Silver nitrate staining improves visual analysis of daily otolith increments

Trika L. Gerard[1] (corresponding Author), and Estrella Malca[2]

[1]NOAA Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, FL 33149, USA, 305-361-4493, 305-365-4103 (Fax). Trika.Gerard@noaa.gov

[2]Cooperative Institute for Marine and Atmospheric Studies, University of Miami, 4600 Rickenbacker Cswy, Miami, FL 33149, USA, 305-361-4295, 305-361-4103 (Fax). Emalca@rsmas.miami.edu

Abstract: Sagittal otoliths in juvenile to sub-adult (62mm-150mm standard length) gray snapper (*Lutjanus griseus*) were analyzed using a modified staining method. Daily growth increments from transversely sectioned otoliths were stained using silver nitrate and fixed using sodium thiosulfate. Stained otoliths showed a noticeable improvement in the resolution of daily increments compared to those not stained. This procedure lends to the enhanced visualization of daily rings and has the potential to be a timely, yet efficient, technique for age and growth analysis of calcium carbonate structures.

[Trika L. Gerard, Estrella Malca. Silver nitrate staining improves visual analysis of daily otolith increments. Journal of American Science 2011;7(1):120-124]. http://www.americanscience.org.

Keywords: silver nitrate, staining, otolith, daily increment, von Kossa

1. Introduction

An essential component of fisheries science is the knowledge and understanding of age and growth of fishes. There are numerous ways to determine age and growth of fishes; however, to discern the age of wild fish, it is imperative to analyze calcified structures. Otoliths are one of these structures and they hold a wealth of information on daily age, size, growth and ontogeny of fishes. They are the most reliable indicators of fish age simply because otoliths are the only structures that consistently record daily events in the early life stages (Campana and Jones, 1992). Otolith microstructure analysis is the preferred means to determine age and growth of fishes because experimental evidence reveals that otolith material is neither re-absorbed nor reworked after deposition (Thorrold and Hare, 2002). Although otolith microstructure increments differ in shape and pattern depending on the fish family and species, one thing remains constant: otoliths serve as a permanent record for changes in environmental conditions and physiological changes experienced by the fish. The literature discussed here refers to reef fish, a category into which gray snapper fit.

Otoliths are calcareous accretions found within the semicircular canals of teleost fishes. They are organized in alternating layers dominated by hydrophobic high-molecular-weight proteins and inorganic calcium carbonates (Thorrold and Hare, 2002). Otoliths are used for balance and hearing of fishes and are useful tools for age determination through analysis of annual and daily increments (Campana, 2001). Daily increments occur as a result of the endogenous circadian rhythm occurring in fish (Campana and Jones 1992). The formation of daily increments takes place under most conditions, including times of food deprivation. In the absence of any somatic growth, deposition is maintained because the endolymph chemistry is buffered from the blood plasma (Thorrold and Hare, 2002). The production of visible daily increments is contingent upon a daily cycle of differing rates of accretion of a protein matrix and crystalline inclusions (Victor, 1991).

Determination of the age of fish has represented a challenge for otolith readers, and various methods have been used to improve visualization of annuli. Successful otolith age determination techniques require the appropriate otolith preparation method. The method of choice is largely dependent on the size of the otolith and the type of analysis to be performed. The most popular preparation method involves simply mounting and clearing small otoliths that are less than 50 µm in diameter (Secor et al, 1992). This method enables adequate resolution of daily increments on otolith microstructure. For larger otoliths, sectioning and polishing is required in order to remove material and expose the core and all increments.

The von Kossa staining method has been utilized in fish age studies for decades, including the use of whole vertebrae, neural arches (McFarlane et al 2002, Rossouw 1984), and in the age determination of not only teleosts but also elasmobranchs (Stevens 1975, Cailleiet et al 1983, Green et al., 2002). In this study, we describe a new method to improve the visualization of otolith increments, thereby making it easier to count during age estimation. This new method is silver nitrate ($AgNO_3$) staining of otoliths, derived from Von Kossa's staining method for calcium. The positive silver ions from $AgNO_3$ bind with the negative carbonate ions (CO_3^{-2}) in Calcium Carbonate ($CaCO_3$).

This bond darkens the increments, or "stains" them, and allows the increments to be viewed much more easily. Previous studies experimented with dyeing, burning and staining techniques (Richter and McDermott, 1990; Bouain and Siau, 1988).

