Validation and Timing of Annulus Formation in Sagittal Otoliths of Alligator Gar

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Abstract: Successful management of fish populations relies on accurate age estimates for calculation of age-based population parameters. Otoliths are considered the most accurate and reliable structure for estimating age of most fish species. Validation studies should be conducted for populations or species that have not been evaluated to ensure otoliths can be aged reliably. Marginal increment analysis is a technique that can be used to validate annulus formation in otoliths. Our objective was to validate that a single annulus was formed yearly and to determine the timing of annulus formation in the sagittal otoliths of Alligator Gar. A total of 152 Alligator Gar were collected from Texoma Reservoir, Oklahoma for marginal increment analysis. We found that a single opaque band (annulus) formed yearly in otoliths of Alligator Gar. Annulus formation occurred during May and the completed band was evident in June. Findings from this study suggest that sagittal otoliths can be used reliably for aging Alligator Gar, since a single annulus forms yearly in this structure. Furthermore, collection of Alligator Gar for age estimation should occur after May to minimize age estimation errors caused by annulus formation timing.

Introduction

Characterizing growth, mortality, and recruitment of fish populations relies on accurate age estimates (Campana 2001). An assumption of fish age estimation is that counted annuli correspond with the number of years since hatch (Buckmeier et al. 2017). Otoliths have been validated for many fish species, and therefore, are typically considered the most reliable and accurate aging structure (Spurgeon et al. 2015). Sagittal otoliths of Alligator Gar (*Atractosteus spatula*) have been validated by injecting fish with oxytetracycline to create a distinct time stamp on the otolith and then counting the number of rings formed post-injection (Buckmeier et al. 2012). However, identification of the annulus was sometimes difficult due to false annuli (growth checks) formation in otoliths of young fish and the constriction of annuli (reduced growth) in otoliths of older fish (Buckmeier et al. 2012). Misinterpretation of annuli can result in inaccurate age estimates, which can produce erroneous age-based population parameters and lead fisheries biologists to make incorrect management decisions (Lai and Gunderson 1987, Beamish and McFarlane 1995, Porta et al. 2018).

Marginal increment analysis is an alternate technique for validating annulus formation in otoliths and has been used to validate that aging structures produce a single annulus yearly and to determine the time of year to sample fish for collection of aging structures (Buckmeier et al. 2017, Porta and Snow 2018, Snow et al. 2018). This method relies on measurements of the translucent zone of the otolith, that when plotted against the month that fish were collected, exhibits an annual sinusoidal pattern where the smallest point (slowest growth) on the curve represents the month that the annulus is formed (Okamura et al. 2013). Our objective was to validate that a single annulus forms yearly in Alligator Gar otoliths collected from Texoma Reservoir, Oklahoma. Additionally, because the timing of annulus formation can differ by fish age, we sought to determine the timing of annulus formation for young (ages 1-15) and older (ages 16-49) Alligator Gar.

Methods

Alligator Gar used for marginal increment analysis were collected via angler donations and direct sampling from Texoma Reservoir during 2017 - 2020. Fish were processed at the Oklahoma Fishery Research Laboratory in Norman, Oklahoma where they were measured for total length (TL, mm) and sagittal otoliths were removed. Following removal, otoliths were cleaned, placed into a small envelope, and allowed to dry for at least 2 weeks prior to processing.

Sagittal otoliths were processed following the methods of Buckmeier et al. (2012). In short, otoliths were ground on a plane perpendicular to the anterior–posterior axis using a rotary tool fitted with a grinding bit (Dremel, Racine WI). The rotary tool was secured to a laboratory bench using a vice. Forceps coated in a rubberized tool dip (Plasti Dip International, Blaine MN) were used to securely hold the posterior portion of the otolith during processing. Otoliths were polished using wetted 2000 grit sandpaper to smooth the surface of the otolith. Age estimation was accomplished by standing the otolith polished side up in a glass dish containing black modeling and the otolith was submerged in water to improve clarity. Otoliths were viewed with a variable-power Olympus SZX16 stereomicroscope capable of 130× magnification (Olympus Corporation, Lake Success, New York), and a single strand fiber-optic filament attached to an external light source was shined through the otolith to illuminate the annuli. An age estimate was assigned to each Alligator Gar by two independent readers. In cases where readers disagreed on an age estimate, a concert read was conducted by both readers and a final age was assigned.

