Proceedings of the Atlantic States Marine Fisheries Commission

Bluefish Ageing Workshop

Norfolk, VA

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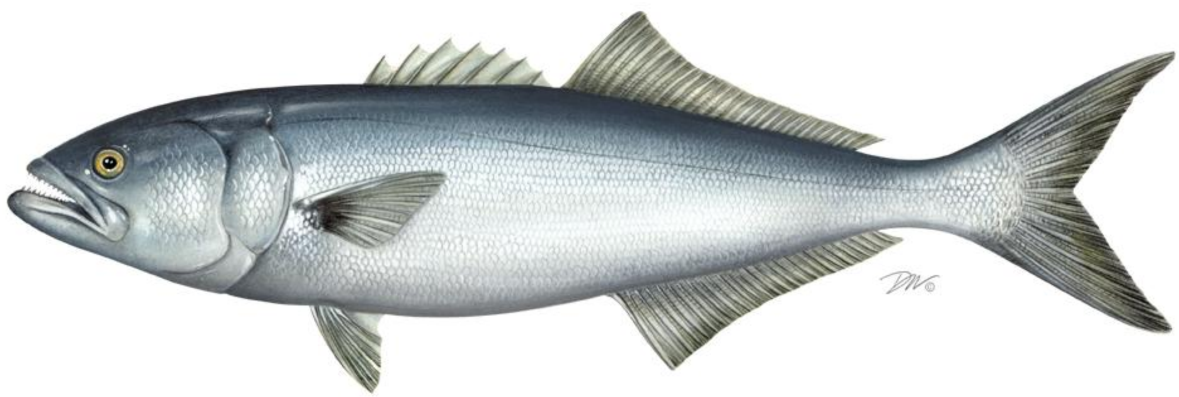

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Acknowledgements

The Atlantic States Marine Fisheries Commission thanks Old Dominion University's Center for Quantitative Fishery Ecology for welcoming workshop participants into their ageing lab for hands-on demonstrations and practice.

The Commission also thanks the individuals who contributed their time and expertise to this project, including Jessica Carroll (FL FWC), Paul Caruso (MA DMF), Joe Cimino (VMRC), Heather Corbett (NJ DFW), James Davies (ODU CQFE), Scott Elzey (MA DMF), Randy Gregory (NC DMF), Hongsheng Liao (ODU CQFE), Debra Parthree (VIMS), Jon Richardson (SC DNR), Nicole Travisono (RI DEM DFW), and Rich Wong (DE FW). In particular, the Commission appreciates the efforts of the former Bluefish Technical Committee chair, Paul Caruso, who initiated the discussion of bluefish ageing and sampling protocols and contributed greatly to organizing and running this workshop.

ASMFC also appreciates the efforts of Commission staff Katie Drew and Mike Waine in organizing the workshop and preparing this report.

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1 Introduction

Bluefish (*Pomatomus saltatrix*) is a highly migratory pelagic species found throughout the temperate coastal regions of the Atlantic and the western Pacific Oceans. It supports a major recreational fishery in the US, with most landings coming from the mid-Atlantic region. Harvest and total catch have declined from a peak in the early 1980s, although total catch (retained and released alive) has been recovering since the late 1990s. The most recent benchmark assessment was peer reviewed in 2005; while the panel agreed that the stock was not overfished, they noted that the results were uncertain and should be used with caution (NEFSC 2005).

A large part of the uncertainty in the assessment came from the age data used in the model (NEFSC 2005). The assessment used scale ages for the early part of the time series (1982 – 1997) and otolith ages for the later part (1998 – 2004). The panel was concerned about discrepancies between scale and otolith ages and the difficulties of ageing bluefish. The assessment was further hampered by gaps in the age-length keys resulting from a lack of samples for certain age and size classes (Figure 1); these gaps were filled by pooling samples across years, which increased uncertainty. Age samples were also geographically limited, coming only from Virginia and North Carolina. The panel recommended that ageing practices be standardized and sampling expanded to overcome these deficiencies in the assessment.

At the behest of the ASMFC Bluefish Board, the Bluefish Technical Committee organized an ageing workshop to establish consistency and a common protocol of best ageing practices across state and university labs that process and read bluefish hard parts. Workshop participants also agreed to discuss the design of a coastwide sampling program intended to expand the geographical range of bluefish age samples and fill the gaps in the age-length key.

The goals of workshop were:

- Share knowledge and methods of sampling, processing, and reading bluefish hard parts
- Develop consensus on best processing and reading practices for bluefish
- Develop recommendations for a coastwide sampling program for bluefish ages
- Prepare a workshop report

2 Current Methods and Sampling Programs

2.1 VMRC/ODU

The Virginia Marine Resources Commission (VMRC) obtains bluefish otoliths from the commercial catch and fishery independent sampling programs. These otoliths are processed and read by Old Dominion University's Center for Quantitative Fisheries Ecology (ODU CQFE; http://www.odu.edu/sci/cqfe/index.htm). ODU chooses a random subsample of otoliths collected in each length bin to age. In 2010, VMRC collected 715 bluefish otoliths and ODU aged 401 of them. This ageing sample size provides CVs of less than 5% and 4% for age composition estimates of Age 1 and 2, respectively. These two ages make up about 80% of the total catch.

ODU uses sectioned otoliths to age bluefish. The sagittal otolith is mounted on a microscope slide and a 0.4 mm section of the core is taken with a low-speed saw. The sections are then baked at 400°C for 2 – 4 minutes until caramel colored. The baked sections are mounted on microscope slides for storage and reading. To increase readability, Flo-Texx is used as mounting medium.

Sectioned otoliths are read under transmitted light. The characteristics described in Robillard *et al.* (2009) are used to identify the first ring and false annuli. Bluefish are assigned a January 1st birth date by convention. The sample date is used to assign the final age. If the sample was taken before the period of ring formation (March to May), the age is the annulus count plus one. If the sample was taken after that, the age is the annulus count.

Each year, readers revisit a reference collection of samples from 2000 to increase consistency across years. Each section is aged by two readers. If the first readings disagree, the readers re-age the fish together. If a consensus cannot be reached, the sample is excluded from further analysis and, if available, another sample from the same length bin replaces it. ODU uses the coefficient of variance (CV) to measure the precision of age assignments; in 2010, between reader ages had a CV of 2.1%. CVs for individual readers within and across years ranged from 1.3% to 4.1%.

