

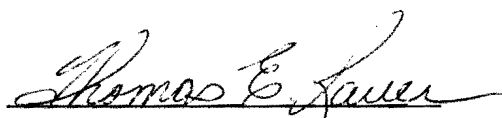
Comparison of Techniques Used to Age Yellow Perch in Southern Lake Michigan

An Honors Thesis (HONRS 499)

by

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A handwritten signature in cursive script, reading "Thomas E. Lauer", written in black ink. The signature is fluid and stylized, with a horizontal line underneath it.

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Abstract

Previous ageing studies compared the precision of yellow perch (*Perca flavescens*) scales to otoliths and scales to opercles and in each study, scales were proven to be less precise. To date, a comparative study between yellow perch otoliths and opercles has not been undertaken. Therefore, the goal of our study was to compare ageing precision of opercles to otoliths, while establishing the best methodology to use for ageing otoliths. Opercles and otoliths were collected from 217 yellow perch in the summer of 2003 from three sites located in southern Lake Michigan. Opercles were read as whole structures, while otoliths were read whole, sectioned, and break and burn. All four methodologies were aged three times by three independent readers. Opercles, sectioned otoliths, and break and burn otoliths were found to be the most precise structures for aging. Further investigation into the ageing of yellow perch improves the efficiency and precision of the ageing process. As a result, better fisheries management practices can be utilized.

Introduction

Development and evaluation of fishery management plans are dependant on the population demographics that require accurate age determinations (Shramm and Doerzbacher 1982). Ages are used to evaluate growth and mortality rates, recruitment, age-size structure calculating potential yields, and to describe and compare fish populations, among other fisheries modeling statistics (Belanger and Hogler 1982, Weisberg and Frie 1987, Van Den Avyle 1993). These methodologies require accurate ageing methods, or management strategies could be misguided or incorrectly applied.

Yellow perch are an important sport and commercial species in Lake Michigan, but have been the subject of some controversy (Francis et. al 1996). Over the years the perch population has fluctuated widely (Wells 1977), showing historic high abundance in the mid-1980's followed by a decline in the early 1990's that has persisted (McComish et al. 2000). This decline in Indiana forced the Indiana Department of Natural Resources (IDNR) to impose quota restrictions on harvest for commercial fisherman in 1995. This harvest strategy was amended two years later when commercial fishing for yellow perch was halted due to the increasing concern for the declining abundance and poor population structure of the yellow perch. At this same time, the IDNR promulgated a change in the creel limit from 25 to 15 fish/day for sport anglers. Many of these management plans had a foundation from the aging process that was thought to be accurate and precise.

Several studies on yellow perch aging have been conducted to find the most precise structure from which to age; these studies have compared scales with opercles

and scales with otoliths (Robillard and Marsden 1996; Baker and McComish 1998). In both cases scales were found to be the least precise structure to age from. However, there are several different methods of aging otoliths, and otoliths have not been compared with opercles. Therefore, in this study we compared three different methods of otolith aging with opercle aging to determine which structure provides the most precise method.

In addition to precision, time required to remove and process structures was also taken into consideration, and is a concern accounted for in few studies (Isermann et al. 2003). Ihde and Chittenden (2002) included processing time as criteria for determining the “best” ageing structure, while Welch et al. (1993) revealed that reading times differed between otoliths, scales, and anal fin rays and spines of striped bass. Processing and ageing times can further aid in determining how convenient and efficient the aging method can be (Hammers and Miranda 1991).

This study has two objectives that address both precision and time efficiency of aging. The first is to determine the most precise ageing methodology for yellow perch using both opercles and otoliths. Otoliths for this study were aged in three different forms: whole otoliths, break and burn, and sectioned otoliths, while opercles were aged whole. Precision refers to the aging reproducibility by more than one reader or by the same reader when reading the structure more than one time. The second objective is to determine which method is most time efficient.

Methods

Field

Yellow perch were collected from three different sites in southern Lake Michigan at depths of 5, 10, and 15 meters. Site M is located approximately 2 km east of Michigan

City Harbor and is characterized by a flat and sandy substrate with some small pebbles. Site G is located approximately 7 km north of Burns Harbor and is also characterized by a smooth and sandy substrate with some small pebbles. Site K is located approximately 3 km west of the harbor at Michigan City. The substrate here was characterized by smooth sand but additionally has clay ridge depressions up to 1 m deep at 5 m depth. A more complete description of the sites is given by Shroyer and McComish (1998).

