Atlantic States Marine Fisheries Commission

Summary of the 2023-2024 Atlantic Menhaden Ageing Sampling Exchange

MAY 2025

Vision: Sustainably Managing Atlantic Coastal Fisheries

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Background and Statement of Problem

In 1955, the NOAA Laboratory in Beaufort, North Carolina, began monitoring the Atlantic menhaden purse-seine fishery for size and age composition of the catch (June and Reintjes 1959). Scales were selected as the ageing tool of choice for Atlantic menhaden due to ease of processing and reading and an age validation study confirming reliable age marks on scales (June and Roithmayer 1960). During the early decades of the Menhaden Program at the NOAA Beaufort Laboratory, scales from individual menhaden specimens were read multiple times by several readers. Since the early 1970s, only a single reader was retained on staff to age menhaden scales. The Beaufort Lab still ages all reduction fishery samples.

In response to an identified need for more biological sampling (i.e., length and age data) by the Atlantic Menhaden Advisory and Technical Committees (TC), Amendment 2 to the Interstate Fishery Management Plan for Atlantic Menhaden (ASMFC 2012) has required jurisdictions from NC to ME to collect biological samples proportional to bait landings since 2013. Jurisdiction contributions to coastwide bait landings in recent years (2021-2023) are summarized in Table 1, with Maine, New Jersey, and Virginia accounting for the vast majority of landings. During the same years, bait landings have accounted for approximately 30% of coastwide menhaden fishery landings. State agencies also collect and age scales and otoliths from their fishery-independent programs, although those have not been used in any of the assessments.

To address future plans for states to age Atlantic menhaden scales from the bait fishery and the research recommendation to conduct an ageing workshop, the ASMFC organized and held a workshop in 2015 (ASMFC 2015). An exchange of scale samples took place and was followed with an in-person workshop to discuss the results. Despite the fact that most participating agers were new to ageing Atlantic menhaden or had never aged the species, agreement between readers was, on average, 73% and increased to 95% within one year. False annuli, poor storage of samples, and damaged scales were common issues identified at the workshop.

Atlantic menhaden scales were also examined at ASMFC's 2017 and 2018 Quality Assurance/Quality Control Fish Ageing Workshops (ASMFC 2017, 2018). Average percent error between agers along the Atlantic coast was 15% in 2017 and 13% in 2018, although many readers had no previous experience ageing Atlantic menhaden.

Since Amendment 2 (ASMFC 2012), the Beaufort Lab has been ageing the bait samples in addition to the reduction samples for the stock assessments (2015, 2017, 2020, 2022, and anticipated 2025). The workload of ageing both the reduction and bait samples is unsustainable and thus the TC recommended *another* ageing workshop and sample exchange to establish ageing protocols so the states could begin ageing their own bait and fishery-independent samples for future stock assessments. Agers from Maine through South Carolina met for an ageing workshop in November 2023 to review otolith and scale ageing protocols, age samples in groups, and review differences in age readings from the group ages. Following the workshop, an exchange set was circulated to the various states' ageing laboratories from December 2023 through February 2025 (Table 1). Agers met via conference call on April 29, 2025 to review the results of the exchange and make recommendations to the TC.

Exchange Objectives and Goals

The goals of the Atlantic menhaden ageing structure exchange are to:

- *Establish methods to prepare and read otoliths and/or scales*
- *Determine the precision and bias of age reading data between different readers and labs or agencies along the coast*
- *Make recommendations to improve and standardize ageing practices*
- *Move bait sample ageing from the Beaufort Lab to the states*

Hard Part Exchange

Sample exchange set description

Agencies and labs were asked to supply paired samples of otoliths and scales that were collected throughout the year representing various lengths and collection sites in their respective waters. Sample preparation methods vary among labs, and participants of the 2023 ageing workshop recommended collecting information on processing and cleaning age samples for context on readability of exchange samples. Details of menhaden age sample preparation in each lab are provided in Appendix 1.

An exchange set was created from the submitted samples for a total of 65 paired samples of whole otolith and scales and 11 additional unpaired scales. While paired samples were prioritized, the unpaired scales in the collection represent fork lengths, collection months, or areas with limited representation in the exchange set so these samples were retained. Samples in the exchange represented Atlantic menhaden of various fork lengths with an average of 242 mm (Figure 1). Samples were collected from various state waters (Figure 2) and collection months (Figure 3).

State and lab participation

Thirteen labs participated in the Atlantic menhaden ageing exchange, see Table 2 for the order the labs received the exchange set. The number of readers participating in the exchange for each lab was one (ME DMR, RI DMF, CT DEEP, NYS DEC, NJ FW, VIMS, VMRC, NC DMF, and SC DNR), two (MA DMF, MD DNR, and NOAA), or three (DE DFW; Table 3), although all labs provided consensus ages for the analysis. Each reader provided their experience level with ageing Atlantic menhaden and the equipment used (Table 3). Additional details of menhaden ageing practices in each lab are provided in Appendix 1. When reading the samples, agers provided annulus count, margin code, and final age for all samples with the exception of some unreadable samples as determined by the individual agers.

Methods

Agreement between readers and between labs was evaluated to provide information on ageing error. A test of symmetry around the diagonal 1:1 line (Evans and Hoenig 1998) was performed using the FSA package (Ogle et al. 2025) where a significant p-value (<0.05) indicates systematic bias between the age readings. Without knowing the true age of the fish, this test does not identify which reader is more accurate, but rather identifies whether there are differences or not. Mean coefficient of variation (CV), percent of exact agreement between readers, and percent agreement within one year was also calculated for each lab and reader to provide a measure of precision. While this does not serve as a proxy for accuracy, it does indicate the level of ease for assigning an age to that ageing structure, the reproducibility of the age, or the skill level of the readers. Generally, CVs of 5% serve as a reference point for determining precision, where greater values would indicate ageing imprecision (Campana 2001). Some previous ASMFC ageing workshops revised the reference value so that acceptable CVs were those less than 10% if the species in question was hard to age.

Prior to a meeting to review exchange results, a participant asked to see the precision results of the age exchange for fish of two different size classes: <260 mm and ≥ 260 mm. Precision statistics were calculated as described above.

Results

Scale Samples

Sample size, CVs (%), Evans & Hoenig's test of symmetry (p-value), exact agreement (%), and agreement within one year (%) were calculated for all consensus ages for the scale samples. Sample size varied from 74 to 76 samples because readers did not provide ages for all samples (Table 4). CVs ranged from 8-29% (average of 20%), with all 78 comparisons being greater than 5% and 75 being greater than 10%, indicating a lack of precision (Table 5). Of the 78 comparisons made, 51 had significant p-values which indicated systematic bias between the readers and labs (Table 6). Exact agreement between readers ranged from 29 - 67% (average of 47%; Table 7) and agreement within one year ranged from 71-99% (average of 86%; Table 8).

Scales were read from 43 fish <260 mm (fork length) and 31-33 (depending on lab) fish ≥ 260 mm. Scales from fish <260 mm had an exact agreement of 52% on average between labs. The mean CV was 22% on average (Table 15). Whereas, for fish ≥260 mm, exact agreement was 40%, on average, and the mean CV was 16% on average. Results generally did not show substantial differences between size groups and size does not appear to be a primary factor of ageing error of scale samples from this exchange.

Otolith Samples

Sample size, CVs (%), Evans & Hoenig's test of symmetry (p-value), exact agreement (%), and agreement within one year (%) were calculated for all consensus ages for the whole otolith samples. Sample size varied from 59 to 64 samples because readers did not provide ages for all samples (Table 9). CVs ranged from 6 - 43% (average of 22%), with all 78 comparisons being greater than 5% and 76 being greater than 10%, indicating a lack of precision (Table 10). Of the 78 comparisons made, 42 had significant p-values which indicated systematic bias between the readers and labs (Table 11). Exact agreement between readers ranged from 24 - 80% (average of 43%; Table 12) and agreement within one year ranged from 64-97% (average of 85%; Table 13).

Otoliths were read from 33-36 fish <260 mm and 26-28 menhaden ≥ 260 mm. The exact agreement of otoliths was 50%, on average, for fish < 260 mm and 37% for fish ≥ 260 mm. The mean CV was 22%, on average for fish < 260mm and 22% for fish ≥ 260 mm. Results generally did not show substantial differences between size groups and size does not appear to be a primary factor of ageing error of otolith samples from this exchange.