2.2 NEAMAP/VIMS

The Northeast Area Monitoring and Assessment Program (NEAMAP) is a cooperative state-federal program that has operated a Near Shore Trawl Survey in the mid-Atlantic Bight and southern New England since fall 2007. The Virginia Institute of Marine Science (VIMS) has been awarded the contract to carry out the survey. It continues and extends the methods of the Chesapeake Bay Multispecies Monitoring and Assessment Program (ChesMMAP) which started in 2002. Bluefish is a "Priority A" species for NEAMAP, meaning that length, weight, sex, maturity state, stomach, and otoliths are collected for 5 individuals from each length bin on each tow. In 2010, NEAMAP measured 2,035 bluefish and collected 516 bluefish otoliths for ageing, the vast majority of which were collected during the fall survey. ChesMMAP has aged 422 bluefish from 2002 – 2009, the majority of which were age-0 and 1.

VIMS uses sectioned otoliths to age bluefish. Otoliths are sectioned using a method similar to ODU's. However, VIMS wet-sands the sections to a thinner width than ODU and does not bake the sections. Annulus counts are adjusted to reflect the timing of sample collection relative to ring formation. Age is assigned as the mode of three independent readings. VIMS has aged 588 bluefish from the fall 2007 survey, and retains a backlog of samples from subsequent cruises.

2.3 MA DMF

The Massachusetts Division of Marine Fisheries (MA DMF) has recently begun a sampling and ageing program for bluefish. Samples come from a combination of commercial and fishery independent sources.

MA DMF uses sectioned otoliths to age bluefish. Otoliths are processed using methods based on ODU's protocols. However, MA DMF bakes the whole otoliths before sectioning until they attain the characteristic caramel color. The baked otoliths are embedded using a silicone bullet mold in West Systems brand two part epoxy. The epoxy is allowed to harden over night before the embedded otoliths are sectioned.

Each section is aged independently by two readers, and re-read if the age assignments disagree. MA DMF uses the CV to measure between reader precision. Their precision is not as high as ODU, but that is to be expected as readers are still in the training phase of the process, and MA DMF anticipates their precision will increase over time.

2.4 RI DEM DFW

The Rhode Island Department of Environmental Management's Division of Fish and Wildlife (RI DEM DFW) collects and ages hard parts for a number of priority species, including striped bass, menhaden, and tautog. RI DEM DFW does not currently sample bluefish for ageing, but it is considering adding it to their list of priority species.

RI DEM DFW has found that collecting otoliths from commercial fisheries is more difficult and expensive than collecting scales, but has had some success with an angler-based program to get tautog opercula, another difficult to sample hard part.

2.5 NJ DFW

The New Jersey Division of Fish and Wildlife (NJ DFW) initiated a sampling program for bluefish in 2010 with the intent of filling gaps in the stock assessment age-length key. NJ DFW has collected otoliths from 219 bluefish from April to November of 2010, primarily from recreational fisheries, including party boats, tournaments, and individual donations of heads or carcasses. Fishery independent sampling programs for other species supplemented these samples with incidentally caught bluefish.

NJ DFW has archived the whole otoliths and as of this workshop has not begun to process or read them.

2.6 NC DMF

North Carolina Division of Marine Fisheries (NC DMF) has collected and aged bluefish scales from 1983 – 1998, and collected and aged otoliths from 1996 – 2000 and from 2006 to the present. From 1996 – 1998, NC DMF collected paired samples of scales and otoliths for a comparison of the two structures (NC DMF 2000). NC DMF did not collect any hard parts for bluefish from 2001 – 2005, when the Bluefish TC switched to a surplus production model for assessment purposes. The SAW/SARC review of that assessment (NEFSC 2004) found a lumped biomass model inappropriate for bluefish and recommended the use of an age-structured model instead. Thus, NC DMF began collecting otoliths for bluefish again in 2006.

From 2006 – 2010, NC DMF has collected a total of 2,806 bluefish otoliths from a combination of commercial, recreational, and fishery independent sampling. Despite training at ODU's lab, NC DMF could not replicate ODU's process to produce readable otolith sections and discontinued processing of annual samples in favor of archiving whole otoliths.

2.7 SEAMAP/SC DNR

The Southeast Area Monitoring and Assessment Program (SEAMAP) is cooperative state-federal program that has operated a fishery independent Shallow Water Trawl Survey in the nearshore waters from Cape Hatteras, NC to Cape Canaveral, FL since 1986. The survey is conducted by South Carolina Department of Natural Resources (SC DNR).

In 2011, SEAMAP added bluefish to the list of species that received a full work-up including the collection of stomachs for diet information and hard parts for ageing. SEAMAP has been collecting otoliths for bluefish. As with the NEAMAP samples, the majority of bluefish samples are small, young fish; this is not surprising in a trawl survey, as older bluefish can easily out-swim a trawl. Given the size range of samples and the time and cost of sectioning otoliths, SEAMAP has been considering using whole otoliths to age their bluefish samples.

2.8 FL FWC

The Florida Fish and Wildlife Commission (FL FWC) does not target bluefish for production ageing, but over a period of ten years has collected and aged otoliths from several hundred bluefish through a combination of commercial and fishery independent sampling.

FL FWC uses a processing method similar to ODU. Otoliths are mounted on tag paper with hot glue and sectioned with a low-speed saw. However, FL FWC uses a four blade saw to take three sections of an otolith at once to increase the probability of getting a clean section of the core.

2.9 NMFS-NEFSC

The NMFS-NEFSC representative, Nicole Calabrese, was unable to attend the workshop at the last minute. She sent a description of NMFS-NEFSC bluefish sampling efforts via e-mail.

The National Marine Fisheries Service Northeast Fisheries Science Center (NMFS-NEFSC) collects bluefish scales on the seasonal research trawl survey. Scale impressions are prepared by placing several scales, sculptured side up, on a heavy base slide of 1-mm thick (0.040 inch) cellulose acetate plastic. A laminated plastic slide, with the soft side down, is then placed over the scales. Another heavy plastic slide (0.65-1 mm thick) is placed on top of the laminated plastic slide, and the whole “sandwich” of slides is rolled through a jeweler's press.

Currently, the NEFSC does not age bluefish, and the scale samples are simply archived. Should the NEFSC begin ageing bluefish, they will follow the protocols set out by ODU.

Table 1 contains a summary of current age sample sources.

3 Best Ageing Practices for Bluefish

3.1 Scales vs. Otoliths

Bluefish have historically been aged with scales. The most recent benchmark assessment for bluefish (ASMFC 2005) used scales during the early part of times series (1982 – 1997) when otoliths were not routinely collected. Scales have fallen out of favor as an ageing structure for a number of species, and there are pros and cons to the use of both hard parts (summarized in Table 2).

The benefits of scales include the ease and non-destructive nature of their collection compared to otoliths, which increases the number of fisheries that can be easily and inexpensively sampled. Scales are also easier than otoliths to process.

Otoliths are easier to read and have a higher rate of agreement between readers than scales (NC DMF 2000, Sipe and Chittenden 2002, Robillard *et al.* 2009). Additionally, scales tended to underestimate ages when compared to otoliths, especially in older, larger fish. Bluefish otoliths may be read whole or sectioned; like scales, however, whole otoliths tend to assign a lower age than sectioned otoliths in older, larger fish. The annual periodicity of otolith ring formation has been validated using relative marginal increment analysis by Robillard *et al.* (2009).