2. Materials and Methods

Juvenile gray snapper (131mm SL) otoliths were embedded in resin and cut with an Isomet low speed saw, resulting in transverse sections approximately 1 mm thick. A 2.943mM of $AgNO_3$ and a 3.162mM of Sodium Thiosulfate ($Na_2S_2O_3$) solution were prepared and a series of Petri dish baths were assembled in the following order: distilled water, $AgNO_3$ solution, distilled water, $Na_2S_2O_3$ solution, and distilled water. Each transverse section was dipped in the distilled water bath, and then placed in the $AgNO_3$ solution for ten minutes. The otolith section was then introduced to the next distilled water bath. Subsequently, otolith sections were individually illuminated with ultraviolet light (15 W) for fifteen minutes. Otoliths were then dipped in $Na_2S_2O_3$ solution for two minutes to allow the silver stain to become affixed to the "stained" otoliths and to remove excess silver (Stevens, 1975). Finally, stained otoliths were dipped in the last distilled water bath before air drying on a flat surface. Stained otolith sections were glued to a microscope slide using thermoplastic glue and were polished using 800 and 1200 grit sandpaper until the otolith core was observed clearly with a compound microscope at 40x magnification. Daily increments were examined while focusing at magnifications of 40-200x.

In order to quantify the impact of staining otoliths, a sagittal otolith was processed, photographed and analyzed, and was subsequently stained and photographed again. Digital micrographs with transmitted light were taken using a compound microscope and Image Pro 4.5 with digital camera Evolution MP at 200x. Lighting was maintained at the same setting and no additional adjustments or enhancements were made to either image. Using Image Pro software, the images were scaled and line profiles were drawn from the edge towards the core while the line was perpendicular to the daily increments. The line profile feature measures the intensity of pixel values along the feature (Image Pro 4.5 user guide, 2002) and can be used to identify peaks or maximum values (darker) and troughs or minimum values (lighter) in otolith age determination. Adjacent minimum and maximum values in intensity across the otolith were used to identify peak and trough locations. The value for each peak and trough was extracted, and then subtracted from the transect mean. To investigate whether the silver nitrate treatment had enhanced the contrast across the treated otolith, and thus magnified the differences between adjacent peaks and troughs, the difference between each peak and the previous trough (or each trough and the previous peak) was calculated. The minimum, maximum and standard deviation of these values were then used to determine if silver nitrate staining enhanced extreme peak and trough values, and if differences between adjacent peaks and troughs increased, as measured by the standard deviation.

3. Results

Silver nitrate treatment appeared to improve the resolution of daily otolith increments (Figure 1). Overall, the line profiles in the stained otolith had higher values for peaks and lower values for the troughs when compared to the unstained otolith. The maximum difference in intensity between adjacent peaks and troughs was also greater in the stained otolith (Figure 2). Standard deviations of differences between adjacent peaks and troughs were higher for the stained otolith, suggesting that silver nitrate treatment had enhanced the contrast between daily growth increments.

4. Discussions

Currently, age determination using otoliths is the most common method for fishes, although the difficulty of counting daily increments varies greatly across different taxa. Although daily increments occur, the length of the increment formation can vary due to several environmental and physiological parameters, thus make daily increment interpretation difficult (Campana and Neilson 1985, Wooton, 1990). The use of silver nitrate to stain juvenile gray snapper otoliths successfully enhanced the visibility of daily increments and improved precision and accuracy when reading otolith increments. Stained daily increments darkened the translucent zones that appear raised under transmitted light while enhancing the contrast of the opaque zones that appear depressed. This allowed for the age of the fish to be more easily determined.

Silver nitrate staining may be used for otolith age determination of different age classes from larval (Green et al., 2002) to adult, and for fishes that inhabit tropical and subtropical environments where daily or annual increments may be difficult to distinguish. This method is inexpensive and has the potential to be implemented for stock assessment studies that monitor the age and growth of large quantities of commercially valuable species.