Comparison between Paired Scales and Whole Otoliths

There were 64 paired scales and whole otolith samples in the exchange, although not all readers aged all samples. Sample size, symmetry test p-values, CVs, exact agreement, and agreement within one year were used to evaluate bias and precision in age readings between paired scale and whole otolith samples (Table 14). These tests identified imprecision (CVs > 10%) for all readers and systematic bias between sets of age determination (Evans & Hoenig $p > 0.05$). Exact agreement varied from 28-58% with an average of 44% for all 13 labs. Agreement within one year varied from 70-97% with an average of 85%. Without a validated ageing method, these tests cannot indicate which structure provides more accurate ages, only that imprecision was detected. Reader age frequency and bias plots can be found in Figure 4-Figure 16.

Discussion

A virtual meeting was held on April 29th, 2025, to review the results of the age exchange and included representation from ME, RI, NY, NJ, CT, DE, VMRC, VIMS, and NOAA. Participants were concerned with the lack of agreement between otolith ages across labs. Participants suggested that some of the ageing bias and imprecision between structures and labs could be due to varying experience with ageing menhaden structures, differences in equipment used to read scales and otoliths, and, for some, lack of experience reading whole otolith rather than sectioned otolith. It was also noted that some samples in the exchange set were damaged or of poor quality, likely due to inexperience with processing and mounting scales or otoliths. Participants suggested that a standardized process for ageing menhaden, including preparation of scales and otolith, should be developed and there should be further discussion on the best equipment to use for ageing.

There are many withstanding uncertainties in the ageing process that need to be addressed before states are comfortable ageing their bait fishery samples rather than sending samples to NOAA for ageing. Overall, many participants indicated that another workshop should be held for participants to collectively work through samples with poor agreement and determine if discrepancies can be resolved to arrive at consensus ages or if issues contributing to poor agreement can be rectified with future samples (e.g., improving preparation methods). This workshop would help to develop a standardized processing and ageing protocol for Atlantic menhaden. Further, participants suggested that an additional ageing reference set should be compiled to facilitate training, but that this reference set would require new, high quality paired age samples with consensus ages determined by participants in advance.

Recommendations from this exchange for Atlantic menhaden are:

- Samples from the current age exchange set that had lower agreement should be re-evaluated to understand why there was disagreement and come to consensus ages to aid in development of consistent ageing standards.
- States should continue collecting scales based on the current compliance standards but should also strive to collect a subset of otoliths from the bait fishery to get paired samples. The exact number of otoliths collected may depend on each lab's capacity, but states should strive for 100 per year. A pre-determined binning structure should be implemented to ensure otoliths are collected from menhaden of various sizes. These paired samples would provide additional

information for comparing ageing structures and maintain a set of otolith samples that could be used in place of scales if otoliths are determined to be better ageing structures in the future.

- A new reference set of scale and otolith paired ages should be maintained for training. This reference set should include consensus ages and digitized copies of each sample. Paired samples in addition to those from the exchange are available from several labs along the coast. ASMFC should organize efforts to evaluate the current exchange set and/or add new paired age samples from labs for the reference set.
- ASMFC should organize an additional ageing workshop which could be used for training on Atlantic menhaden age structure reading. Specifically, this workshop will provide consensus ages for the exchange sets and facilitate more conversation regarding best practices for otolith/scale preparation, equipment to use for reading ages, and how to count annuli (i.e., determining the first ring).
- Due to potential uncertainties with NOAA staffing and prioritization, states should age their own scale samples collected from bait fisheries before sending them to Beaufort.

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Tables

Table 1. Atlantic coast jurisdiction contributions to coastwide menhaden bait fishery landings averaged over recent two year time blocks.

Jurisdiction	2021-2022	2022-2023
ME	18.32%	22.73%
NH	3.75%	3.86%
MA	7.79%	5.53%
RI	1.60%	0.32%
CT	0.18%	0.21%
NY	1.68%	0.75%
NJ	35.44%	36.21%
DE	0.03%	0.04%
MD	2.36%	2.21%
PFRC	2.34%	2.32%
VA	26.05%	25.13%
NC	0.37%	0.56%
SC	0.00%	0.00%
GA	0.00%	0.00%
FL	0.10%	0.13%

Table 2. Schedule of the Atlantic menhaden sample exchange.

Date	Ageing Lab
December 4-15, 2023	MA DMF
December 18, 2023 – January 5, 2024	DE DFW
January 8-19, 2024	RI DMF
January 22 – February 2, 2024	CT DEEP
February 5-16, 2024	VIMS
February 26 – March 8, 2024	SC DNR
April 1-12, 2024	NYS DEC
April 15-26, 2024	ME DMR
May 6-17, 2024	NJ FW
June 3-14, 2024	VMRC
June 17-28, 2024	NC DMF
July 1-12, 2024	MD DNR
September 3-November 18, 2024	NOAA Beaufort

Table 3. Level of experience reading Atlantic menhaden ages by reader and the equipment used. Lighting is specific to scopes unless otherwise noted.

Lab	Reader	Experience Level	Equipment	Lighting
ME DMR	Lisa Pinkham	Some	Olympus SZ61 microscope, Infinity 2 Lumenera camera, Infinity Analyze 7 picture software	Optical Analysis Corporation/Schott light – adjustable external source
MA DMF	Kara Duprey	None	Microfiche (scales); stereoscope (otoliths)	Reflected (otoliths)
	Scott Elzey	Some		
RI DMF	Nicole Lengyel Costa	Experienced	Microfiche (scales); Leica stereoscope (otoliths)	Reflected (otoliths)
CT DEEP	Kelli Mosca	Experienced	Microfiche (scales); Zeiss microscope (otoliths)	Transmitted (otoliths)
NYS DEC	Caitlin Craig	Some	Microfiche (scales); Nikon Stereo Microscope SMZ-U (otoliths)	Transmitted (otoliths)
NJ FW	Jamie Darrow	Some	Microfiche – Eyecom 3000 (scales); Leica M165 c Stereo microscope, Leica Camera – MC 190 HD (otoliths)	FLT 13.8v – 25w bulb (scales); Leica Ring Light – LED5000 RL 80/40, TL5000 Ergo Transmitted light base (otoliths)
DE DFW	Alexandria Hoffman	Some	Microfiche (scales); Zeiss compound microscope (otoliths)	Transmitted
	Audrey Ostroski	Some		
	Colt Williamson	Some		
MD DNR	Katie Messer	Experienced	Microfiche (scales); compound microscope (otoliths)	Transmitted
	Harry Rickabaugh	Experienced (scales); none (whole otoliths)		
VIMS	Jameson Gregg	Experienced	Stereoscope	Transmitted
VMRC	Hank Liao	Some	Nikon stereoscope	Transmitted
NC DMF	Sara Pace	None	Olympus microscope	Transmitted (scales); Reflected (otoliths)
SC DNR	Jonathan Tucker	None	Nikon 10x stereo microscope	Transmitted (scales); Reflected (otoliths)
NOAA	Amanda Rezek	Experienced (scales); some (otoliths)	Olympus stereoscope, cellSens imaging software	Transmitted LED light
	Jessica Branscome			

Table 4. Sample size of the scale samples in the Atlantic menhaden exchange by state/lab. There were 76 scales samples in the exchange but not all readers aged all samples.

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	76	76										
RI	75	75	75									
CT	76	76	75	76								
NY	76	76	75	76	76							
NJ	75	75	74	75	75	75						
DE	76	76	75	76	76	75	76					
MD	76	76	75	76	76	75	76	76				
VIMS	76	76	75	76	76	75	76	76	76			
VMRC	76	76	75	76	76	75	76	76	76	76		
NC	76	76	75	76	76	75	76	76	76	76	76	
SC	76	76	75	76	76	75	76	76	76	76	76	76
NOAA	76	76	75	76	76	75	76	76	76	76	76	76

Table 5. **Mean coefficients of variation (CVs) between readers for Atlantic menhaden scale samples. CVs greater than or equal to 10% indicate ageing imprecision between readers.**

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	23											
RI	22	13										
CT	26	8	9									
NY	24	17	19	18								
NJ	19	15	11	15	23							
DE	11	22	19	24	25	19						
MD	25	19	20	20	23	14	27					
VIMS	23	15	16	13	18	19	23	24				
VMRC	22	18	16	18	22	18	26	22	20			
NC	23	22	17	21	29	18	21	20	25	22		
SC	23	14	14	15	18	14	25	21	19	19	21	
NOAA	23	23	18	23	27	14	24	9	26	25	16	24

Table 6. Symmetry test p-values for the Atlantic menhaden scale comparisons using Evans & Hoenig's test. Significant p-values (α<0.05) are bolded and indicate systematic bias between the readers and labs.