The consensus of the group was that sectioned otoliths (Figure 2) are the best structure with which to age bluefish.

In order to reduce processing time, the group also recommended that a length cut-off be used to determine age-0 fish. Fish smaller than 20 cm fork length would not be aged but instead assigned age-0; fish larger than the length cut off would be aged. The exact value of the length cut-off should be reviewed periodically and adjusted if changes in length-at-age are noted. The most recent bluefish stock assessment used a fork-length threshold of 25 cm to identify young-of-the-year fish in fishery independent surveys.

3.2 Otolith Processing Protocols

The group agreed that ODU's processing methods should be the default practice. For bluefish, ODU mounts sagittal otoliths on microscope slides with Crystalbond and takes 0.4 mm sections of the core using a low-speed saw (Figure 3). These sections are then baked at 400^{o}C for 2 – 4 minutes until they are caramel-colored. The baked sections are then mounted on a microscope slide with Flo-Texx for reading and storage.

FL FWC, MA DMF and VIMS use sectioning protocols similar to ODU's. FL FWC uses a multi-blade saw to take three sections at once. MA-DMF bakes the otoliths prior to sectioning. VIMS takes slightly thinner sections and does not bake them at all. At the workshop, examples of sectioned otoliths from all four labs were compared, and all methods were found to produce readable samples (Figure 4).

3.3 Otolith Reading Practices

3.3.1 Identifying annuli

Otolith sections are read with transmitted light using a dissecting microscope, without knowledge of the fish's length.

The first annulus is often more diffuse and smudgy than subsequent annuli (Figure 5). Robillard *et al.* (2009) identified crenulations on the dorsal edge of the section that were commonly associated with the first annulus and could be used to help identify it.

Double rings are sometimes seen in older fish, characterized by two distinct opaque zone in close proximity that join to form a single origin, usually at the succal groove or the outer edge of the otolith. If the two opaque zones do not join and remain distinct, they are counted as two annuli. If they do join together, they are counted as a single annulus.

3.3.2 Assigning an age

Bluefish spawn from April through August, and lay down annuli in their otoliths from March to May, but by convention, they are assigned a birthdate of January 1 (Figure 6). To assign a final age, the relative timing of these events must be considered.

If no translucent material has been laid down after the last opaque zone (i.e., the last annulus forms the edge of otolith section), the final age is the annulus count. If growth has occurred since the formation of the last annulus, information on the date of sample collection is used to assign a final age to an individual. If the sample was collected between January 1 and February 28, the final age is assigned as the annulus count plus one. If the sample was collected between June 1 and December 31, the final age is the annulus count.

If the sample is collected during the period of ring formation (March – May), the reader must use professional judgment to determine whether ring formation for the year has occurred or not yet, based on the width of the translucent zone laid down after the last opaque zone and when the fish was caught during the ring formation period.

3.3.3 Measuring precision and repeatability

High precision does not guarantee that age assignments are accurate or unbiased. However, it provides important information on the reliability of age data and should always be reported with the age data. The coefficient of variance (CV) is the preferred statistic (Campana 2001). It is defined as:

$$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(X_{ij}-\overline{X_j}\right)^2}{R-1}}}{\overline{X_j}} \qquad (1)$$

where CV_j is the CV of the j^{th} fish, X_{ij} is the age assigned to fish j by reader i, R is the total number of readings of fish j, and $\overline{X}_j$ is the mean age assigned to fish j. The CV should be calculated after the first round of independent readings and before a consensus is reached on disagreements. The mean CV can be used to assess precision and repeatability of age assignments both between readers and within a single reader across repeated readings.

The higher the CV, the lower the precision, and labs should strive to minimize their CV. It is difficult to assign a benchmark for acceptable precision, since it depends on the species and the structure in question, as well as the experience of the readers. A CV of 5% is a commonly used target (Campana 2001), and ODU has achieved CVs lower than this, indicating it is reasonable for bluefish.

4 Coastwide Sampling Program Design

The most recent stock assessment of bluefish used age data from two states: North Carolina for the early part of the time series (1982 – 1997) and Virginia for the later part of the time series (1998 – 2004) (NEFSC 2005). Virginia accounted for approximately 4% of the total coastwide harvest of bluefish from 1998 – 2008 and yet supplied all of the age data for those years in the assessment.

Additionally, the age-length keys used in the assessment had gaps due to a lack of samples in certain size classes. Fishery dependent length sampling of bluefish shows a bimodal pattern, with few samples in the 50-60cm size range, and the age samples used to develop age-length keys do not adequately cover the entire size range of the fisheries (Figure 1). These gaps had to be filled by pooling data across years.

The 2005 peer review of the stock assessment highlighted both of these issues as sources of uncertainty. In 2010, the Bluefish TC recommended that a coastwide sampling program be developed to expand the geographical range of sampling and to fill in gaps in the age-length key. The TC identified the states that had accounted for more than 5% of the total bluefish harvest (commercial and recreational) from 1998 – 2008 (Massachusetts, Rhode Island, Connecticut, New York, New Jersey, and North Carolina) and recommended that they be responsible for providing a number of samples based on their contribution to the total landings.

Workshop participants revisited this issue, and recommended that a pilot program be developed to determine the optimum sample size for a coastwide age-length key and test the feasibility of state-level sampling combined with regional level ageing. Sampling allocation was reduced and simplified so that each of the key states plus Virginia would be responsible for providing 100 bluefish ages per year (50 from the spring and 50 from the fall). The importance of sampling from as wide a range of sizes as possible was stressed. States without the capabilities to effectively age bluefish could cooperate with ageing labs in other states to process and age the samples they collect.

The methods described in Quinn and Deriso (1999) will be used to determine the appropriate sample size for a two-stage proportional sampling design for a coastal age-length key bluefish. To estimate the age composition of a given age, $\widehat{\theta_a}$, with a specified degree of precision, CV_a, a total of A age samples are needed, where A is defined as:

$$A = \frac{V_a}{\widehat{\theta}_a^2 CV_a^2 - \frac{B_a}{L}} \quad (2)$$

L is the number of length samples collected, and V_a and B_a represent the variance within and between each length bin, l, respectively:

$$V_a = \sum_l \frac{L_l}{L} \cdot \frac{A_{la}}{A_l} \cdot \left(1 - \frac{A_{la}}{A_l}\right) \quad (3)$$

$$B_a = \sum_l \frac{L_l}{L} \cdot \left(\frac{A_{la}}{A_l} - \widehat{\theta_a}\right) \tag{4}$$

A_{la} is the number of age a fish in length bin l, and A_l is the total number of fish in length bin l.

