Attempts to Enhance the Visibility and Contrast of Presumed Growth Marks on Sagittal Otoliths from Wahoo, *Acanthocybium solandri*, from the Northern Gulf of Mexico and Bimini, Bahamas

JAMES S. FRANKS, JAIME L. SHEA,
NANCY J. BROWN-PETERSON, MELANIE S. GRIGGS,
and KIRSTEN M. LARSEN
The University of Southern Mississippi
Institute of Marine Sciences
Gulf Coast Research Laboratory
P.O. Box 7000
Ocean Springs, Mississippi 39566-7000 USA

ABSTRACT

In the western Atlantic Ocean wahoo, *Acanthocybium solandri*, occur from New Jersey to Columbia, including Bermuda, the Bahamas, the Caribbean Sea, and the Gulf of Mexico. Although wahoo support recreational and commercial fisheries throughout the Western Central Atlantic, adequate life history data, including estimated size-at-age, for stock assessments are lacking. Sagittal otoliths from wahoo (935 - 1,803 mm FL) caught in the recreational fishery from the northern Gulf of Mexico during May - September 1997 and 1998 and at Bimini, Bahamas in November 1997 and 1998 were examined to determine their potential use for age estimation. Whole sagittae and transverse thin-sections of sagittae viewed under transmitted and reflected light at 25 - 40x magnification generally revealed a series of ridges and faint opaque marks on the posterior portion of the distal surface of all whole sagittae and vague opaque marks on thin-sections from some sagittae. Attempts to enhance the visibility and contrast of the vague opaque marks (presumed growth zones) on thin-sectioned sagittae included application of histological stains (Toluidine Blue, Eosine Y, Neutral Red, and Analine Blue), etching with 5% EDTA followed by application of histological stains, etching and staining with mixtures of 1% acetic acid and histological stains, and digestion with proteinase K buffer. Etching and staining techniques did not delineate growth zones nor did they expose any "concealed" annual marks. Distinct opaque bands were observed only on sectioned sagittae from the two largest fish in the sample prior to the application of etching and staining agents, and a 2-h application of acidified Neutral Red provided the greatest visibility and contrast between presumed growth zones on those two sagittae.

KEY WORDS: *Acanthocybium solandri*, age, otoliths

INTRODUCTION

Wahoo, *Acanthocybium solandri*, are large, oceanic, pelagic fish of the family Scombridae and are distributed worldwide in tropical and subtropical waters (Collette and Nauen 1983). Wahoo seasonally extend into temperate waters. In the south Atlantic Ocean wahoo occur from New Jersey to Columbia, including Bermuda, the Bahamas, the Caribbean Sea and the Gulf of Mexico (Robins et al. 1986). Wahoo are considered a migratory species throughout much of the Western Central Atlantic (WCA) region (Hunte and Mahon 1985), although little is actually known about its movements and seasonal migratory patterns. Rivas (1951) reported that wahoo migrate seasonally through the Florida Straits and along the Gulf Stream and are particularly abundant along the north coast of Cuba during the winter. Wahoo are caught year-round in the northern Gulf of Mexico (Gulf), but apparently are most abundant there during spring through fall. Wahoo appear to be most abundant off Bimini, Bahamas during fall through winter.

Wahoo support important commercial (Luckhurst et al. 1997) and recreational fisheries in the Gulf of Mexico, south Atlantic Ocean, and Caribbean Sea (Franks et al. In press). Wahoo are a highly prized game fish at Bimini where prestigious wahoo fishing tournaments are held annually. Wahoo are not managed in the territorial waters of the United States or the Bahamas.

Despite their importance, little is known about the age and growth of wahoo from the WCA. Hogarth (1976) examined various life history aspects of wahoo off North Carolina and reported estimated ages for his specimens based upon examination of whole sagittal otoliths. Luckhurst et al. (1997) examined the microstructure of sagittal otoliths from wahoo collected off Bermuda and reported the presence of presumed daily growth increments. Franks et al. (In press) examined dorsal fin spines from wahoo captured in the northern Gulf and at Bimini, Bahamas to determine their potential as ageing structures.