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	**0.00**											
RI	**0.00**	0.05										
CT	**0.00**	0.50	**0.00**									
NY	**0.00**	0.34	**0.01**	0.88								
NJ	**0.02**	**0.00**	**0.02**	**0.00**	**0.00**							
DE	0.56	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**						
MD	**0.01**	**0.00**	0.14	**0.00**	**0.00**	0.78	**0.02**					
VIMS	**0.00**	0.42	0.05	0.55	0.62	**0.00**	**0.00**	**0.00**				
VMRC	**0.01**	0.05	0.63	**0.01**	**0.02**	0.39	**0.03**	0.34	0.11			
NC	0.88	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	0.52	**0.04**	**0.00**	**0.01**		
SC	**0.00**	0.34	0.22	0.09	0.25	**0.00**	**0.00**	**0.01**	0.14	0.17	**0.00**	
NOAA	1.00	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.14	**0.02**	**0.00**	**0.01**	0.79	**0.00**

Table 7. Percent exact agreement between readers for the Atlantic menhaden scale samples. Shading indicates level of agreement where dark gray is highest agreement and white is the lowest agreement.

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	36											
RI	39	55										
CT	29	66	61									
NY	32	53	48	46								
NJ	49	53	62	47	37							
DE	62	39	49	36	33	53						
MD	45	51	52	47	39	64	42					
VIMS	30	57	51	62	45	51	39	43				
VMRC	45	45	49	43	43	51	33	50	43			
NC	43	43	52	41	37	55	51	53	39	43		
SC	37	49	47	43	49	53	34	45	39	42	50	
NOAA	50	43	56	36	38	65	49	67	38	43	66	43

Table 8. **Percent agreement within one year between readers for the Atlantic menhaden scale samples. Shading indicates level of agreement where dark gray is highest agreement and white is the lowest agreement.**

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	79											
RI	88	87										
CT	72	93	95									
NY	75	92	81	91								
NJ	96	88	97	91	84							
DE	99	80	91	75	71	95						
MD	91	91	88	88	86	96	87					
VIMS	82	88	92	92	95	85	78	86				
VMRC	82	82	85	86	89	85	82	87	88			
NC	91	78	92	78	72	92	93	86	82	79		
SC	79	87	88	88	87	87	78	87	92	82	75	
NOAA	97	82	92	83	75	97	96	93	82	83	96	83

Table 9. **Sample size of the otolith samples in the Atlantic menhaden exchange by state/lab. There were 64 otolith samples in the exchange but not all readers aged all samples.**

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	61	64										
RI	59	61	61									
CT	61	64	61	64								
NY	61	64	61	64	64							
NJ	59	61	61	61	61	61						
DE	61	64	61	64	64	61	64					
MD	61	64	61	64	64	61	64	64				
VIMS	61	64	61	64	64	61	64	64	64			
VMRC	61	63	61	63	63	61	63	63	63	63		
NC	61	64	61	64	64	61	64	64	64	63	64	
SC	61	64	61	64	64	61	64	64	64	63	64	64
NOAA	59	62	61	62	62	61	62	62	62	61	62	62

Table 10. Mean coefficients of variation (CVs) between readers for Atlantic menhaden otolith samples. CVs greater than or equal to 10% indicate ageing imprecision between readers.

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	20											
RI	16	12										
CT	20	13	12									
NY	19	18	14	21								
NJ	33	26	20	21	26							
DE	23	20	18	24	12	26						
MD	20	22	12	20	14	23	18					
VIMS	16	15	6	13	16	22	19	15				
VMRC	28	28	23	30	22	27	27	19	28			
NC	43	40	35	41	34	38	36	34	38	38		
SC	28	21	18	25	20	24	18	21	20	28	35	
NOAA	21	12	8	12	14	19	16	12	11	23	33	17

Table 11. Symmetry test p-values for the Atlantic menhaden otolith comparisons using Evans & Hoenig's test. Significant p-values (<0.05) are bolded and indicate systematic bias between the readers and labs.

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	0.15											
RI	**0.01**	0.09										
CT	0.15	0.70	0.10									
NY	**0.00**	**0.03**	0.37	0.11								
NJ	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**							
DE	**0.00**	**0.00**	**0.01**	**0.00**	0.09	0.18						
MD	**0.00**	0.13	0.18	0.10	0.89	**0.01**	0.11					
VIMS	**0.02**	0.13	0.51	0.48	0.27	**0.00**	**0.02**	0.08				
VMRC	**0.00**	0.09	0.09	0.07	0.88	**0.04**	0.34	0.42	0.06			
NC	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.38	0.08	**0.00**	**0.00**	**0.04**		
SC	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	0.14	0.12	**0.01**	**0.00**	0.27	0.56	
NOAA	**0.00**	**0.02**	**0.03**	**0.00**	0.22	**0.00**	0.19	0.20	**0.02**	0.67	**0.01**	**0.03**

Table 12. **Percent exact agreement between readers for the Atlantic menhaden otolith samples. Shading indicates level of agreement where dark gray is highest agreement and white is the lowest agreement.**

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	30											
RI	44	61										
CT	34	58	59									
NY	33	50	59	45								
NJ	24	33	52	38	41							
DE	26	44	46	38	59	31						
MD	38	41	61	48	56	44	44					
VIMS	43	55	80	63	55	46	47	56				
VMRC	25	33	38	29	38	43	29	40	30			
NC	26	33	41	36	44	43	38	44	39	32		
SC	28	45	51	36	48	39	56	47	45	32	41	
NOAA	34	63	67	61	55	44	52	63	63	36	44	53

Table 13. Percent agreement within one year between readers for the Atlantic menhaden otolith samples. Shading indicates level of agreement where dark gray is highest agreement and white is the lowest agreement.

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	87											
RI	88	87										
CT	89	89	95									
NY	84	88	95	86								
NJ	64	80	87	85	84							
DE	80	77	92	81	95	93						
MD	80	78	93	88	92	92	92					
VIMS	90	86	95	92	91	85	86	89				
VMRC	67	70	79	71	83	75	78	87	73			
NC	67	77	79	77	80	74	83	77	77	75		
SC	77	72	85	77	91	93	95	91	84	81	83	
NOAA	81	82	97	92	95	97	95	95	92	85	84	87

Table 14. Sample size (n), Evans & Hoenig p-value, mean CV, exact agreement, and agreement within one year for paired Atlantic menhaden scale and whole otolith samples. Significant p-values (<0.05) are indicated with an asterisk.

Lab/State	n	p-value	CV (%)	Exact Agreement (%)	Agreement within 1 yr (%)
ME DMR	61	0.000*	25	28	77
MA DMF	64	0.810	16	41	86
RI DMF	60	0.407	12	57	90
CT DEEP	64	0.023*	15	50	88
NYS DEC	64	0.000*	23	30	73
NJ FW	61	0.000*	24	41	85
DE DFW	64	0.830	14	56	95
MD DNR	64	0.961	24	45	86
VIMS	64	0.049*	14	58	91
VMRC	63	0.418	31	35	70
NC DMF	64	0.019*	38	31	84
SC DNR	64	0.000*	24	38	77
NOAA	62	0.361	15	56	97

Table 15. Mean coefficients of variation (CVs) between readers for Atlantic menhaden scale samples from fish <260mm fork length (a.) and ≥260mm fork length (b.). CVs greater than or equal to 10% indicate ageing imprecision between readers.

a.)

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	24											
RI	27	11										
CT	29	8	7									
NY	23	22	21	21								
NJ	25	14	14	16	26							
DE	14	23	26	25	26	27						
MD	31	24	27	28	28	19	38					
VIMS	25	17	17	14	22	22	24	32				
VMRC	25	18	16	19	25	19	31	27	22			
NC	26	18	18	17	28	19	25	20	24	21		
SC	25	16	15	15	23	15	30	29	21	21	18	
NOAA	33	25	25	26	30	19	36	6	31	29	20	29

b.)