ODU has performed this analysis on its own sample collection, using the most recent 5 years of sampling as a pilot study. They found that 280 samples provided a CV of 5% for age-2 fish, the most common age in their samples, and increasing the sample size by 100 reduced the CV for age-2 by less than 1%.

Not all states have resources to devote to production ageing of bluefish, while the marginal cost to other states, though not negligible, is small. This pilot study would also allow the states to determine the cost and feasibility of sharing ageing responsibilities, as well as explore options for funding mechanisms.

5 Conclusions and Recommendations

Workshop participants were satisfied with the level of consistency in ageing protocols across the states.

1. **Sectioned otoliths are the preferred structure with which to age bluefish.**

   Although the ease of collection and preparation of scales offers several advantages over sectioned otoliths, otoliths are more readable and do not under-age fish at older ages.

2. **The processing and reading methods of ODU and Robillard *et al.* (2009), as described in detail in Appendix 2 to this report, should be accepted as the default methods for ageing bluefish.**

   Variations in processing methods are acceptable as long as they produce consistently readable sections.

3. **A digital reference collection should be assembled and an ageing exchange should be organized.**

   Each lab that currently ages bluefish should contribute digital images of their otolith sections to a coastwide reference collection. These images should include samples from the full range of sizes, ages, and section quality/readability of samples, and submissions should include both annotated and unmarked copies of each image. Annotations should indicate each mark that is counted as a true annulus as well as any unusual features such as edge type or false annuli.

The annotated images will be compiled into a reference collection for training purposes, and the unmarked images will be used for a digital ageing exchange. The unmarked images will be circulated among labs to be aged, and consistency across labs will be assessed. Should precision be low in the digital exchange, an exchange of actual hard parts will be arranged.

4. A pilot coastwide sampling program for bluefish should be undertaken in 2012.

The states that account for more than 5% of total coastwide bluefish harvest (recreational and commercial combined) for the 1998 – 2008 period should commit to providing a minimum of 100 bluefish ages (50 from January through June, 50 from July through December) for the 2012 year. These states are: Massachusetts, Rhode Island, Connecticut, New York, New Jersey, and North Carolina. Virginia should continue its current sampling regime for bluefish and provide that same minimum 100 samples as the other states.

Every effort should be made to cover the full range of bluefish sizes with these samples. States that cannot age bluefish may send their whole otolith samples to another state with ageing capacity during this pilot study.

The results of this pilot study should be used to determine the optimum sample size and geographic range for a long-term coastwide sampling program for bluefish. These design recommendations will be sent to the Bluefish TC and SASC for approval before being submitted to the Bluefish Management Board for action.

Bluefish support a major, coastwide recreational fishery and are apex predators in the nearshore ocean waters. This species demands a rigorous assessment with the best possible data. Consistent, high quality age data are a critical component of this assessment, and this workshop is a valuable contribution to that data stream. However, more extensive sampling and long-term monitoring are also required, and this is only the first step towards that goal.

6 Literature Cited

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7 Tables and Figures

Table 1: Summary of current age sample sources.

State/Survey	Recreational	Commercial	Fishery-Independent
VA		X	X
NEAMAP			X
MA		X	X
RI	No bluefish sampling at present		
NJ	X		X
NC	X	X	X
SEAMAP			X
FL		X	X
NEFSC	No bluefish sampling at present		

Table 2: Pros and cons of scales and otoliths for ageing bluefish.

	Scales	**Otoliths**
Pros	• More cost effective • Easier to collect • Non-destructive • More gears sampled • Impressions last indefinitely • Scales are durable • Simple preparation • Processing time is much shorter • Individual sample size can be greater than 2	• More readable • Can be used over entire age range • Higher agreement between and within readers • Durable • Whole otoliths are faster to read than scales for young fish but may introduce bias (i.e., you know what the upper age cut-off is for reading a whole otolith)
Cons	• Precise up to age 3 or 4; harder to read at older ages • Underages fish at older ages • Less readable than otoliths over all • Low agreement between & within readers • Reading time is higher • Possibility of regeneration, but not a common problem; extra scale samples from same fish can account for this	• Storage slightly more difficult; must be clean and dry • More difficult to extract from fish • More expensive to collect and process • More time-consuming to collect and process • Restricts gear types • Destructive sampling

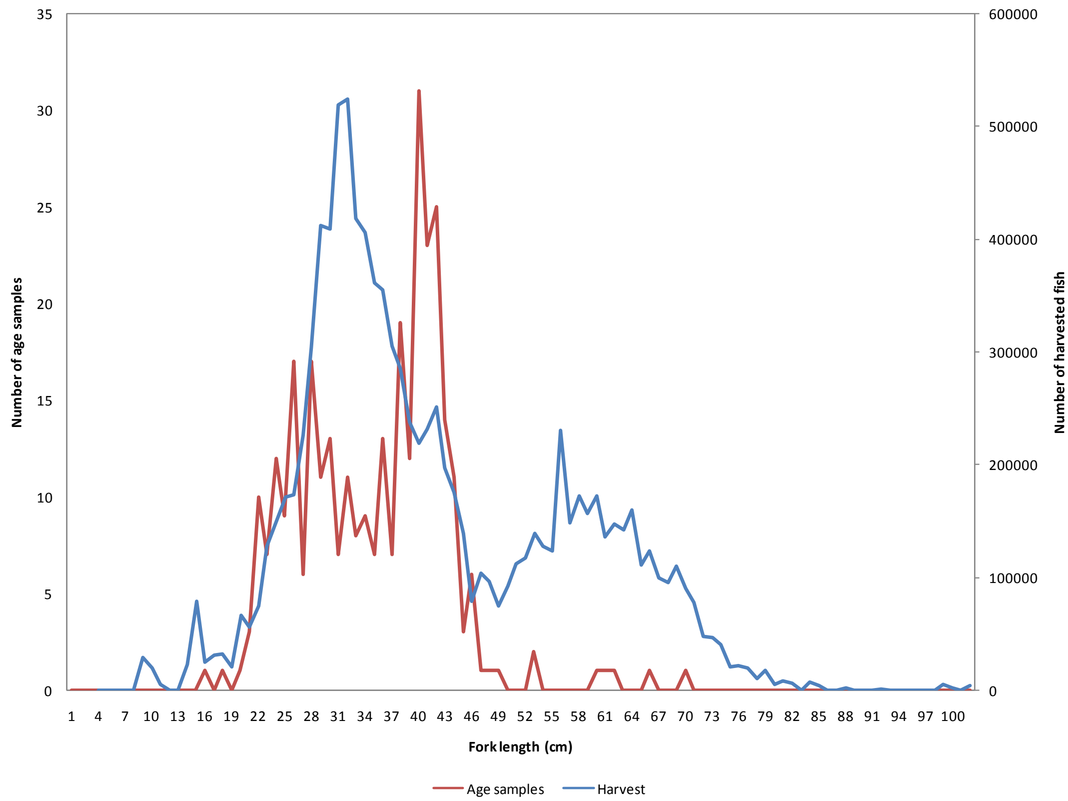

Figure 1: Length frequencies of bluefish harvest and age samples for 2004.