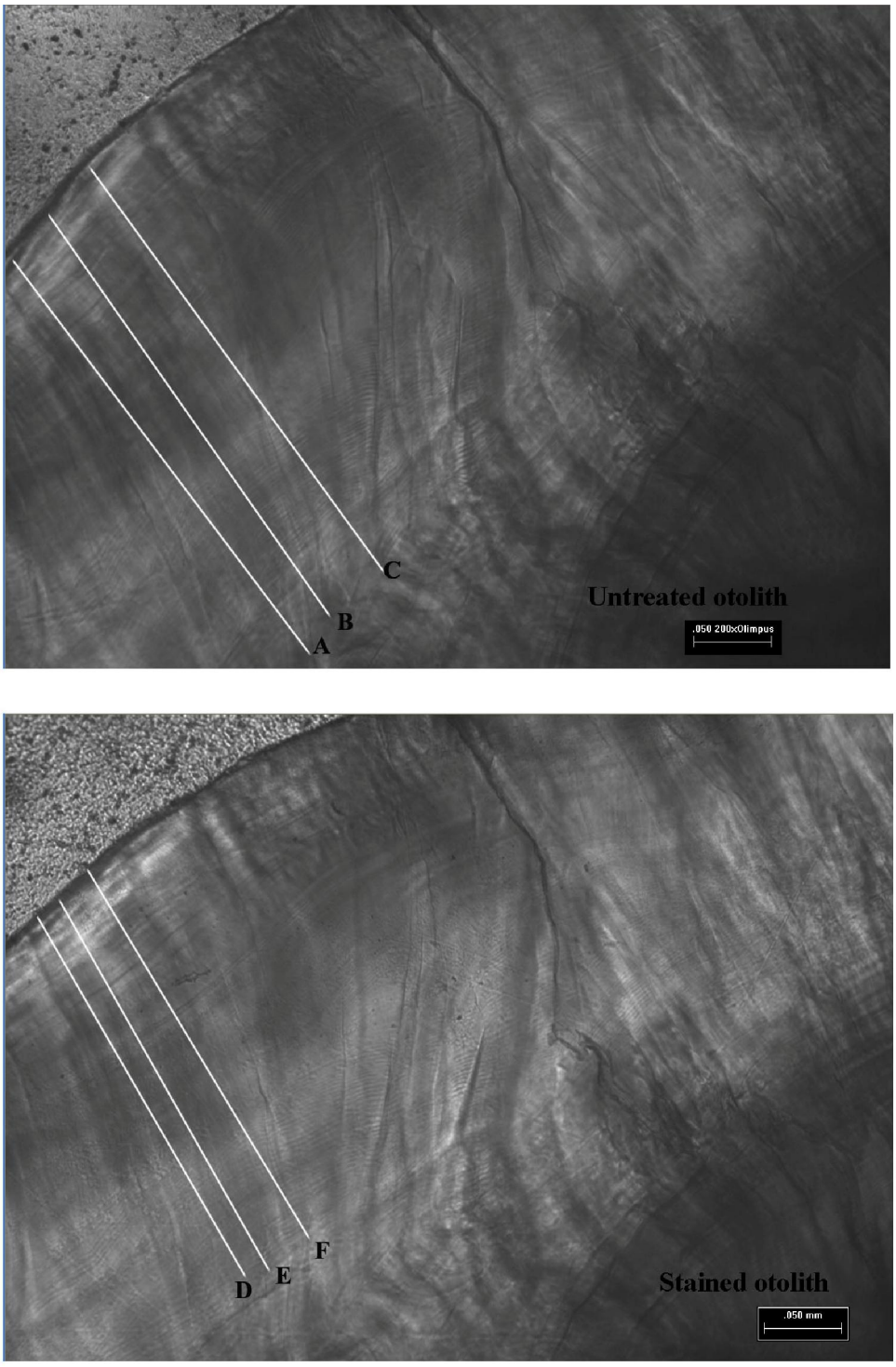
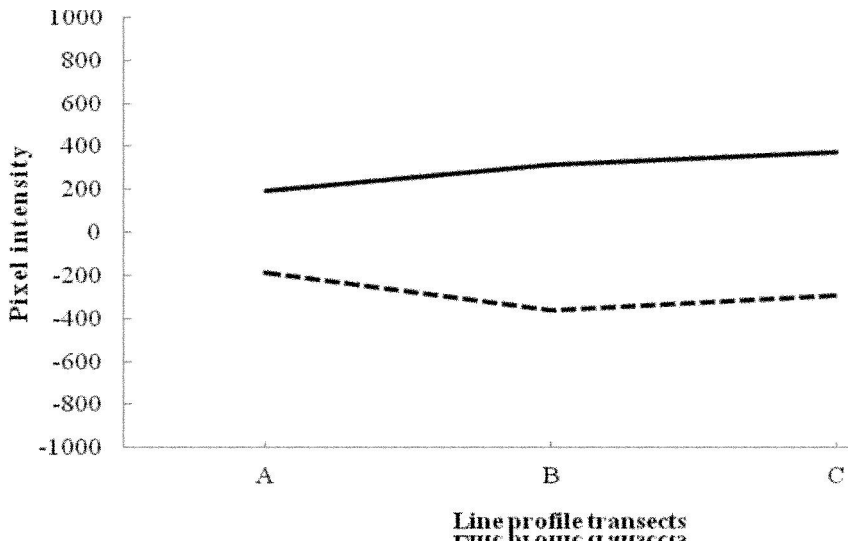


Figure 1: Digital micrographs of one transverse section of a Gray Snapper otolith with lines showing the line profiles used for comparing the intensities of daily increments of untreated (top) and stained with silver nitrate (bottom).

a)



b)