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	22											
RI	14	16										
CT	22	9	12									
NY	25	10	17	12								
NJ	12	16	7	15	19							
DE	7	22	10	22	24	9						
MD	16	12	10	10	17	7	13					
VIMS	20	12	14	11	12	14	21	13				
VMRC	19	19	17	16	17	16	20	14	17			
NC	19	29	16	28	30	16	15	20	27	25		
SC	20	11	13	14	10	13	19	11	15	17	25	
NOAA	11	21	10	21	23	7	9	13	19	19	12	17

Table 16. Mean coefficients of variation (CVs) between readers for Atlantic menhaden otolith samples from fish <260mm fork length (a.) and >260mm fork length (b.). CVs greater than or equal to 10% indicate ageing imprecision between readers.

a.)

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	21											
RI	15	6										
CT	23	12	11									
NY	19	17	17	23								
NJ	35	22	24	18	26							
DE	24	18	23	25	11	28						
MD	18	19	12	21	16	24	21					
VIMS	15	10	6	14	18	27	23	18				
VMRC	26	24	20	28	20	26	24	14	26			
NC	44	35	36	40	34	35	34	35	40	34		
SC	31	17	22	26	25	27	22	27	24	27	34	
NOAA	20	5	6	10	15	19	18	12	11	19	32	17

b.)

	ME	MA	RI	CT	NY	NJ	DE	MD	VIMS	VMRC	NC	SC
MA	18											
RI	17	19										
CT	16	15	14									
NY	20	19	10	18								
NJ	31	33	16	25	26							
DE	23	23	10	21	12	22						
MD	22	25	12	18	12	20	15					
VIMS	18	21	6	12	13	16	14	12				
VMRC	32	34	26	33	25	29	30	26	30			
NC	40	46	34	41	34	42	38	34	35	42		
SC	24	27	13	24	14	19	13	13	16	28	36	
NOAA	22	22	11	15	13	19	13	12	11	27	34	16

Figures

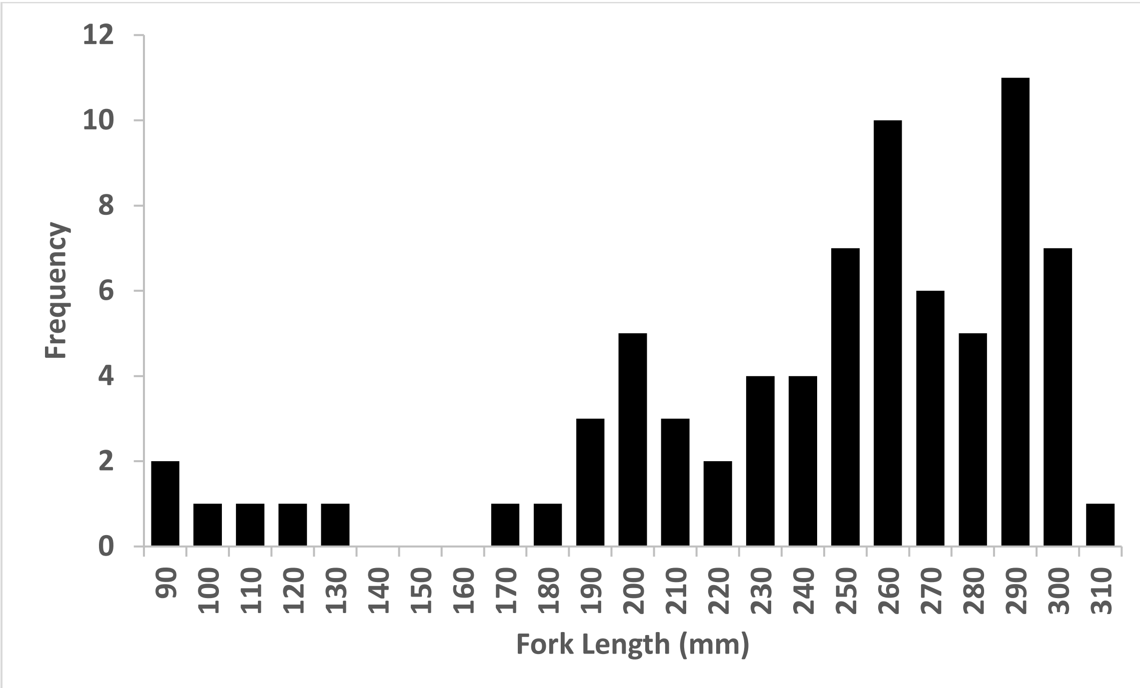

Figure 1. **Length frequency of Atlantic menhaden in the workshop set.**

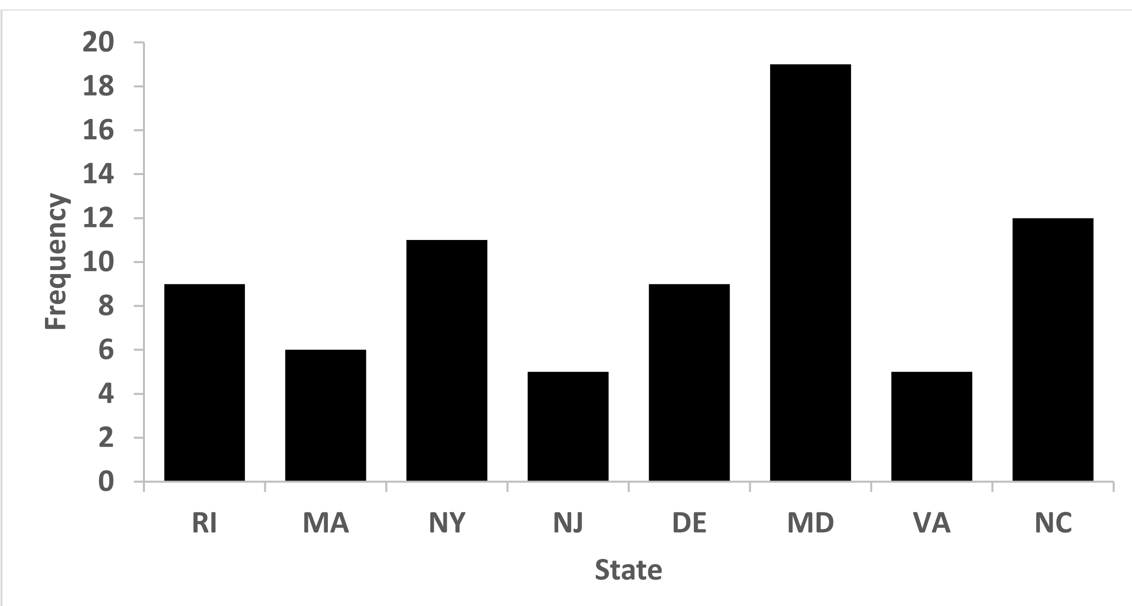

Figure 2. **Number of samples collected in state waters in the workshop set.**

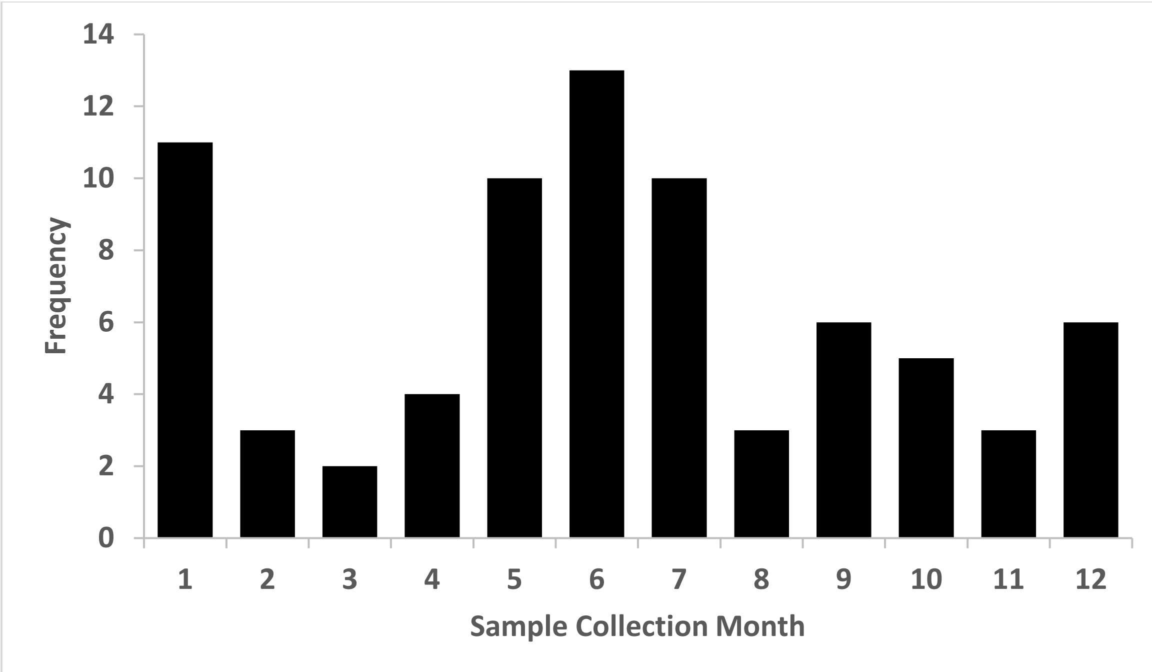

Figure 3. Number of samples collected by month in the workshop set.