Sampling was conducted bimonthly at each station from 2 June to 20 August 2003 using nighttime bottom trawling and multi-filament gillnetting. The semi-balloon bottom otter trawl consisted of a 4.9 m head rope and a 5.8 m foot rope with a stretch-mesh of 38.1 mm that was lined with 12.7 mm stretch mesh cod end. Bottom trawling was conducted at the 5 m depth contour immediately following sunset and consisted of 6 each 10 minute tows, for a total of 60 minutes of trawling per night. The multifilament nylon gill nets contain three different alternating mesh sizes: 51, 64, and 76 mm stretch-mesh which were repeated 3 times creating 9 panels spanning 137 m with a height of 1.8 m. Gillnets were set parallel to shore at both 10 and 15 m depth contours before sunset and were retrieved the next morning for 12 ± 2 hours of fishing. After capture all fish were placed on ice for processing. Lauer et al. (2004) provides a more complete description of collection methods.

Processing took place within 18 hours of capture, typically the following morning. All fish were identified to species and yellow perch total lengths (mm) and weights (g) were measured. Opercles were removed from the left side of the yellow perch and placed into coin envelopes. Both sagitta otoliths were removed from the same fish with ventral entry just posterior of the brachistegal rays by cutting through overlaying

tissue using a pair of pliers. Otoliths were removed with forceps and placed in a glass vial for storage. A total of 217 fish were processed ranging in size from 90 to 350 mm. Twenty-seven 10 mm length classes were established and contained 5 to 10 fish each.

Laboratory Analysis

Yellow perch ($n = 217$) used in the analysis were placed into one of 27 different 10 mm size groupings ranging in length from 90 to 350 mm. At least 5, but not more than 10 fish were placed in each size grouping. If more than 10 fish were collected within a particular size grouping, fish used in the analysis ($n = 10$) were selected randomly. Fish were also eliminated if otoliths or opercles were damaged or missing.

Four different structures were analyzed in the ageing study, three otolith configurations as well as whole opercles. Whole otoliths were read without further preparation after extraction as little extraneous tissue that could obscure the reading was observed. The second configuration, break and burn otoliths, were patterned after the techniques used by Robillard and Marsden (1996). Initially, the otolith was broken perpendicular to the sulcus through the core using thumbnail pressure and the posterior half was sanded down using 600 grit sandpaper for a flat reading surface. If the posterior half did not contain the core, the anterior portion was used. The sanded surface was then placed under a flame for several seconds to enhance the annuli. The otolith was then placed into a petri dish of clay for support, burnt side up, for aging. The third aging procedure required the otolith to be sectioned by a saw. However, because the otolith was too small to be held by the saw jig, it was embedded in epoxy to provide size and strength for cutting. After this mounting, otoliths were cut using a Buehler Isomet low speed saw into 50 micron sections through the core. Individual sections were then placed

between two slides for reading, with a minimum of two sections prepared for each otolith. Lastly, opercles were boiled for five minutes in water and brushed clean with a toothbrush to remove any remaining soft tissue that was attached to the bone structure.

Aging for all tissue types was done under a dissecting microscope using reflected light on a black background. An external light source was used for added illumination. Opercles, whole otoliths, and break and burn otoliths were additionally placed in Type B immersion oil. Sectioned otoliths were viewed dry.

The four methods were aged by three different readers. In addition, each reader aged each specific method three times. The structures were initially randomized for each reader and when a reader began aging a particular method (e.g., whole otoliths), they continued with the same technique until all fish of that type were aged. For successive readings, fish were again randomized. Reading took place over several days. At no time did the same reader age the same tissue more than once on any given day.

Reader experience aging these tissues varied. Reader one was very experienced in ageing opercles, and had never aged otoliths. Reader two had limited experience with ageing opercles and had also never aged otoliths. Reader three had no prior ageing experience.

