used for estimating ages of freshwater fishes. Our second objective was to determine if these structures contained discernible annuli. We had no known-age Silver Carp, so validation of age was impossible. Thus, we determined between-reader precision of each structure. Lastly, we tested for any potential bias associated with these structures.

METHODS

Fish collection.—During fall 2011, 120 Silver Carp were collected from the Illinois; Missouri; Ohio; and upper, middle, and lower Mississippi rivers (20 fish from each river or section) using daytime electrofishing. Total length (mm) and weight (g) of each fish were measured; scales, opercles, vertebrae, pectoral fin rays, postcleithra, and asterisci and lapilli otoliths were removed and placed in appropriate containers.

Removal and processing of aging structures.—Scales were removed from an area directly behind the tip of the pectoral fin and above the lateral line (Shefler and Reich 1977). Scales were placed in coin envelopes, air dried, and 10 indiscriminately selected scales were subsequently pressed onto acetate slides using a roller press. Scale impressions on acetate slides were evaluated under a microfiche reader.

The right opercle and opercular assembly was removed from each fish. Skin was removed from all sides. Opercles were placed in individually marked bags and frozen to avoid decomposition or desiccation. Prior to counting annuli, opercles were boiled for approximately 1 min to remove any excess tissue. Occasionally, a bristled brush was used to scrub off excess soft tissue. Opercles were then viewed under transmitted fluorescent light with the naked eye as described by Phelps et al. (2007).

The first hard ray of the pectoral fin was removed from the left side of every fish when possible; the right side was used when not possible. Tissue around the pectoral fin rays was removed and three 0.8-mm-thick subsections were removed from the anterior portion of the pectoral fin ray with a Buehler Isomet low-speed saw and then secured on microscope slides. Sections were examined under a dissecting microscope $(10-40 \times \text{magnification})$ with transmitted light (as described by Phelps et al. 2007).

The first vertebra of each fish was extracted (methods of Deters et al. 2011) and placed in boiling water to remove

tissue. Vertebrae were subsequently air dried and placed into 125-mL plastic bottles filled with 2% sodium hypochlorite solution, sealed, shaken, allowed to soak for 1 h, cleaned, soaked for 15-20 min in distilled water, air dried for 24 h, and then read with a dissecting microscope ($4-10 \times$ magnification).

The postcleithrum was removed by dissecting tissue along each side of this structure. Once extracted any excess muscle tissue was removed with a scalpel. Three transverse sections were then taken from the middle of the postcleithrum using a fine jeweler's saw (Johal et al. 2000). These sections were ground and polished using a carborundum stone and fine-ground glass to a thickness of 0.3-0.5 mm using water as a lubricant and mounted on glass slides in DPX glue to be viewed under a dissecting microscope ($10-40 \times 2000$).

Asterisci otoliths were removed from the lagena ventrally (as per Secor et al. 1991). Asterisci were cleaned, air dried, mounted in clear epoxy, and transversely sectioned along the dorsoventral plane through the nucleus with a low-speed saw. To ensure that the nucleus was included, several 0.4-mm-thick sections within this area were cut. Sections of asterisci were examined under a dissecting microscope ($10-40 \times \text{magnification}$) with transmitted light as documented for Common Carp *Cyprinus carpio* (Brown et al. 2004).

Lapilli otoliths were removed by sectioning through the supraoccipital bone using a hacksaw. The cut was made in line with the gap between the preopercle and the opercle. Lapilli were removed with forceps from the posterior portion of the skull. These removal procedures were similar to lapilli removal in Channel Catfish *Ictalurus punctatus* as explained by Buckmeier et al. (2002) and Long and Stewart (2010). Specifically, we found that using forceps to grip lapilli instead of epoxying them to a slide provided a more efficient method. Lapilli were sanded with 400-grit wetted sandpaper from the anterior side in order to get close to the nucleus. At that point, fine wetted sandpaper (1,000-grit) was used to reach the nucleus. The lapillus's reading surface was burnt golden brown with a candle. Next, the lapillus was placed posterior side down in putty and a drop of immersion oil was applied. Annuli were viewed under a dissecting microscope $(4-10 \times)$ with a fiber optic filament (1-mm-diameter tip) connected to a light source. The movable fiber optic filament facilitated identification of all annular rings.