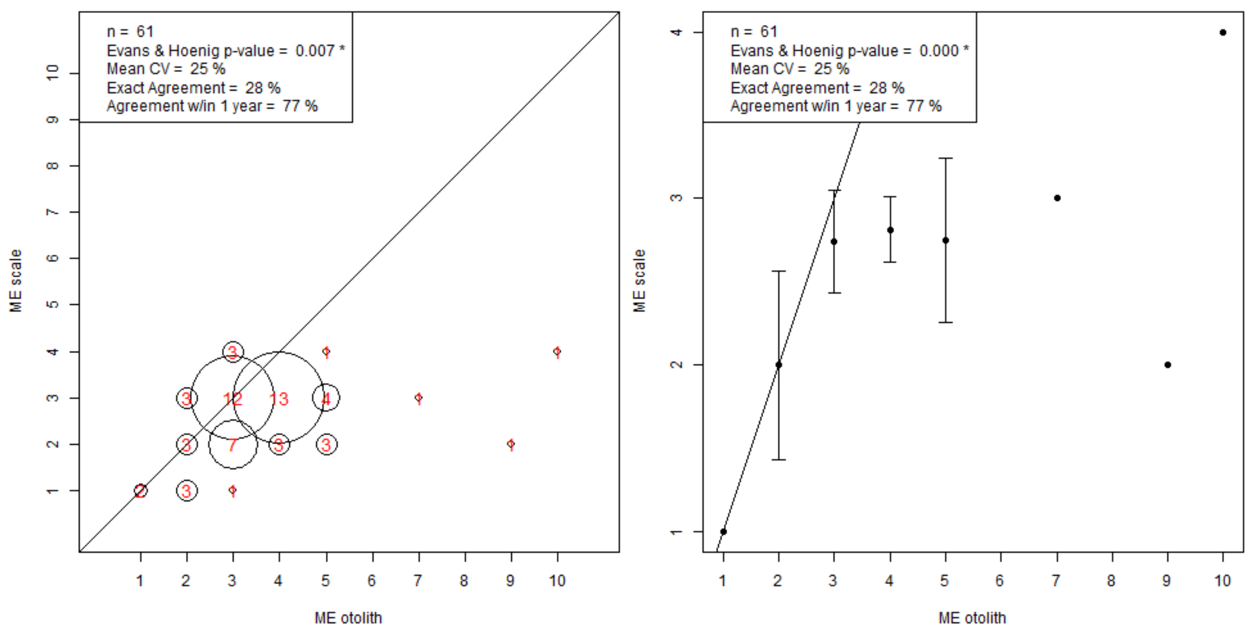

Figure 4. Age frequency (left) and age bias (right) plots for ME Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals.

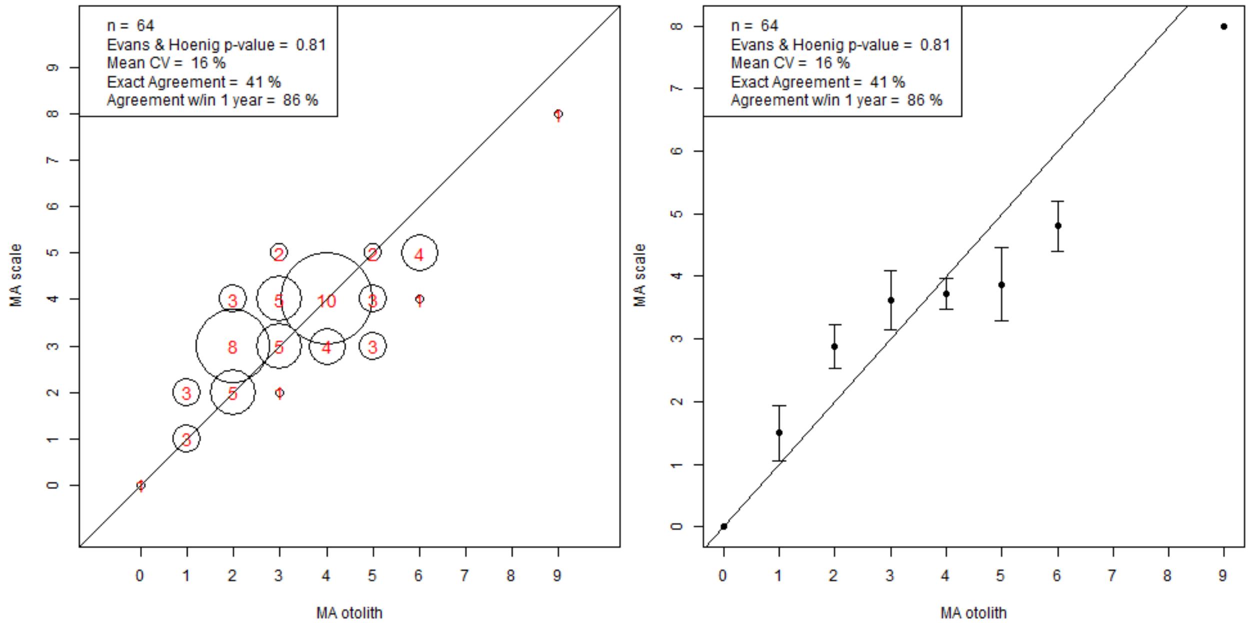

Figure 5. **Age frequency (left) and age bias (right) plots for MA Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals.**

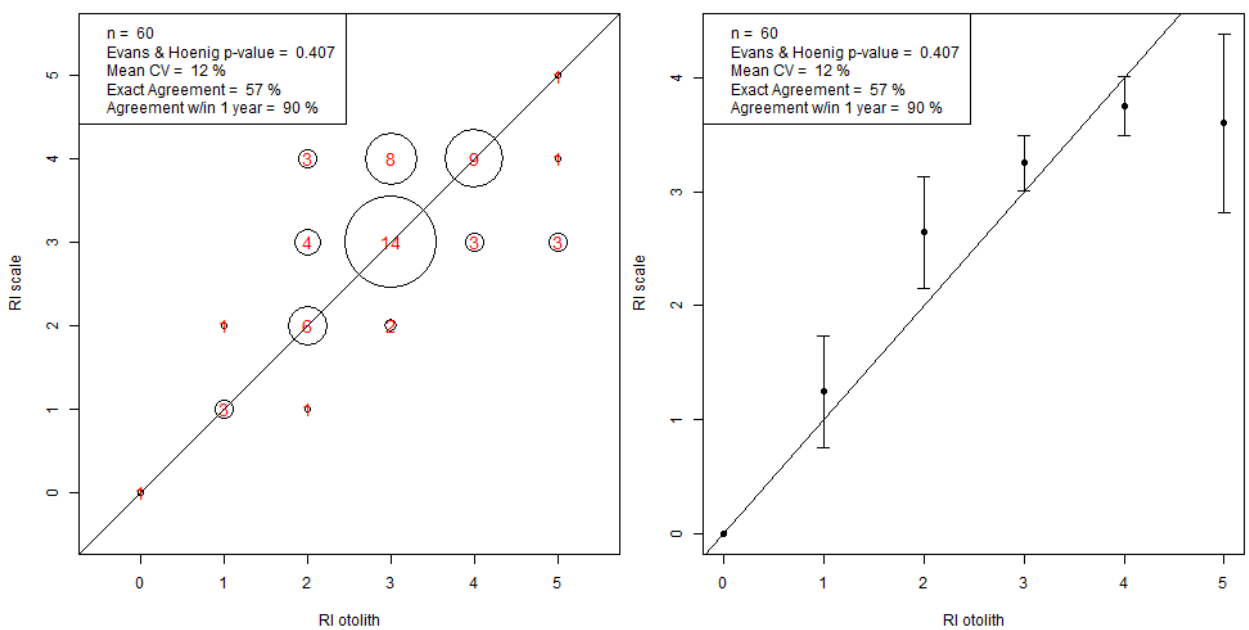

Figure 6. **Age frequency (left) and age bias (right) plots for RI Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals .**