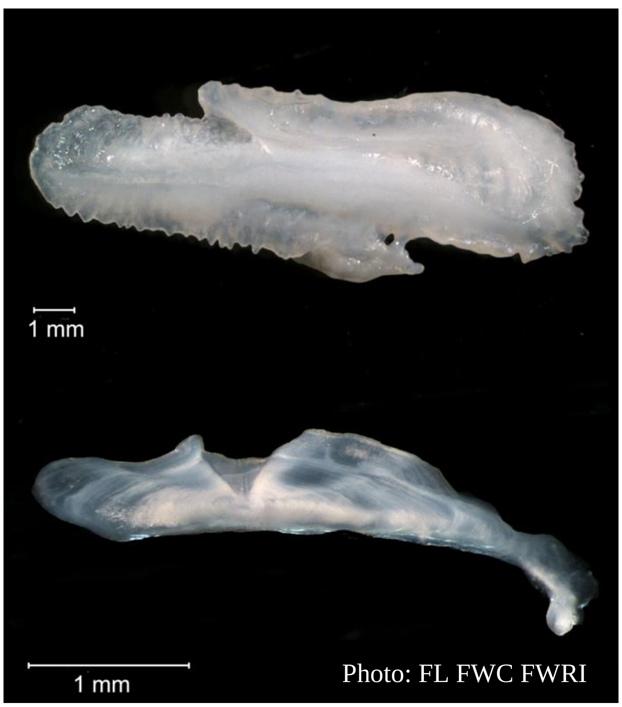

Figure 2: A whole bluefish otolith (top) and transverse cross-section (bottom).

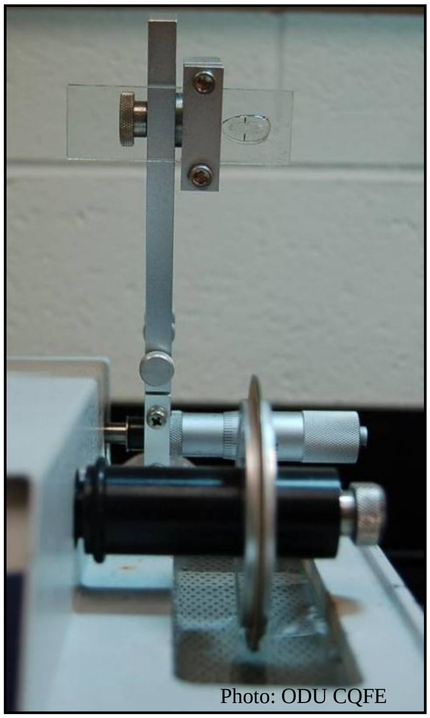

Figure 3: Low speed saw with otolith mounted on microscope slide for sectioning.

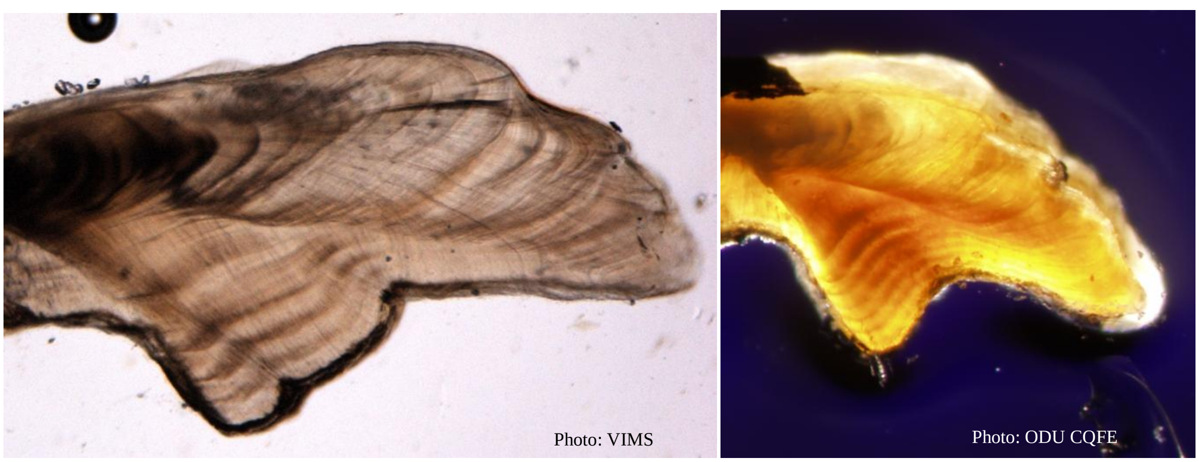

Figure 4: Unbaked (left) and baked (right) otolith sections both revealed readable annuli.

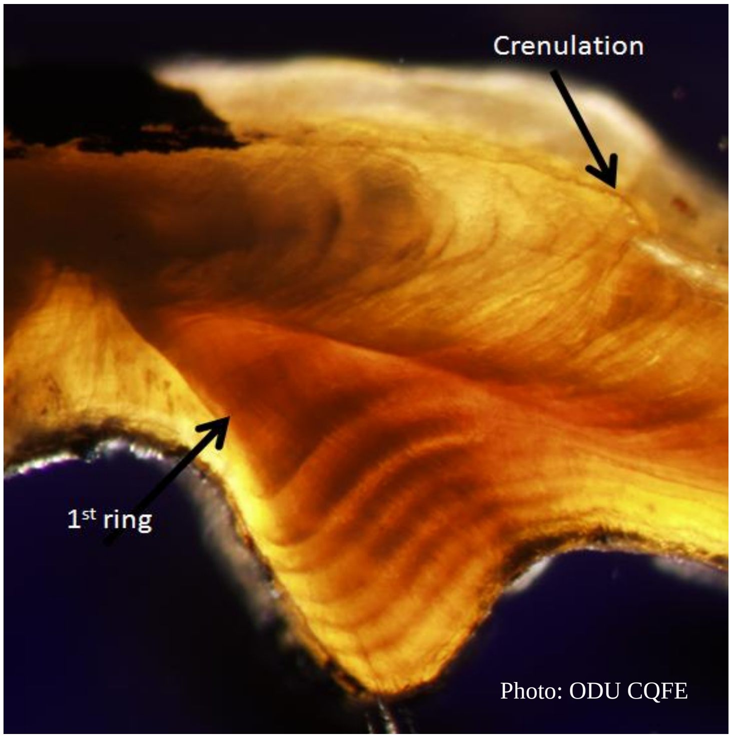

Figure 5: Sectioned otolith from an 8-year old bluefish with the first annulus and its associated crenulation indicated.