Preliminary evaluation by us of transverse, thin-sectioned sagittal otoliths from adult wahoo revealed vague, opaque areas that potentially represented annular marks. Recent advances in otolith research have demonstrated that chemical treatment such as etching and digestion enhance the visibility of growth marks in difficult to interpret whole and sectioned otoliths (Richter and McDermott 1990, Shiao et al. 1999). Thus, in an effort to further examine the potential of wahoo sagittal otoliths as ageing structures, we conducted a small-scale experimental study to determine the effectiveness of specific etching, digestion, and staining techniques to enhance the visibility and contrast of vague, opaque marks (possible annual growth bands), as well as possibly expose "concealed or obscured" annual growth marks, on transverse thin-sections of wahoo sagittal otoliths.

MATERIALS AND METHODS

Sample collection

We sampled wahoo caught by recreational hook-and-line gear in the northcentral Gulf (June - September 1997, and May - June 1998) and, to a lesser extent, at Bimini, Bahamas (November 1997 and 1998). Wahoo from the northern Gulf were caught off the states of Louisiana, Mississippi, Alabama, and Florida in an area located north of lat. 29° and between long. 86°W and long. 89°W from waters that ranged 100 to 600 meters deep. Specimens from Bimini were caught in nearby Atlantic waters. We sampled specimens dockside and at fishing tournaments and recorded pertinent biological data for most specimens. Sagittal otoliths were extracted from specimens (57 males; 110 females), cleaned with water, air-dried, placed in vials with a collection number, and archived until processed.

Selection and Processing of Sagittal Otoliths

Left sagittae were selected from a sample of adult males (n = 20, 1,100 - 1,390 mm fork length) and females (n = 20, 1,000 - 1,803 mm fork length). Otoliths were embedded in epoxy resin and thin-sectioned transversely through the core at thicknesses ranging from 0.3 - 0.6 mm using a Buehler Isomet low-speed saw with a diamond blade. Sections were viewed under a dissecting microscope at 25 - 40x magnification using transmitted and reflected light to document any opaque marks.

Treatment of Otoliths

Based largely upon studies by Richter and McDermott (1990), Deree (1999), and Shiao et al. (1999), we selected two etching agents, one digestion agent, and four histological stains of various characteristics for our work. Our study consisted of five separate experiments with sectioned sagittae:

i) staining with a 1% solution of four histological stains only (Table 1);
ii) etching and staining with an acidified solution (1% acetic acid) of each of the four histological stains;
iii) etching with 5% ethylenediaminetetraacetate (EDTA);
iv) etching with 5% EDTA, followed by applications of each of the four stains, and
v) digestion by proteinase K buffer (PKb) (Table 2). Times of exposure to etching agents and stains varied somewhat, depending upon the particular experiment, and ranged from a few minutes to several days.

Proteinase K (buffered) was selected for its potential to degrade the fibrous proteins within thin-sections of sagittae. Components of the PKb mixture were diluted from stock solution (Table 2). Sagittae were digested with 0.2 ml PKb in 1.5 ml Eppendorf vials at 45°C with gentle shaking for periods of time which

ranged from 0.25 - 48 hours. Samples were periodically removed from the PKb to assess the progress of digestion. Freshly prepared PKb was used for each digestion.

Otolith sections were examined with both transmitted and reflected light following staining, etching, and digestion techniques. Appraisals and general conclusions on the appearance and general morphology of sectioned sagittae were based upon the observations of three readers.

RESULTS

Untreated Otoliths

Observations of whole sagittal otoliths from adult wahoo under a dissecting microscope at 24 - 40x magnification using both transmitted and reflected light generally revealed a series of "ridges" (or "steps") and other features on the posterior region (postrostrum) of the distal surface. The proximal surface, which contained the sulcus, displayed a smoother exterior. The rostrum and antirostrum displayed a series diminutive marks (some conspicuous, some vague) along the surface of their entire length. Transverse sections typically revealed intensely opaque dorsal and ventral lobes with faint, opaque marks. Some sections appeared exceedingly crystalline. With the exception of two otoliths, "distinctive" opaque bands were not observed on any sections.