Marginal increment analysis was performed by measuring the width of the hyaline zone following the most recently deposited opaque band (annulus) on the ventral edge of the otolith (Clayton and Maceina 1999, Blackwell and Kaufman 2012, Porta and Snow 2017, Snow et al. 2018). Three measurements were taken at similar points along the ventral edge of each otolith and the average of the three measurements was used as the marginal increment distance for each fish. Otoliths were measured (mm) using an Olympus DP74 digital camera attached to the stereomicroscope and cellSens entry imaging software (Olympus Corporation, Lake Success, New York). Marginal increment measurements were separated into two age groups, young fish (ages 1-15) and older fish (ages 16-43), and the mean increment distance was graphed by month. Marginal increment data were evaluated by age classes to determine if timing of annulus formation varied with age.

Results and Discussion

A total of 152 Alligator Gar ranging 590 - 2,310 mm TL were collected for marginal increment analysis (Figure 1). Alligator Gar age estimates ranged from 1 to 43 years old, which resulted in 107 fish in the young age group and 45 fish in the older age group. The marginal increment measurements indicated that a single annulus was formed yearly during May in the sagittal otoliths of Alligator Gar from Texoma Reservoir for both age classes (Figure 2). An annulus was fully completed in June, as indicated by deposited translucent material beyond the annulus. The marginal increment method has also been used to validate annulus formation in otoliths of Bluegill (*Lepomis macrochirus*; Hales and Belk 1992), Gizzard Shad (*Dorosoma cepedianum*; Clayton and

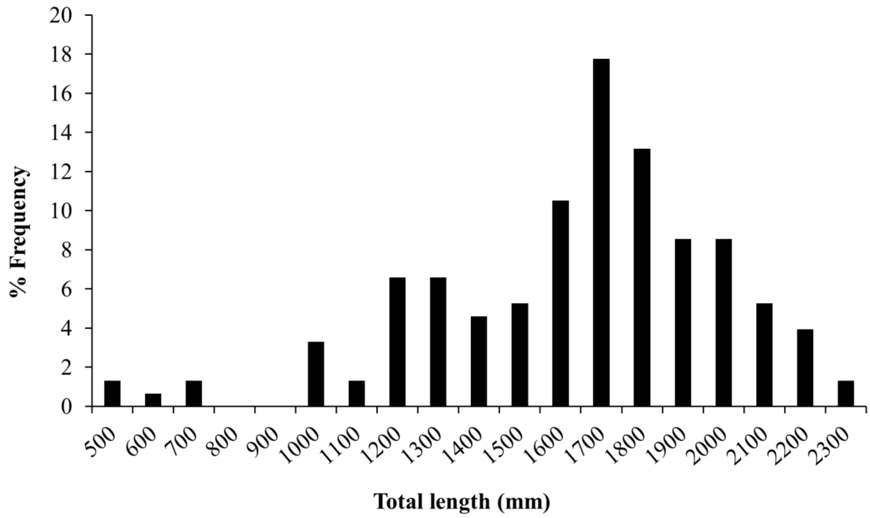

Figure 1. Length frequency distribution of Alligator Gar collected from Texoma Reservoir, Oklahoma for marginal increment analysis

Maceina 1999), Redband Trout (*Oncorhynchus mykiss gairdneri*; Schill et al. 2010), Saugeye (*Sander vitreus and S. Canadensis*; Snow et al. 2018), Spotted Suckers (*Minytrema melanops*; Strickland and Middaugh 2015), White Perch (*Morone Americana*; Porta and Snow 2017), and Yellow Perch (*Perca flavescens*; Blackwell and Kaufman 2012).