Year	Year One											
Assigned birth date	1-Jan											
Biological birth date						1-Jun						
Spawning period						Spawning Period						
Annulus formation				Annulus Formation								
Month	J	F	M	A	M	J	J	A	S	O	N	D
Number of rings				0	0	0	0	0	0	0	0	0
Biological age				0	0	0	0	0	0	0	0	0
Year grouping				0	0	0	0	0	0	0	0	0

Year	Year Two											
Assigned birth date	1-Jan											
Biological birth date						1-Jun						
Spawning period						Spawning Period						
Annulus formation				Annulus Formation								
Month	J	F	M	A	M	J	J	A	S	O	N	D
Number of rings	0	0	0	1	1	1	1	1	1	1	1	1
Biological age	0	0	0	0	0	1	1	1	1	1	1	1
Year grouping	1	1	1	1	1	1	1	1	1	1	1	1

Year	Year 3											
Assigned birth date	1-Jan											
Biological birth date						1-Jun						
Spawning period						Spawning Period						
Annulus formation				Annulus Formation								
Month	J	F	M	A	M	J	J	A	S	O	N	D
Number of rings	1	1	1	2	2	2	2	2	2	2	2	2
Biological age	1	1	1	1	1	2	2	2	2	2	2	2
Year grouping	2	2	2	2	2	2	2	2	2	2	2	2

Figure 6: Relative timing of assigned birth date, spawning, and annulus formation for bluefish.

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Appendix 2: Protocols for sectioning and reading bluefish otoliths

1.1 Sectioning Otoliths

1.1.1 Standard Protocol

The following protocol is based on the one developed by Old Dominion University's Center for Quantitative Fisheries Ecology (ODU CQFE). Other labs have successfully sectioned bluefish otoliths with slightly different methods; these alternatives are listed at the end of this section.

Step 1: Create a sectioning slide.

Heat a quarter of a stick of Crystalbond™ 509 in a heat-resistant dish, such as a Stender dish, over a hot plate until it melts slightly and becomes easy to manipulate.

Place a microscopy slide (1in x 3in x 1.2mm) on the hot plate. Use an applicator stick to evenly spread a thin layer of semi-liquid Crystalbond onto the slide, then place a second slide on top. Press the two slides together with the applicator stick to distribute the Crystalbond evenly between the two slides.

Remove the pair of slides from the hot plate and let them cool. This double-thick slide is your sectioning slide.

Step 2: Mount the whole otolith on a sectioning slide.

When the sectioning slide is completely cooled, use the applicator stick to place a dab of semi-liquid Crystalbond in the center of the right hand side of the sectioning slide. The amount of Crystalbond used should be approximately equal to the volume of the otolith.

Place the otolith, distal side down, on this base of Crystalbond. The dorsal and ventral sides of the otolith should be parallel to the long side of the slide and perpendicular to the short side. Apply a layer of Crystalbond to the tips of the cauda and ostia of the otolith to secure the otolith in place.

Allow the Crystalbond to cool and harden completely before proceeding.

Step 3: Mark the core of the otolith to guide the cutting path of the saw.

The nucleus of the otolith should be included in the section. The core is located at the intersection of the ostium and the caudal section of the otolith. With a pencil, draw a straight line over the core, perpendicular to the long edge of the slide (Figure 1). The saw blades should cut along either side of this guide line when sectioning.

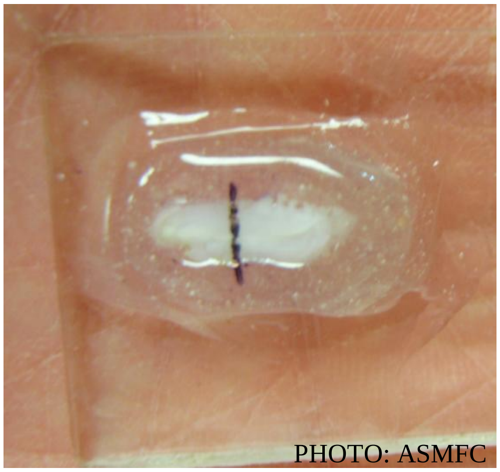

Figure 1: Bluefish otolith mounted on sectioning slide. Black line indicates the core to guide sectioning.

Step 4: Section the otolith.

ODU CQFE uses a Buehler® IsoMet™ low speed saw with two Norton® Grinding Wheels separated by a 0.4mm spacer to section bluefish otoliths. Water is used in the saw lubricating pan.

Secure the sectioning slide with the mounted otolith in the chuck of the saw's support arm (Figure 2). Lower the section onto the blades to check the alignment of the otolith. The guide line should fall between the blades and be parallel to them. Use the micrometer to adjust the position of the mounted otolith relative to the blades.

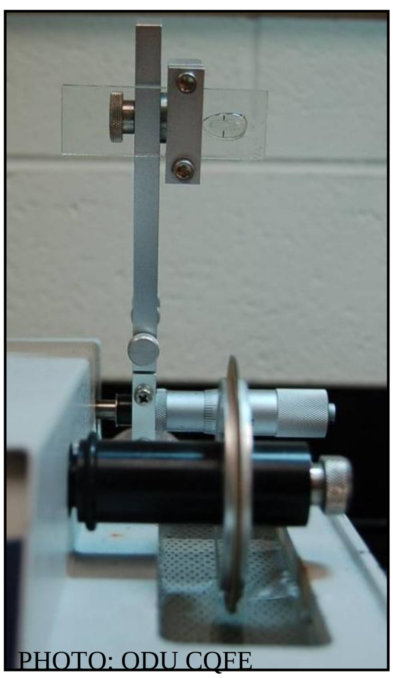

Figure 2: Mounted otolith secured to saw arm for sectioning.

When the slide is secured and the otolith properly aligned, return the support arm to the upright position, so that it is not touching the blades. Start the saw at low speed (3 or 4 on the IsoMet). Once the blades are spinning, gently lower the arm and bring the otolith into contact with the blades (Figure 3). After a few seconds, once the blades have cut a groove into the otolith, you can increase the saw speed somewhat (7 or 8 on the IsoMet is acceptable for bluefish otoliths, although they are fragile).

Increasing the saw speed and/or adding weight to the support arm will decrease the cutting time, but increase the risk of damage to the otolith or the blades.

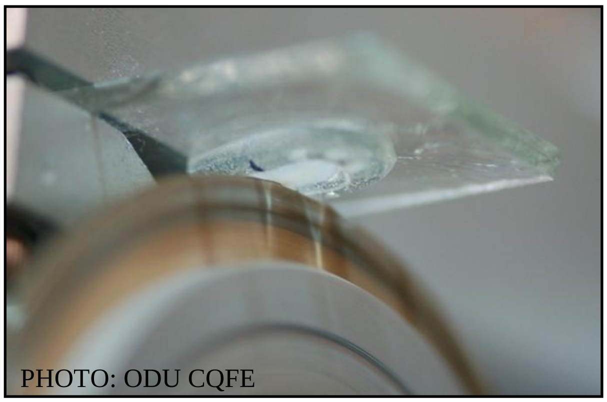

Figure 3: An otolith being cut by a low-speed saw.