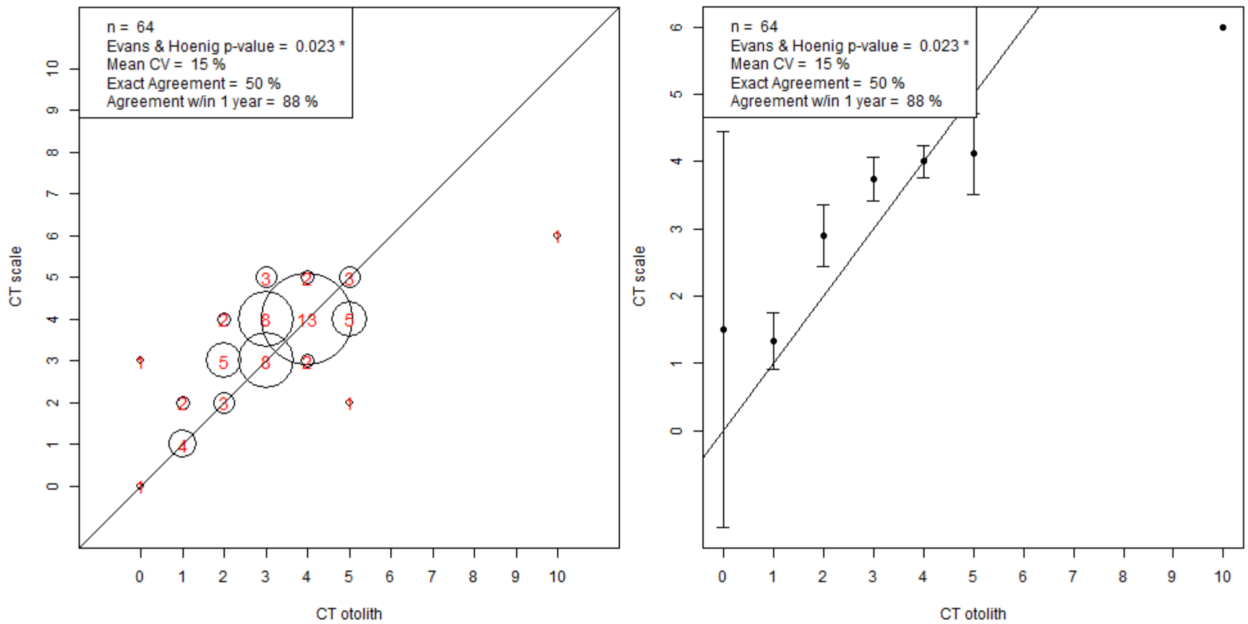

Figure 7. Age frequency (left) and age bias (right) plots for CT Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

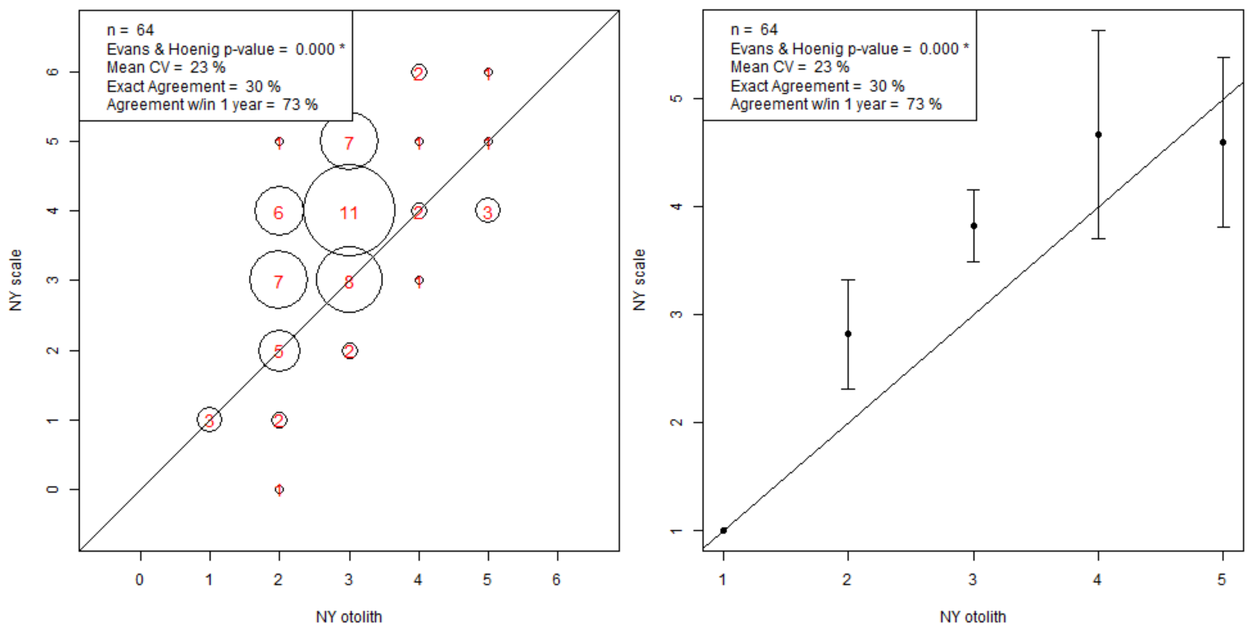

Figure 8. Age frequency (left) and age bias (right) plots for NY Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

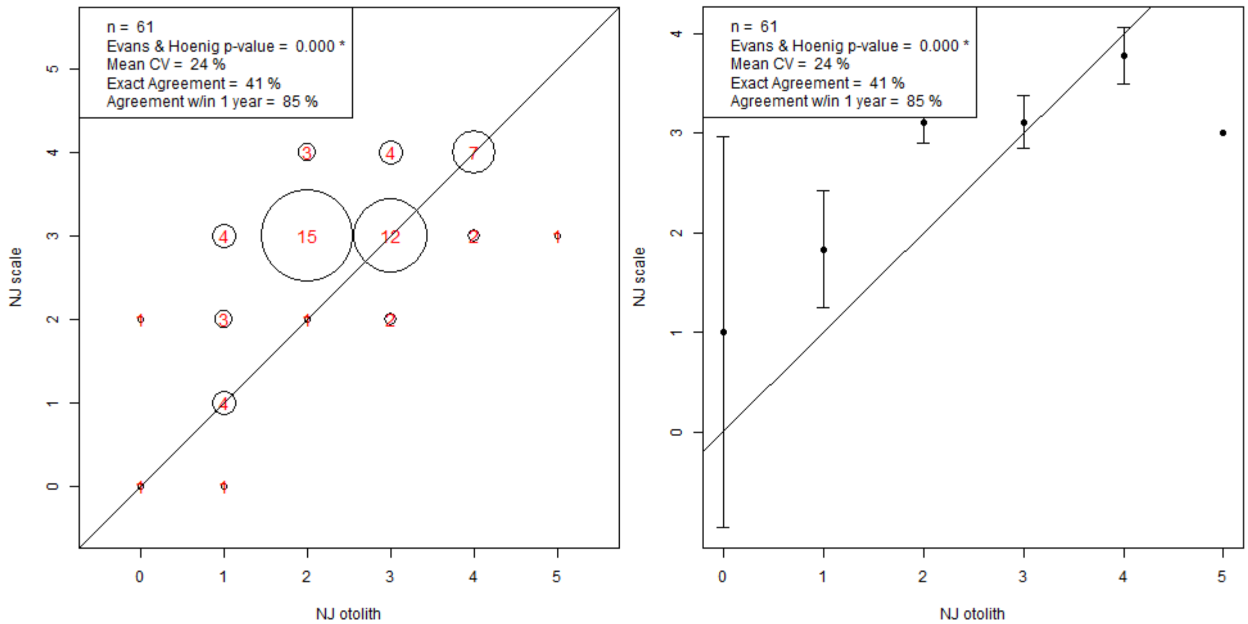

Figure 9. Age frequency (left) and age bias (right) plots for NJ Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