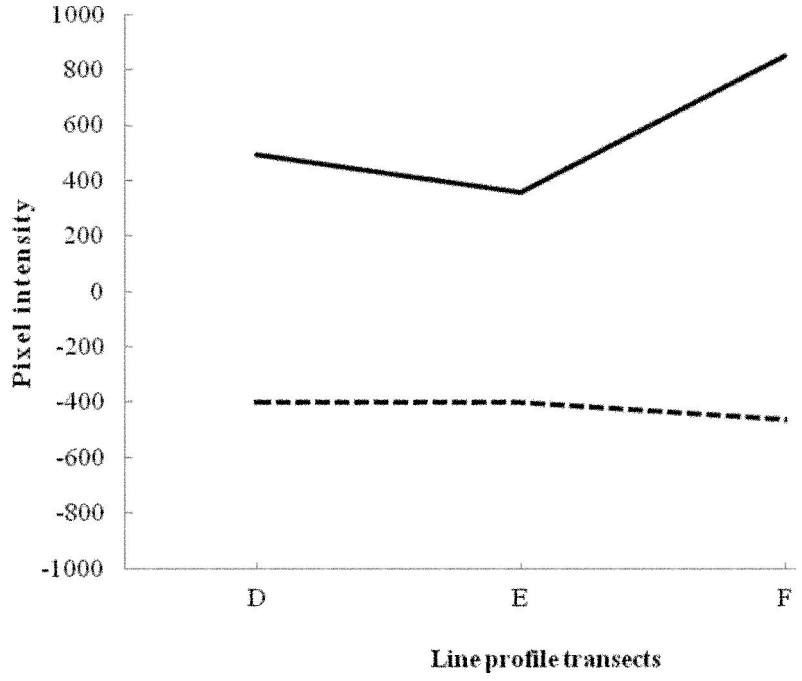


Figure 2. a) Line graph plotting the maximum values (solid line) and minimum values (dashed line) of pixel intensity of untreated line profile transects A, B & C. b) Line graph plotting the maximum values (solid line) and minimum values (dashed lien) of pixel intensity of silver nitrate stained transects D, E &F.

Acknowledgements

We thank the NOAA Southeast Fisheries Science Center, Early Life History Unit, for their assistance in sample collection and processing. Dr. B. Muhling and S. Privoznik provided constructive comments. This study was funded in part by the NOAA Educational Partnership Program and the South Florida Science Program.

Corresponding Author:
Dr. Trika Gerard
NOAA Southeast Fisheries Science Center
75 Virginia Beach Drive
Miami, FL 33149, USA
305-361-4493, 305-365-4103 (Fax)
Email: trika.gerard@noaa.gov

References

1. Bouain A, Siau Y. A new technique for staining fish otoliths for age determination. Journal of Fish Biology. 1988;32(6):977-978.
2. Cailliet GM, Martin LK, Kusher D, Wolf P, Welden BA. Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. NOAA Technical Report NMFS. 1983;8:157-165.
3. Campana SE. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology. 2001;59(2):197-242.
4. Campana SE Jones C. Analysis of otolith microstructure data. In: Stevenson DK, Campana SE, eds. Otolith microstructure examination and analysis. Canadian Special Publication of Fisheries and Aquatic Sciences. 1992;117:73-100.
5. Campana SE, Neilson JD. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences. 1985;42(5):1014-1032.
6. Green BS, Reilly SM, McCormick MI. A cost-effective method of preparing larval fish otoliths for reading using enzyme digestion and staining. Journal of Fish Biology. 2002;61(6):1600-1605.
7. McFarlane GA, King JR, Saunders MW. Preliminary study on the use of neural arches in the age determination of bluntnose sixgill sharks *Hexanchus griseus*. Fishery Bulletin. 2002;100:861-864.
8. Richter H, McDermott JG. The staining of fish otoliths for age determination. Journal of Fish Biology. 1990;36(5):773-779.
9. Rossouw GJ. Age and growth of the sand shark, *Rhinobatos annulatus*, in Algoa Bay, South Africa. Journal of Fish Biology. 1984;25(2):213–222.
10. Secor D, Dean JM, Laban E. Otolith removal and preparation for microstructural examination. In: Stevenson DK, Campana SE, eds. Otolith microstructure examination and analysis. Canadian Special Publication of Fisheries and Aquatic Sciences. 1992;117:19-57.
11. Stevens JD. Vertebral rings as a means of age determination in the blue shark (*Prionace glauca* L.). Journal of the Marine Biological Association of the United Kingdom. 1975;55(3):657-665.
12. Thorrold SR, Hare JA. Otolith applications in reef fish ecology. In: Sale PF, ed. Advances in the Ecology of Fishes on Coral Reefs. Academic Press, Inc. San Diego, California, USA. 2002:243-264.
13. Victor B. Settlement Strategies and Biogeography of Reef Fishes. In: Sale PF, ed. The Ecology of Fishes on Coral Reefs. Academic Press, Inc. San Diego, California, USA. 1991:231-260.
14. Wooton RJ. Ecology of Teleost Fishes. Chapman & Hall. New York, New York, USA. 1990.

12/2/2010