It should take 1 to 2 minutes to complete the cut. Once you can see that the blades have gone completely through the otolith and are visible through the glass of the sectioning slide, gently lift the support arm so the otolith is no longer in contact with the blades and turn the saw off.

The cut section should remain between the two halves of the otolith; remove it carefully with tweezers. However, the section can sometimes become stuck between the blades of the saw or be knocked loose into the lubricant pan. If the section is missing from the sectioning slide, check those locations. You may have to remove the blades from the saw arm to retrieve the section.

Step 5: Bake the otolith.

ODU CQFE uses a Barnstead/Thermolyne 1400 Small Benchtop Muffle Furnace to bake their otoliths.

Heat the oven to 400^{o}C.

Place each section to be baked into a well on a ceramic spot plate. Remember to log which sample goes in which well.

Place the plate inside the oven and bake for 2 minutes. Remove the plate from the oven and examine the sections. They should be a caramel color. If there has not been enough color change, return the sections to the oven for another 30 seconds. Continue to bake in 30 second intervals until the caramel color is achieved. There should not be any charring or large black spots on the sections; carefully scrape away any charring with tweezers.

Step 6: Mount the sections for reading and storage.

Check the quality of the section under a microscope before mounting. A correctly cut section should have no chips or imperfections that obscure the view of the core, sulcal groove, or annuli. The sulcal groove should meet the core to form a triangle so that all the annuli can be seen from the core to the edge of the otolith (Figure 4). If the sulcal groove does not form a point, the cut was made too close to the ostium. If it forms a twisted, tornado-like point, the cut was made too far from the ostium.

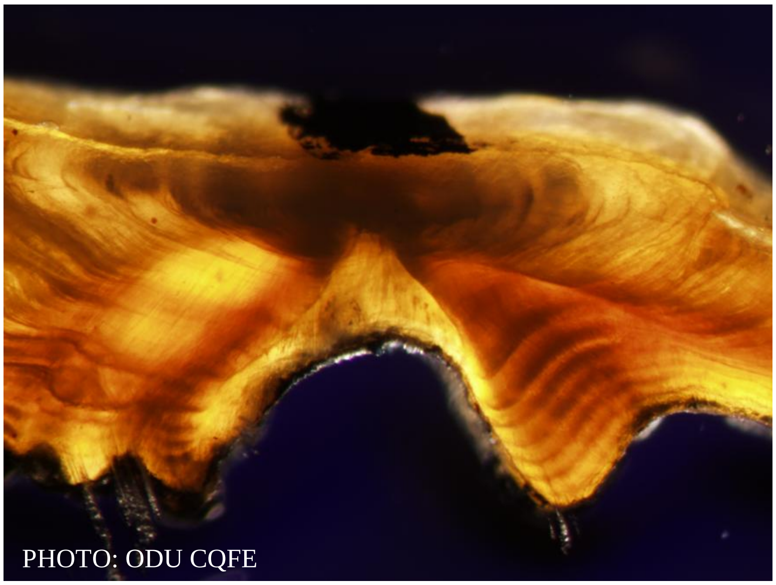

Figure 4: Correctly cut section from a bluefish otolith showing desired triangular point to sulcal groove.

If the quality of the section is not adequate, another section must be taken. You may use the same otolith, or the second otolith of the same fish. If a good section cannot be obtained from the same fish, replace that fish with another from the same length bin where available.

If the section quality is acceptable, select the best, most readable side. Scrape off any residual Crystalbond with tweezers and ensure the section sits level. Place the section with the best side facing upwards on a microscope slide. Use an eye-dropper to put a small amount of Flo-Texx® liquid slip cover on top of the section to protect it. Spread the Flo-Texx around with a circular motion and pop or move any bubbles away from the section with tweezers. Allow the Flo-Texx to air-dry for several hours until completely solidified.

Remember to label the slides with the sample number prior to storage (Figure 5).

Now that you have an acceptable section from that otolith, the remaining halves can be removed from the section slide. Clean them with tweezers and a Kimwipe® so that no Crystalbond remains on them. When they are clean and dry, return them to their storage container.

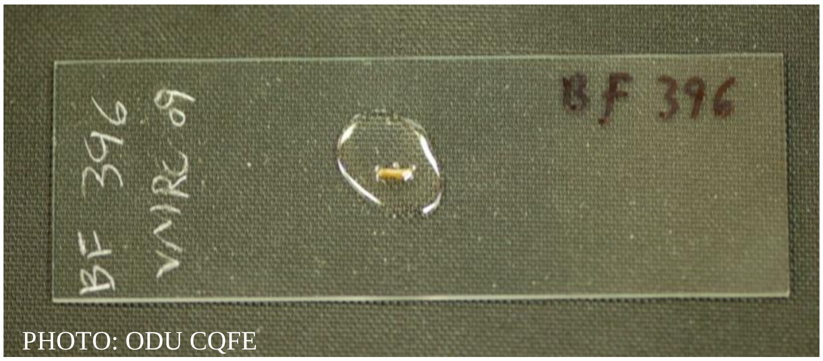

Figure 5: Bluefish otolith section mounted on a slide for reading and storage.

1.1.2 Alternative methods

Massachusetts Division of Marine Fisheries bakes their otoliths whole, before sectioning them, until the whole otolith reaches the desired caramel color. The whole otoliths are then embedded in West Systems brand two part epoxy using a silicone bullet mold. The epoxy is allowed to harden overnight before the embedded otoliths are sectioned.

Florida Wildlife Research Institute mounts whole otoliths on tag paper with hot glue for sectioning. Sections are taken with a 4-bladed saw that produces three sections simultaneously, to increase the likelihood of sectioning the core. FWRI does not bake the otolith sections.

Virginia Institute of Marine Science takes sections in a similar manner to ODU CQFE but then wet-polishes their sections to a slightly thinner width and does not bake the sections.

At the Bluefish Ageing Workshop (Norfolk, VA, May 4-5, 2011), example sections produced by all four techniques were examined and found to be readable. Variations on this protocol are acceptable, as long as readable sections are consistently produced.

1.2 Reading Otoliths

1.2.1 Identifying annuli

Otolith sections are read with transmitted light using a dissecting microscope, without knowledge of the fish's length.

The first annulus is often more diffuse and smudgy than subsequent annuli. Robillard *et al.* (2009) identified crenulations (rounded protrusions) on the dorsal edge of the section that were commonly associated with the first annulus and could be used to help identify it (Figure 6, Figure 7).