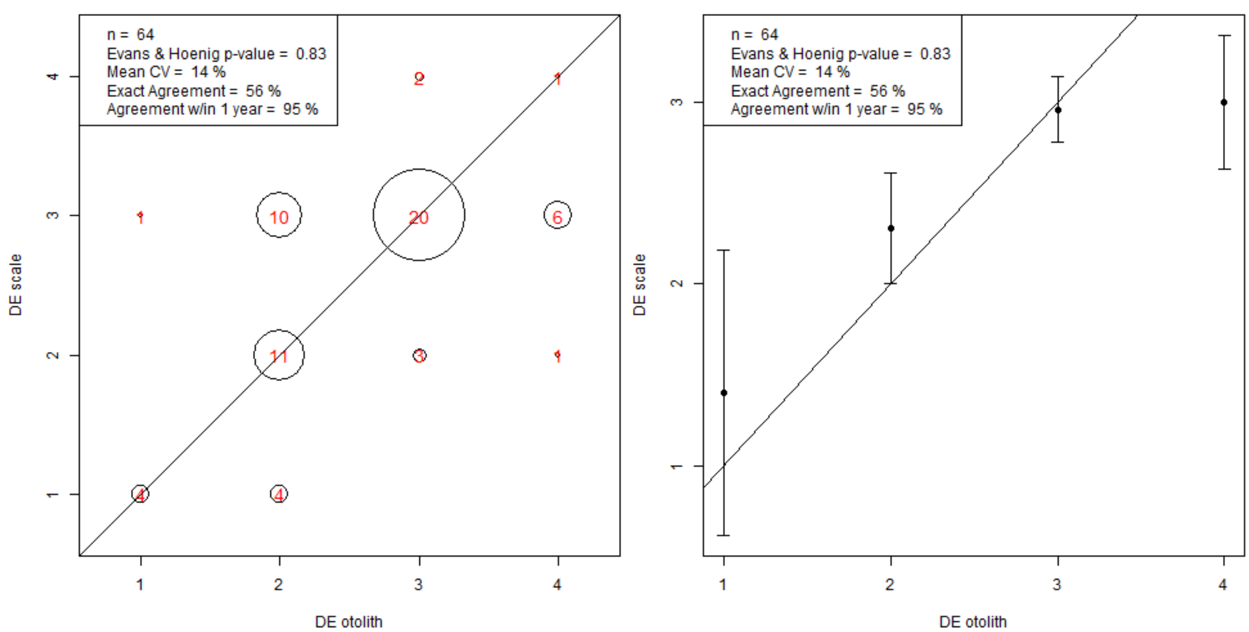

Figure 10. Age frequency (left) and age bias (right) plots for DE Atlantic menhaden scale and whole otolith age determinations In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

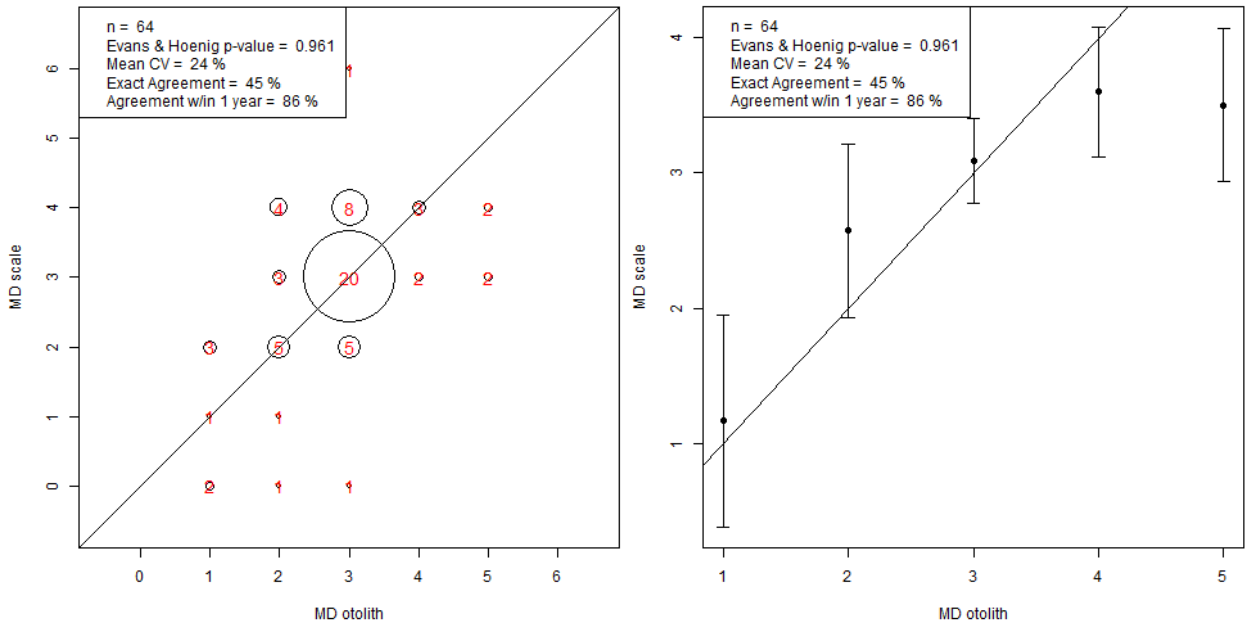

Figure 11. **Age frequency (left) and age bias (right) plots for MD Atlantic menhaden scale and whole otolith age determinations In the age bias plots, dots are the mean age and error bars are 95% confidence intervals**

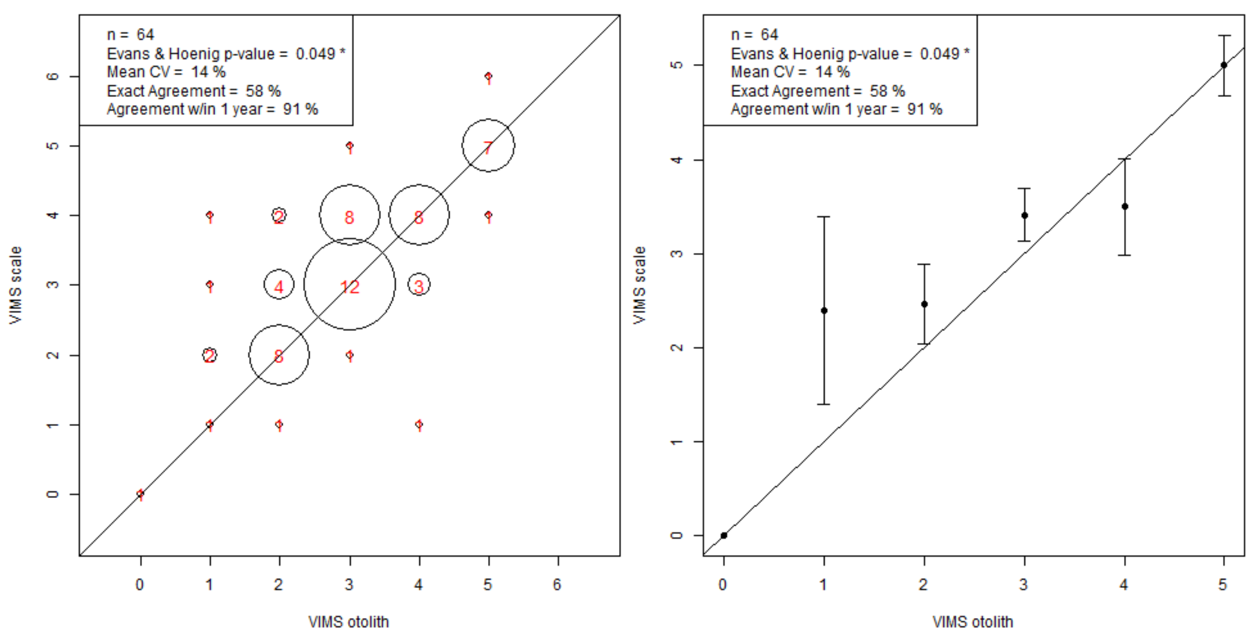

Figure 12. **Age frequency (left) and age bias (right) plots for VIMS Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals**