Before ageing began, all readers were given a tutorial on ageing different methodologies of otoliths and opercles. Practice structures were given to the readers to use and criteria for determining annuli were established. Ageing criteria for otoliths followed Beamish (1979), where annuli are defined as the translucent (hyaline) zones between opaque zones. In break and burn otoliths, annuli were defined as black rings (Christensen 1964). Opercle annuli criteria followed LeCren (1947), where annuli are

determined as the transition between broad opaque summer growth and narrow translucent winter growth. Finally each reader recorded the time it took to age a specific method (e.g., sectioned otoliths).

After all age determinations had been made, two different statistical tests were performed on the data to discriminate differences in precision. First, a coefficient of variation (CV) was used to determine the reproducibility of ageing between readers. This statistic is calculated by dividing the standard deviation of the ages obtained by the three readers by the mean age. The lower the CV the more precise the structure age is said to be, with a high CV indicating less precision (Chang 1982). Unfortunately, this analysis does not discriminate among the calculated CV values using variance, and hence, only statistical suggestion can be made. However, we can compare the four methods using the calculated CV and a single factor ANOVA. In this second analysis, an individual CV was calculated for each structure (e.g., whole otoliths) using the three age determinations from reader 1. Similarly, a CV for the same tissue (whole otoliths) was additionally calculated for readers 2 and 3. With these three CV values for an individual tissue, a mean CV value along with a standard deviation could then be calculated. By applying this technique to all four methods (whole otoliths, break and burn otoliths, sectioned otoliths, and opercles), values were generated that would allow single factor ANOVA analysis with the objective to determine whether any of the four tissue CV values were significantly different from the other three. Lastly, a Tukey-Kramer multiple comparisons test was used to determine specific differences

In addition to precision measurements, mean viewing and processing times were calculated. Mean viewing time is the time it took one reader to age one structure, while

processing time is the time it took to remove, clean and prepare an individual structure. Processing time and mean viewing time were added together for a total processing time and were recorded in seconds.

Results

Age determinations were made on 217 fish using 4 different methodologies. Some additional fish were collected, but were not used as selected structures were not readable due to missing or broken sections. When an unreadable or missing structure was discovered, the entire fish was discarded to ensure equal sample sizes for the four methods. The 217 fish utilized for the project ranged in size from 90 to 350 mm total length, with a minimum of 5 fish in each 10mm length class.

Coefficient of variation (CV) was variable among methodologies and readers (Table 1). Reader one, the most experienced reader, had the lowest CV for opercles, break and burn, and sectioned otoliths. Reader 2 had the highest CV for whole otoliths, with the least experienced reader, Reader 3, having the lowest CV for whole otoliths.

When the average CV was computed for each structure and tested for significance using an ANOVA, the model showed significant differences existed in precision. The break and burn otoliths had the lowest CV, but were not significantly different from opercles or sectioned otoliths. Whole otoliths have the highest CV overall, and were the least precise of all structures (Table 2, $P < 0.001$).

A reader comparison was carried out to test for differences in skill level and abilities, using an ANOVA to compare reader CV values. We found that readers differed in their abilities when reading individual structures. For example, reader one was

significantly more precise when reading opercles. However, when all four structures were combined, no differences were found among readers.

Processing and reading times were variable in the study (Table 3). Whole otoliths were the quickest to prepare and age (99 s per structure) followed by opercles. Break and burn and sectioned otoliths had similar viewing and preparation times and took the longest to age. However, no statistical differences were found in reading time between the four structures ($F_{3,23} = 1.22$, $P = 0.329$).

Discussion

Yellow perch have been aged by fin spines, scales, opercles, and sagittal otoliths (LeCren 1947; Robillard and Marsden 1996; Baker and McComish 1998; Niewinski and Ferreri 1999). However, this study was the first to compare otoliths with opercles, the two structures that have been identified as the most precise structures when compared to scales (Robillard and Marsden 1996; Baker and McComish 1998), and fin spines (Niewinski and Ferreri 1999). Although precision is usually the most important consideration when ageing, trauma including mortality, time of processing, and potential to measure growth rates through back-calculation measurements cannot be ignored. Resource managers need to base their decisions on the efficiency of laboratory processing and ageing time or other such characteristics, since ultimately the method chosen needs to be realistic, inexpensive, quick and easy to evaluate and interpret (Belanger and Hogler 1982). This study compared three different otolith ageing configurations, including whole otoliths, break and burn otoliths (Christensen 1964) and sectioned otoliths (Robillard and Marsden 1996).