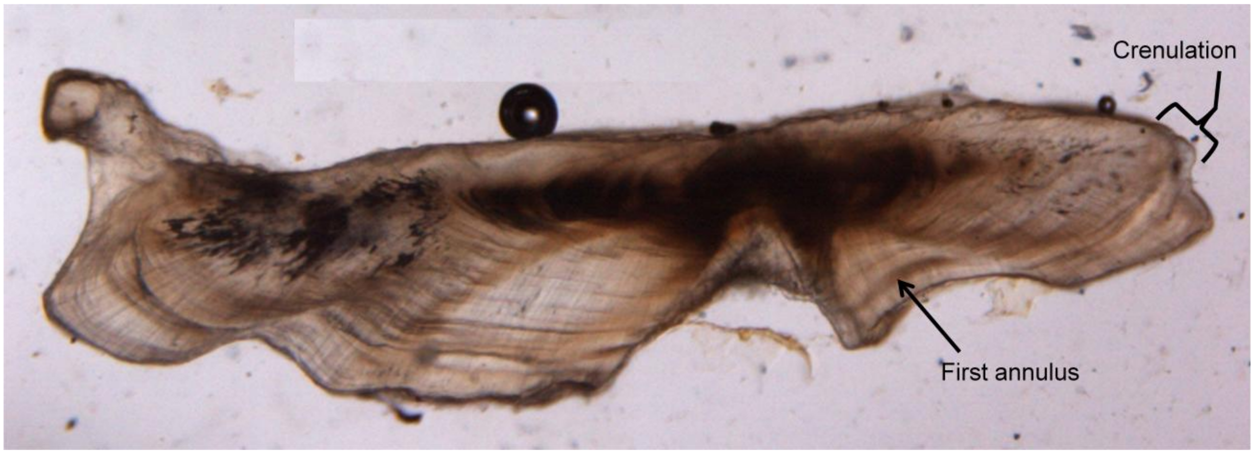

Figure 6: Sectioned otolith from an age-1 bluefish with first annulus and its associated crenulation indicated

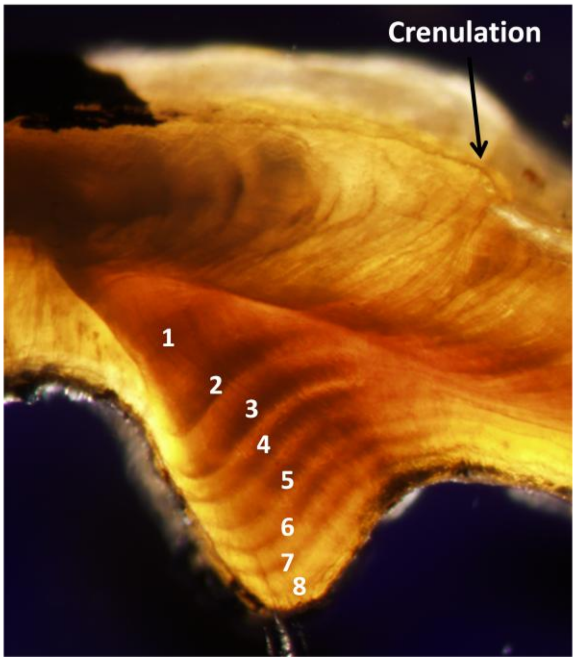

Figure 7: Sectioned otolith from an 8-year old bluefish with annuli and age-1 crenulation indicated.

Double rings are sometimes seen in older fish, characterized by two distinct opaque zone in close proximity that join to form a single origin, usually at the succal groove or the outer edge of the otolith. If the two opaque zones do not join and remain distinct, they are counted as two annuli. If they do join together, they are counted as a single annulus.

1.2.2 Assigning an age

Bluefish spawn from April through August, and lay down annuli in their otoliths from March to May, but by convention, they are assigned a birthdate of January 1 (Figure 8). To assign a final age, the relative timing of these events must be considered.

If no translucent material has been laid down after the last opaque zone (i.e., the last annulus forms the edge of otolith section), the final age is the annulus count. If growth has occurred since the formation of the last annulus, information on the date of sample collection is used to assign a final age to an individual. If the sample was collected between January 1 and February 28, the final age is assigned as the annulus count plus one. If the sample was collected between June 1 and December 31, the final age is the annulus count.

If the sample is collected during the period of ring formation (March – May), the reader must use professional judgment to determine whether ring formation for the year has occurred or not yet, based on the width of the translucent zone laid down after the last opaque zone and when the fish was caught during the ring formation period.

Year	Year One											
Assigned birth date	1-Jan											
Biological birth date						1-Jun						
Spawning period				Spawning								
Annulus formation			Annulus									
Month	J	F	M	A	M	J	J	A	S	O	N	D
Number of rings				0	0	0	0	0	0	0	0	0
Biological age				0	0	0	0	0	0	0	0	0
Year grouping				0	0	0	0	0	0	0	0	0

Year	Year Two											
Assigned birth date	1-Jan											
Biological birth date						1-Jun						
Spawning period				Spawning								
Annulus formation			Annulus									
Month	J	F	M	A	M	J	J	A	S	O	N	D
Number of rings	0	0	0	1	1	1	1	1	1	1	1	1
Biological age	0	0	0	0	0	1	1	1	1	1	1	1
Year grouping	1	1	1	1	1	1	1	1	1	1	1	1

Year	Year 3											
Assigned birth date	1-Jan											
Biological birth date						1-Jun						
Spawning period				Spawning								
Annulus formation			Annulus									
Month	J	F	M	A	M	J	J	A	S	O	N	D
Number of rings	1	1	1	2	2	2	2	2	2	2	2	2
Biological age	1	1	1	1	1	2	2	2	2	2	2	2
Year grouping	2	2	2	2	2	2	2	2	2	2	2	2

Figure 8: Relative time of spawning, annulus formation, and assigned birthdate in bluefish.

1.2.3 Measuring precision and repeatability

Each otolith should be aged by at least two readers. The precision of age assignments should be measured with the coefficient of variance (CV) (Campana 2001). It is defined as:

$$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(X_{ij} - \overline{X_i}\right)^2}{R-1}}}{\overline{X_j}} \qquad (1)$$

where CV_j is the CV of the j^{th} fish, X_{ij} is the age assigned to fish j by reader i, R is the total number of readings of fish j, and $\overline{X_j}$ is the mean age assigned to fish j. The CV should be calculated after the first round of independent readings and before a consensus is reached on disagreements. The mean CV can be used to assess precision and repeatability of age assignments both between readers and within a single reader across repeated readings.

References

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197–242.

Robillard, E., C.S. Reiss, and C.M. Jones. 2009. Age-validation and growth of bluefish (*Pomatomus saltatrix*) along the East Coast of the United States. Fisheries Research 95: 65-75.