Table 1. Histological stains and staining techniques.

Stain	Color	Specificity
Aniline Blue	Blue	Collagen, cartilage
Eosin Y	Red (Orange)	Cytoplasm
Neutral Red	Red	Embryonic tissues
Toluidine Blue	Blue	Metachromic nuclear stain

Methods applied to each of the stains tested:
1. Sectioning and staining in 1% stain
2. Sectioning and staining in 1% stain in 1% acetic acid
3. Sectioning, etching with EDTA, and staining in 1% stain

Source: Richter and McDermott (1990); Deree (1999)

Treated Otoliths

Histological stains only — Otoliths from 10 fish were used in this experiment. Application of the four stains to mounted and unmounted (not on slides) sections did not enhance vague opaque bands and did not reveal any obscured or concealed growth marks. Sections stained for < 0.5 hours appeared faintly stained, and those stained for 0.5 - 2.0 hours became darkened without any observable contrast in cross-section features.

Table 2. Composition of Proteinase K buffer.

	Stock solution concentration	Usage	Volume final Concentration
Proteinase K		10 mg	1 mg/ml
Tri-HCl (pH = 8.0)	1 M	0.1 ml	0.01 M
SDS (Sodium dodecyl sulphate)	20%	0.5 ml	1%
NaCl	5 M	0.02 ml	0.01 M

Total volume of PKb is brought to 10 ml using distilled water.
Source: Shiao et al. (1999)

Acidified histological stains — Otoliths from 20 fish were used in this experiment. Application of the four acidified (1% acetic acid) histological stains to mounted and unmounted sections produced poor results. Applications < 1 hour resulted in minimal etching and faint staining which did not enhance the opaque marks and showed no contrast in any section features. Applications >1 hour resulted in the progressive destruction of sections, characterized by darkened, ambiguous cross-section features.

EDTA — Otoliths from 10 fish were used in this experiment. Etching of sections with EDTA from 0.1 - 1 hours produced no discernable enhancement of cross-section features, and presumably "obscured" bands were not revealed. Etching for >1 hour essentially destroyed the sections.

EDTA and histological stains — Otoliths from 10 fish were used in this experiment. Etching with EDTA from 0.1 - 1 hours, followed by staining with the four histological stains from 1 - 3 hours did not enhance the contrast or visibility of features on mounted sections. Unmounted sections were not treated with EDTA and histological stains.

Proteinase K buffer — Otoliths from nine fish were used in this experiment. Digestion by PKb did not enhance any opaque marks on sections, nor did the treatment reveal any "obscured" annular bands. Digestion with PKb for 48 hours radically altered the surface morphology of sections and rendered them unreadable.

Enhancement of bands: the exceptions — The exceptions to all sagittae examined in our study were those from the two largest fish in our sample (females; 1,780 and 1,803 mm FL). Those otoliths provided thin-sections that

displayed narrow, moderately conspicuous opaque bands on their ventral lobe that were not observed on any other sagittae. These bands were distinctly different in their morphology and physical location on sections than were the faint, opaque markings on sections that we originally considered as possible age marks.

Although the bands on the two sagittae were discernable prior to any chemical treatments, etching and staining did moderately enhance their visibility and contrast against the background opaqueness of the ventral lobe. Among the treatments used with the two otoliths, application of acidified Neutral Red for a period of two hours produced the most obvious enhancement of opaque bands. Sectioned sagittae indicated the existence of 5 and 6 opaque bands for the 1,780 and 1,803 mm FL specimens, respectively. Since all sagittae were processed identically, we are puzzled by the appearance of distinctive opaque bands only on sagittae from the two large fish and by their absence on all other sagittae examined. We questioned: were the opaque marks representative of annular growth, and why were they not visible on thin-sections from all sagittae examined?