A single annulus formed in otoliths of young and old Alligator Gar. Formation of a single annual mark in the sagittal otoliths of Alligator Gar validates this structure for aging this species. Other methods, including oxytetracycline marking (Buckmeier et al. 2012, Buckmeier and Smith 2020) and bomb radiocarbon dating (Daugherty et al. 2020) have been used to validate sagittal otoliths of Alligator Gar to age-31 and > age-60, respectively. Buckmeier et al. (2012) found that false annuli are sometimes formed in Alligator Gar sagittal otoliths, therefore we utilized the marginal increment measurement technique because this method can identify the presence and timing of false annuli formation (Snow et al. 2018). False annuli were not observed in the sagittal otoliths of Alligator gar from Texoma Reservoir, suggesting that this structure can be used reliably to assign age estimates to fish in this population since only one opaque band (annulus) forms yearly.

Annulus formation occurred in the sagittal otoliths of Alligator Gar during May for both age classes, and annual marks were apparent by June. Buckmeier et al. (2012) observed annulus formation in sagittal otoliths of Alligator Gar in May. Buckmeier and Smith (2020) determined that annulus formation in sagittal otoliths of juvenile Alligator Gar was completed in June, but could not be observed until additional material was added to the otolith beyond the newly formed annulus. Similarly, we could not discern the annulus from the edge of the otolith until translucent material associated with new growth had been deposited beyond the annulus, which occurred after May. Therefore, we recommend that Alligator Gar should be collected for aging purposes during summer following formation of the annulus. Collection during this time will reduce age estimation errors caused by the timing of annulus formation. This study adds to the body of literature confirming annulus formation in the sagittal otoliths of Alligator Gar. Additionally, this study provides fisheries managers with information regarding the timing of annulus formation, which is important when collecting Alligator Gar to understand their population dynamics.

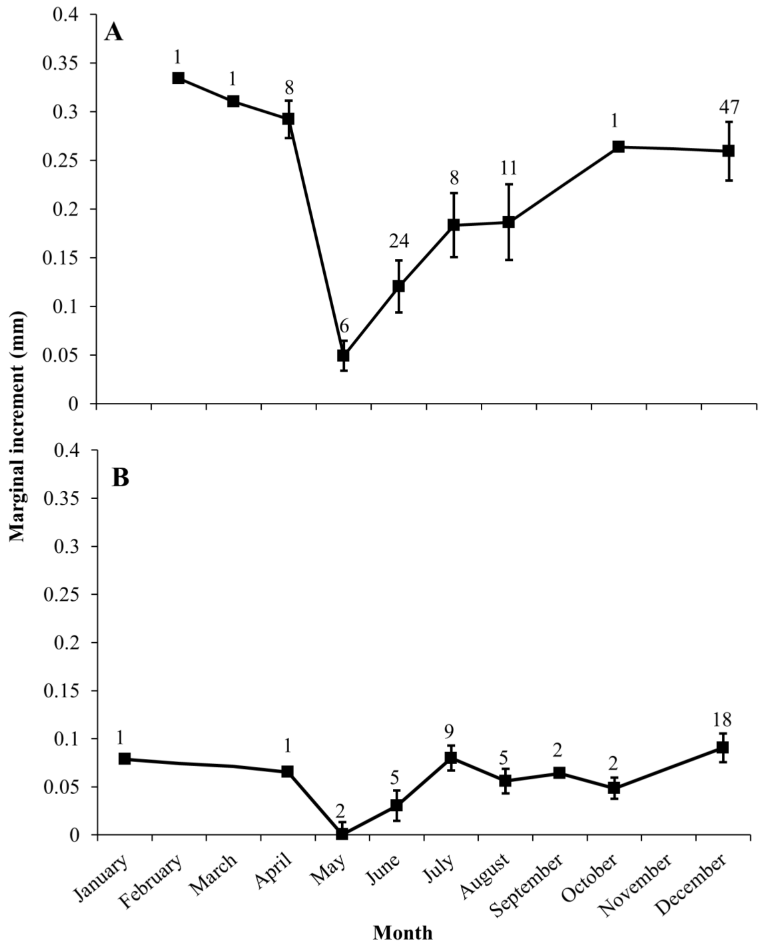

Figure 2. Mean monthly marginal increment measurements for (A) young Alligator Gar (ages 1-15) and (B) older Alligator Gar (ages 16-43) using sagittal otoliths. Error bars represent the standard error of the mean. Numbers above each point represent the monthly sample size.

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