Structural analysis requires the visual assessment of growth rings, something that was difficult for the yellow perch whole otoliths. The relative large size and girth of the otolith precluded light transmission and made reading difficult, particularly when compared to sectioned and break and burn configurations. Therefore, it was understandable that ageing whole otoliths was more challenging and resulted in less precision by the readers.

Because the structure analysis indicated opercles, break and burn otoliths, and sectioned otoliths were not significantly different in precision, alternative reasons for choosing the tissue configuration could be used as (i.e. time). In this case, whole otoliths had the lowest time, but gave the most imprecise results, therefore we eliminated them as not acceptable to use for aging. All other structure times were not significantly different with regard to time.

Structure preparation times may reflect experience with the method. Opercles have been aged at Ball State University for three decades (pers. comm., T. Lauer) and processing time is well established. The high preparation times for break and burn, and sections were likely due to a lack of familiarity. This is the first time for both break and burn, and sections to be aged in this laboratory, and we suspect that lab processing time would decrease for these procedures with more experience.

Disadvantages exist with all ageing structures. Break and burn otoliths may introduce variability in reading as broken surfaces reflect different angles of light (Stransky et al. 2001). To minimize the variability, we rotated the otolith in different directions, if needed, before making an age determination. In addition, otoliths crowd annuli on the edge of the structure and the entire otolith does not grow proportionally

(Beamish and McFarlane 1987). Finally, in several studies opercles have been found to underestimate ages when compared to otoliths (Powers 1983, Beamish and McFarlane 1987, Robillard 2000).

Some structures have obvious advantages. Preparation of both break and burn otoliths and sectioned otoliths took longer than any other structure in this study (333 and 336 seconds respectively). Fortunately, the long preparation time provided a high degree of precision when compared to other methods in the walleye (*Sander vitreum*) (Belanger and Hogle 1982). The distinct annular rings that break and burn and sectioned otoliths have are reflected in the viewing times, as these two structures were the quickest to age (73 and 66 s respectively). Stansky et al. (2001) also felt that the sectioned otoliths had better readability than break and burn otoliths.

Validation of hard parts proves the accuracy of the technique used, and provides a more exact age make-up of the population. Validation is often neglected in ageing studies and could lead to incorrect management (Beamish and McFarlane 1983). Both opercles and otoliths of yellow perch have been validated. Robillard (2000) validated otoliths of yellow perch on Lake Michigan using natural marks on the structure. In two years, 1988 and 1993, annuli were uncharacteristically narrow due to low summer water temperatures. Using these natural markers Robillard (2000) proved that otoliths in Lake Michigan yellow perch form one opaque and one translucent zone per year. Opercles have been validated through tetracycline markers in walleye and marginal increment analysis in European perch (*Perca fluviatilis*), although these studies were not conducted in Lake Michigan (Craig 1974, Babaluk and Campbell 1987). The validation suggests that the ages obtained from the structures are accurate. Although, to obtain true accuracy,

a wide scale mark-recapture study would need to be implemented on Lake Michigan. A study of such magnitude would face obstacles of money and manpower. Also, tagging studies have been carried out on Lake Michigan with lake trout (*Salvelinus namaycush*) yielding a 2-14% recapture rate (Schmalz and Hansen 2002, 2004). One study conducted on yellow perch on southern Lake Michigan in the early 1980's recaptured less than 2% of the total fish marked (McComish and Meade 1981). With low recapture rates it would be difficult to obtain true accuracy of the structures.

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Table 1. Coefficient of variation for readers by method.

Method	Reader 1	Reader 2	Reader 3
Break and Burn Otoliths	8.7	14.2	11.1
Opercles	6.9	14.1	14.6
Sectioned Otoliths	11.2	13.9	11.9
Whole Otoliths	17.2	27.3	13.8

Table 2. Average coefficient of variation by structure. Tukey-Kramer multiple comparison test results are listed.

Method	CV	Tukey
Break and Burn Otoliths	11.3	a
Opercles	11.8	a
Sectioned Otoliths	12.4	a
Whole Otoliths	19.5	b

Table 3. Average time (s) for readers by structure. Preparation time is laboratory preparation.

Method	Preparation	Ageing	Total
Whole Otoliths	0	99	99
Opercles	90	89	179
Break and Burn Otoliths	260	73	333
Sectioned Otoliths	270	66	336