DISCUSSION

Etching and digestion agents and histological stains used in our study did not enhance the visibility of vague, opaque features observed in cross-sections of most wahoo sagittae, nor did the treatments reveal the existence of possibly obscured growth zones in any sections. Distinctive opaque bands on sagittal sagittae from the two largest wahoo in our sample were enhanced somewhat when treated with acidified Neutral Red, and it is presumed that the etching agent (acetic acid) reacted with surface $CaCo_3$, leaving the protein to react with the stain.

Shiao et al. (1999) reported positive results using PKb on cross-sectioned otoliths from two marine fish. They observed that enhancement of large otolith increments, including annuli, often required several hours exposure to PKb to ensure visual enhancement, at least for otoliths which are amenable to PKb treatment. Shiao et al. (1999) further noted that PKb may not be suitable for use with highly calcified otoliths with scanty amounts of proteins. Perhaps wahoo sagittae qualify for this category.

Richter and McDermott (1990) observed that different types of otoliths require different methods of etching and staining. The chemical agents and procedures used in our study were not effective for use with wahoo otoliths, even though essentially identical techniques greatly enhanced the contrast and visibility of vague daily marks and annular bands on otoliths from species examined by Richter and McDermott (1990), Deree (1999), and Shiao et al. (1999). Admittedly, those researchers did observe growth bands, albeit vague, on most otoliths prior to chemical treatments, and, in retrospect, this is probably a

prerequisite for the effective use of etching agents to enhance age-growth features. However, etching is common practice for revealing growth marks, some often totally obscured, on difficult to age otoliths (Haake et al. 1982, Secor et al. 1991).

Perhaps further experimentation with:

i) different concentrations of etching agents and stains used in our study;
ii) entirely different etching agents and stains; and
iii) different exposure times, would result in more favorable results.

Conversely, perhaps there are no etching, digestion or staining procedures available to enhance or reveal annular growth marks on cross-sectioned wahoo sagittae. This assumes that wahoo annular growth is expressed within sagittal otoliths in the form of alternating opaque and translucent bands.

Preliminary estimates of wahoo age based on evaluation of first dorsal fin spine cross-sections (Franks et al., In press) suggested a relatively high growth rate for wahoo, particularly for their first year of life, which is consistent with Hogarth's (1976) findings based upon assessments of whole sagittal otoliths.

The oldest two fish in our spine assessment study were presumed to be 5 and 6 years of age, Incidentally, those were the same two fish that revealed 5 and 6 bands on their sectioned sagittae in this study. Ultimately, detailed examination of whole otoliths (Luckhurst et al. 1997) may represent the best procedure for estimating the age of wahoo.

Although our research did not advance the knowledge of wahoo age and growth, our findings might be of some use to those concerned with age and growth of wahoo from the WCA. Continued efforts by researchers ultimately will result in accurate estimates of age and an understanding of growth rates, life span, and age structure of the catch. Knowledge of wahoo age and other critical life history aspects, as well as improved documentation of the recreational catch and commercial landings within the WCA, are crucial for wahoo stock assessments and the development of future management strategies throughout the region.

ACKNOWLEDGMENTS

We are indebted to numerous recreational fishers along the northcentral Gulf coast and at Bimini who allowed us to sample their catch of wahoo. We also express our deep appreciation to the directors and officials of several Gulf of Mexico sport fishing tournaments for the opportunity to sample wahoo. We specifically recognize Bobby Carter, Bill McClellan, Danny Pitalo, Laurie Osley, Bill Killduff, Jack and Deona Holmes, Jim Roberson, and Ron Cabassa for their tremendous help and support. We express our sincere gratitude to our friend Raul Miranda, tournament director for the Bimini Big Game Fishing Club, for his advocacy of our research and exceptional hospitality during our