For each structure, we recorded the time required to remove the structure (hereafter referred to as removal time). We also recorded the time required to process each structure for estimating age (hereafter referred to as processing time). For removal and processing times, we used times from two individuals to simulate variability due to differences in skill level among personnel (Isermann et al. 2003). Overall processing times were calculated by summing the times required to remove and process each individual structure for each fish. In order to estimate age for structures, annuli were recorded independently by two experienced readers that had no knowledge of fish length, estimated age of other structures, or source river. The age estimated

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by each reader was recorded and later analyzed for betweenreader precision. If age estimates differed between readers for an individual structure, both readers viewed the structure together until a consensus was reached. If consensus between readers could not be reached, the structure was removed from further analysis.

Statistical analyses.—Differences in removal, processing, and overall processing times among aging structures were examined using a one-way analysis of variance (ANOVA) and pairwise comparisons were evaluated with Tukey's honestly significant difference (HSD) test (all pairwise procedures; $\alpha = 0.05$). Following the evaluation of processing times, precision between readers was determined via CV (i.e., 100 × SD/mean; Chang 1982). Furthermore, we calculated between-reader agreement rates (exact agreement and agreement ± 1 year) for each structure, under the supposition that structures displaying easily discernible annuli would result in high agreement rates between readers. To determine if the CV calculated between-readers varied among and between structures we used Kruskal-Wallis ksample tests (Welch et al. 1993). Percent agreement between structures was used to compare age determinations (Phelps el al. 2007). To determine if bias occurred between structures, age-bias plots were generated (Campana et al. 1995; Buckmeier et al. 2002).

RESULTS

The additional four structures (i.e., lapilli, postcleithra, pectoral fin rays, and vertebrae) had apparent annular marks. Removal times were significantly different among structures with discernible annuli (ANOVA: $F_{3,476} = 47.91$, P < 0.01). Furthermore, Tukey's HSD indicated several pairwise differences (Table 1). Pectoral fin rays required the shortest time for removal (39.6 s), while lapilli (46.1 s) and postcleithra (46.0 s) showed similar results, and vertebrae required the longest time (56.2 s; all pairwise comparisons P < 0.05).

TABLE 1. Mean removal, processing and total processing times associated with the use of lapilli, postcleithra, pectoral fin rays, and vertebrae to estimate the age of Silver Carp collected from the Illinois, Missouri, Mississippi, and Ohio rivers during the fall 2011 via boat electrofishing. Standard errors are reported in parentheses. Values for a given statistic with different letters indicate significant differences between structures (Tukey's HSD test; all pairwise procedures, P < 0.05).

	Elapsed time (s)		
Structure	Mean removal (SE)	Mean processing (SE)	Total processing (SE)
Lapilli	46.1 (1.22) y	251.2 (4.77) z	297.2 (5.04) z
Postcleithra	46.0 (1.30) y	246.1 (5.57) z	292.1 (5.92) z
Pectoral fin rays	39.6 (0.79) z	352.9 (7.54) y	389.8 (7.64) y
Vertebrae	56.2 (1.18) x	537.0 (11.23) x	593.1 (11.51) x

TABLE 2. Mean coefficients of variation between reader age assignments and reader agreement rates associated with the use of lapilli, postcliethra, pectoral fin rays, and vertebrae to age Silver Carp collected from the Illinois, Missouri, Mississippi, and Ohio rivers during the fall 2011. Mean coefficients of variation with different letters indicate significant differences between structures (all pairwise procedures, P < 0.05).

Structure	Average CV (SE)	Exact reader agreement (%)	Reader agreement ±1 year (%)
Lapilli	4.22 (0.83) z	76	98
Postcleithra	9.73 (1.27) y	58	91
Pectoral fin rays	10.22 (1.56) y	57	93
Vertebrae	15.73 (2.56) y	51	85

Processing times were significantly different among structures (ANOVA: $F_{3,476} = 312.32$, P < 0.01), and several pairwise differences existed. Processing times were similar between lapilli (251.2 s) and postcleithra (246.1 s); however, pectoral fin rays (352.9 s) and vertebrae (537.0 s) resulted in longer times, the latter representing the greatest time (all pairwise comparisons; P < 0.05). Overall, total processing times were significantly different among structures (ANOVA: $F_{3,476} = 315.14$, P < 0.01). Lapilli (297.2 s) and postcleithra (292.1 s) required the least time for overall processing, while pectoral fin rays (389.8 s) and vertebrae (593.1 s) overall processing times were greater overall (all pairwise comparisons; P < 0.05).