research in Bimini. We are very grateful to Walter Grater, USM Institute of Marine Sciences (USM-IMS), for his valued advice and help with the laboratory experiments conducted during this study. For their extremely valuable assistance in collecting biological samples from wahoo, we offer our sincere thanks to T. J. Becker and USM-IMS colleagues Amber Garber, Niki Garber, David Geter, Chuck Blend, Don Barnes, Read Hendon, Jude LeDoux, Jan Welker, and Gerry Griggs. We acknowledge Captain Gary A. Caputi, publisher of *Big Game Fishing Journal*, C. M. "Rip" Cunningham and Deborah Hood of *Salt Water Sportsman* magazine, and Jim Brown of the International Game Fish Association for their encouragement and support of our research. This work was made possible by support from the Mississippi Gulf Coast Billfish Classic (The Bisbee World Billfish Series), USM-IMS, Damon Chouest and the Chouest family of Galliano, LA, particularly the Chouest Fishing Team, and Bob Brown with Red Barron Pizza, Atlanta, GA.

LITERATURE CITED

Collette, B. B. and C. E. Nauen. 1983. FAO species catalogue. Vol. 2. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos, and related species known to date. *FAO Fish. Synop.*125, 2:137.

Deree, H. L. 1999. Age and growth, dietary habits, and parasitism of the fourbeard rockling, *Enchelyopus cimbrius*, from the Gulf of Maine. *Fish. Bull.* 97:39-52.

Franks, J. S., N. J. Brown-Peterson, M.S. Griggs, N.M. Garber, J.R. Warren, and K. M. Larsen.. (In press). Potential of the first dorsal spine for estimating the age of wahoo, *Acanthocybium solandri*, from the northern Gulf of Mexico, with comments on specimens from Bimini, Bahamas. *Proc. Gulf and Carib. Fish. Inst.* Vol 52.

Haake, P. W., C. A. Wilson, and J. M. Dean. 1992. A technique for the examination of otoliths by SEM with application to larval fishes. Pages 12-15 in: C. F. Bryan, J. V. Conner, and F. M. Truesdale (eds), Proceedings of the Fifth Annual Larval Fish Conference. LSU Press, Baton Rouge, LA.

Hogarth, W. T. 1976. *Life history aspects of the wahoo, Acanthocybium solandri (Cuvier and Valenciennes) from the coast of North Carolina*. Ph.D. Dissertation, North Carolina State University, Raleigh.107 pp.

Hunte, W. and R. Mahon. 1985. A preliminary investigation of the migration of oceanic pelagic fish in the Western Central Atlantic. *Proc. of the fourth session of the working party on assessment of marine fishery resources.* Paipa, Columbia, Oct-Nov, 1984. FAO Fish. Rep. No. 327.

Luckhurst, B. E., J. M. Dean, M. Reichert, M. Cameron, S. Manuel, and T. Trott. 1997. Use of microstructure analysis of the sagittal otoliths for age estimation of the wahoo, *Acanthocybium solandri*, from Bermuda. *Proc. Gulf Carib. Fish. Inst.* **49**:64-70.

NMFS. 1996. Fisheries of the United States. Current Fishery Statistics, 1996. Natl. Mar. Fish. Serv. (NMFS), U. S. Dep. Commer., Washington, D.C.

Richter, H. and J. G. McDermott. 1990. The staining of fish otoliths for age determination. *J. Fish Biol.* **36**:773-779.

Rivas, L. R. 1951. A preliminary review of the western North Atlantic fishes of the family scombridae. *Bull. Mar. Sci.* **1**(3):209-230.

Robins, C. R. and G. C. Ray. 1986. *A field guide to Atlantic coast fishes: North America.* Houghton Mifflin Company, Boston, MA. 354 pp.

Secor, D. H., J. M. Dean, and E. H. Laban. 1991. Manual for otolith removal and preparation for microstructural examination. Publ. by the Electric Power Research Institute and the Belle W. Baruch Institute for Marine Biology and Coastal Research, p. 85.

Shiao, J. C., C. S. Tzeng, C. L. Leu, and F. C. Chen. 1999. Enhancing the contrast and visibility of daily growth increments in fish otoliths etched by proteinase K buffer. *J. Fish. Biol.* **54**:302-309.