Between-reader precision was significantly different among structures (Kruskal–Wallis; $\chi 2=21.37$, df = 3, 476, P<0.01) and between structures (all comparisons P<0.05) with discernible annuli. Lapilli resulted in higher between-reader precision than postcleithra, pectoral fin rays, and vertebrae, as indicated by the significantly lower CV between readers (all pairwise comparisons P<0.05; Table 2). Of estimates from lapilli, 76% agreed exactly among readers, and 98% of the estimates were \pm 1 year (Table 2). The percent exact agreement among readers for postcleithra, pectoral fin rays, and vertebrae was less than 60%, but 85–91% of the estimates agreed within 1 year (Table 2).

Lapilli resulted in an estimated age range of 2–10 years, pectoral fin rays ranging 2–9 years, postcleithra 2–8 years, and vertebra 2–7 years. Because of the significantly lower between-reader precision and the potential drawbacks of pectoral fin rays, postcleithra, and vertebrae, all between-structure age comparisons were evaluated using lapilli as the primary age determinant (Table 2; Figure 1). These comparisons revealed that lapilli exhibited 78% agreement with pectoral fin rays, 49% agreement with postcleithra, and 53% agreement with vertebrae. In terms of agreement \pm 1 year to lapilli, percentages were high for pectoral fin rays (97%), postcleithrum (91%), and vertebra (87%). However, age-bias plots for all structures revealed that these

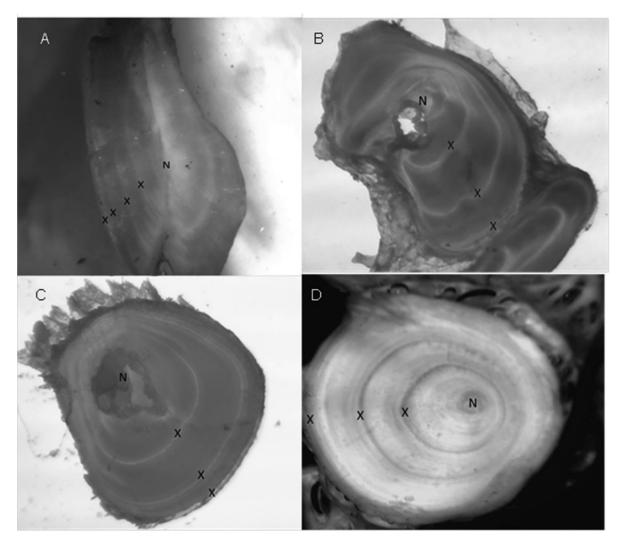


FIGURE 1. Aging structures of a Silver Carp (#106) collected via boat electrofishing from the Illinois River during the fall 2011, showing (\mathbf{A}) lapillus and 1-year disagreements (i.e., underestimate) between lapillus and the alternative aging structures: (\mathbf{B}) pectoral fin ray, (\mathbf{C}) postcleithrum, and (\mathbf{D}) vertebra. N = nucleus of the aging structure, X = individual annuli on the aging structure. Note erosion of the central lumen for the pectoral fin ray and postcleithrum, and the missing first annuli on the vertebra.

discrepancies consistently had lower estimates of Silver Carp age than did lapilli (Figure 2).

DISCUSSION

Otoliths are known as valid and precise structures for accurately aging multiple freshwater fish species and are typically preferred over scales, fin sections, and vertebrae (Heidinger and Clodfelter 1987; Welch et al. 1993; Buckmeier et al. 2002; Isermann et al. 2003; Maceina et al. 2007; Phelps et al. 2007). However, agencies have continued to use alternative aging structures (i.e., not otoliths) because of supposedly faster total processing times and no required sacrifice of fish (Isermann et al. 2003; Maceina et al. 2007). Over the course of our evaluation, total processing times were similar for lapilli and postcleithra, but pectoral fin rays and vertebrae required significantly more

time. Other studies have also found that otoliths had similar (Buckmeier et al. 2002; Isermann et al. 2003) or faster overall processing times than alternate aging structures (Kocovsky and Carline 2000; Stolarski and Hartman 2008).

We found that pectoral fins rays, postcleithra, vertebrae, and lapilli contained discernible annuli. Prior to this study, Silver Carp otoliths were considered fragile and opaque, and therefore difficult to read (Kamilov 1984; Johal et al. 2000). This corresponds to our findings for asteriscis. In contrast, annuli on lapilli were clearly distinguishable and quick to process (Figure 2a). Similar outcomes have been reported for validated Channel Catfish lapilli otoliths (Buckmeier et al. 2002; Long and Stewart 2010).

Lapilli had higher between-reader precision than alternative aging structures. Also between-reader agreement rates for lapilli resulted in a higher percentage of exact agreement and

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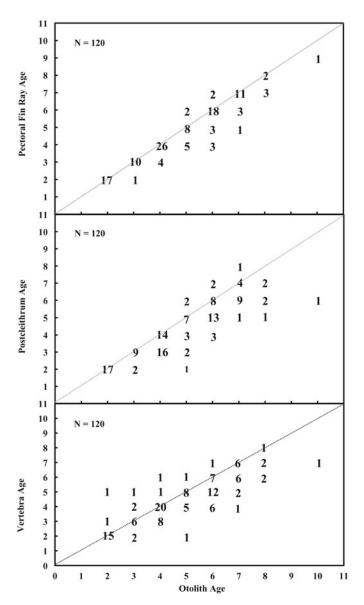


FIGURE 2. Age bias plots for pectoral fin rays, postcleithra, and vertebrae ages (in years) compared with lapilli for Silver Carp collected in fall 2011 from the Illinois, Missouri, Mississippi, and Ohio rivers via boat electrofishing. Dashed line indicates a 1:1 line (i.e., perfect agreement) and numbers indicate the frequency of that observation.

agreement ± 1 year than alternative aging structures. This seems to be a common theme within the literature, in which otoliths are more precise than alternative aging structures (Welch et al. 1993; Isermann et al. 2003; Maceina et al. 2007; Phelps et al. 2007).

Pectoral fin rays and vertebrae tended to underestimate age more than otoliths (Welch et al. 1993; Isermann et al. 2003; Maceina et al. 2007; Phelps et al. 2007); however, to our knowledge this is the first age comparison for postcleithra. Similar to these studies, we found each of these structures underestimated the age of Silver Carp more than lapilli; however, the differences in age estimates were typically ± 1 year of age. We noted that

even though discrepancies existed between lapilli and alternative aging structures, the differences were typically ± 1 year of lapilli age. However, we did not have any known-age fish in this study. Pectoral fin rays had the higher agreement (i.e., ± 1 year) than lapilli. Other studies also documented the precision of pectoral fin rays for other fish species such as the closely related Bighead Carp Hypophthalmichthys nobilis (Nuevo et al. 2004) and Common Carp (Phelps et al. 2007). Fin rays exhibited high agreement (± 1 year) with lapilli. However, age was typically underestimated with fin rays, perhaps due to erosion of the central lumen, thereby confounding identification of the first annulus in the fin section (Figure 1b; Muncy 1959; Mayhew 1969; Isermann et al. 2003).

To our knowledge no studies have compared postcleithra with other aging structures for estimating age of Silver Carp. A simple evaluation was completed by Johal et al. (2000), which noted discernible rings using postcleithrum. In our study, postcleithrum also showed high agreement with lapilli (i.e., ± 1 vear) but typically displayed underestimated ages more than lapilli. This discrepancy of 1 year could possibly be explained by erosion of the central lumen (similar to pectoral fin rays), which was documented for the postcleithrum in this study (Figure 1c). Vertebrae age discrepancies (compared to lapilli) may have been a result of difficulty locating the first annuli (Figure 1d). Similarly, two independent fisheries studies noted discrepancies over the identification of the first annulus when using vertebrae (Francis and Stevens 2000; Natanson et al. 2002). Difficulties in locating the first annulus for alternative structures may be compounded due to the protracted spawning exhibited by Silver Carp, potentially leading to even more difficulty in locating the first annulus (Lohmeyer and Garvey 2009).

To our knowledge, this study is the first to use lapilli for estimating age of Silver Carp. Alternatively, if sacrifice of this species is undesired, we recommend that pectoral fin rays should suffice based on fairly high agreement with lapilli, especially ± 1 year (Sikstrom 1983; Nuevo et al. 2004; Phelps et al. 2007). However, caution should be taken to ensure proper management decisions using pectoral fin rays. Similar discrepancies existed between lapilli and the lethal alternative aging structures (i.e., postcleithra and vertebrae). However, the accuracy of lapilli otoliths for the use of estimating ages of Silver Carp is yet to be validated. Nevertheless, taking into account previous fish-aging studies, evaluation of overall processing times, assessment of between-reader precision, between-reader agreement rates, and bias involved with alternative structures, we recommend that lapilli otoliths should be used to estimate age of Silver Carp.

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