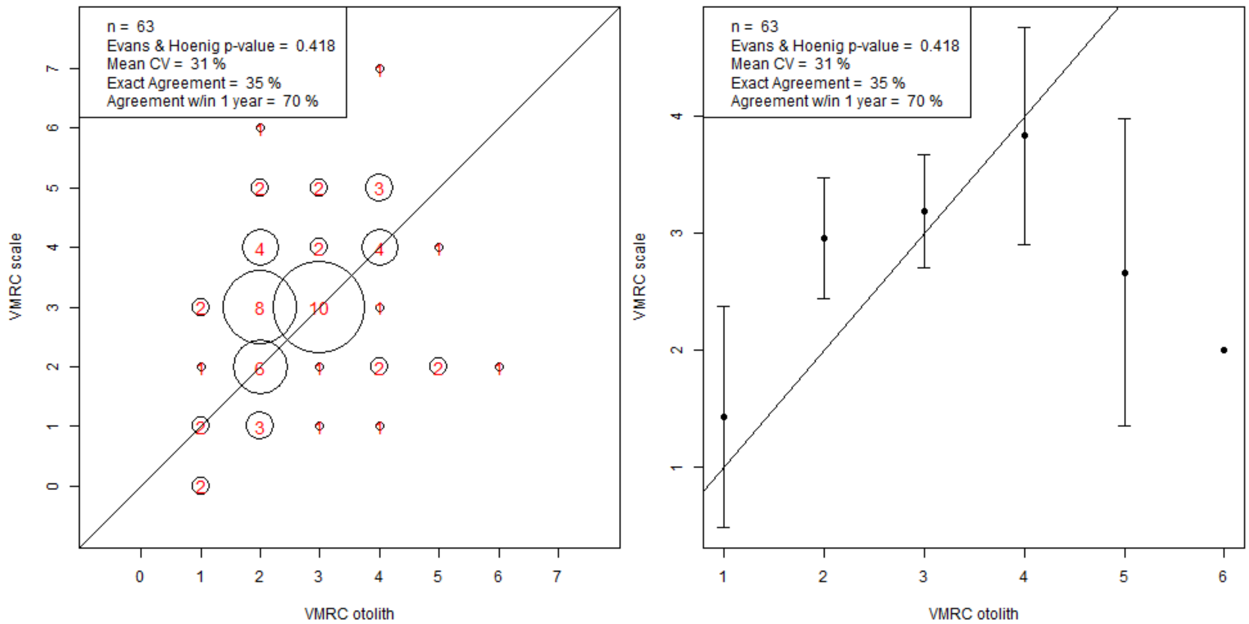

Figure 13. Age frequency (left) and age bias (right) plots for VMRC Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

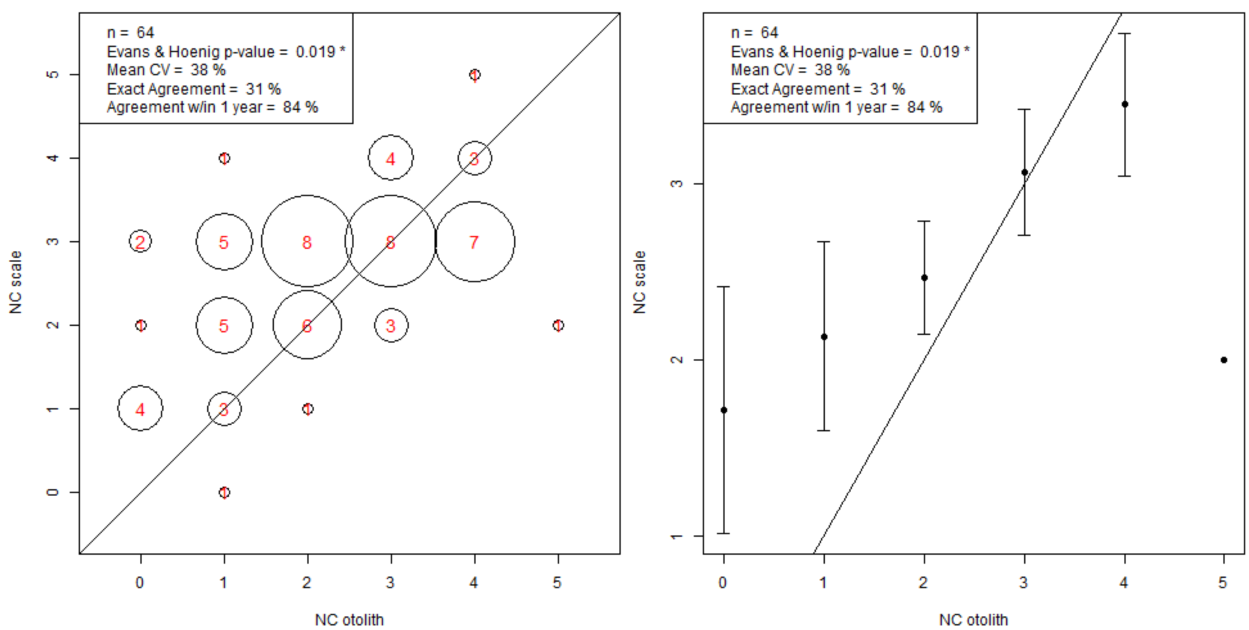

Figure 14. Age frequency (left) and age bias (right) plots for NC Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

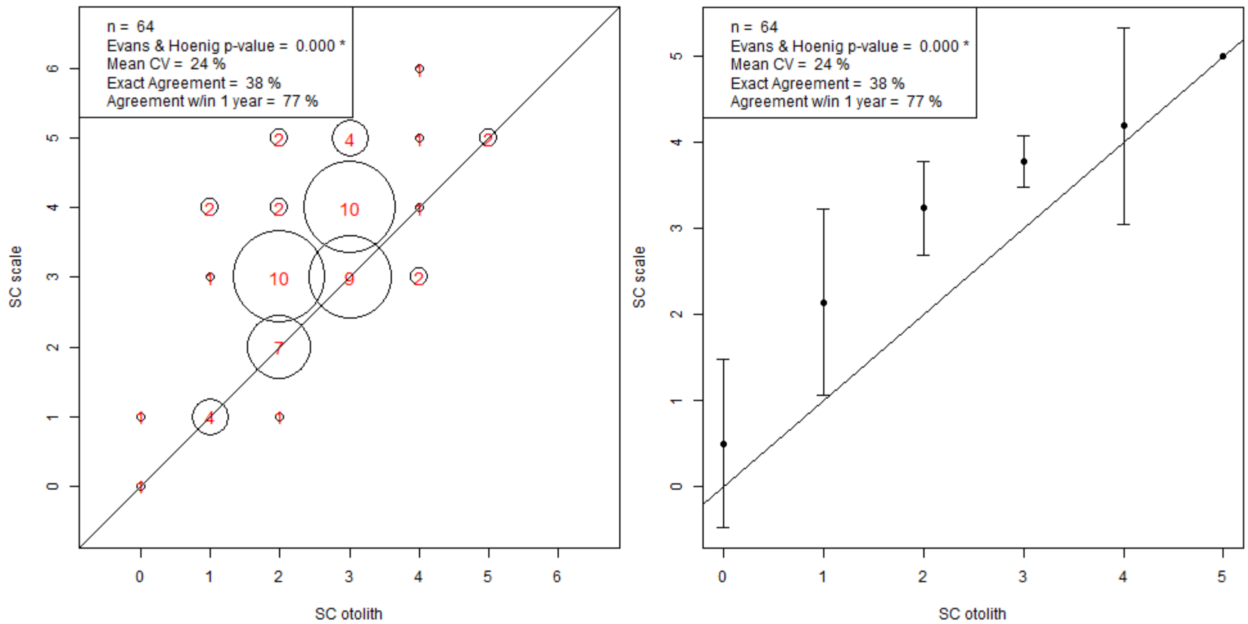

Figure 15. **Age frequency (left) and age bias (right) plots for SC Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals**

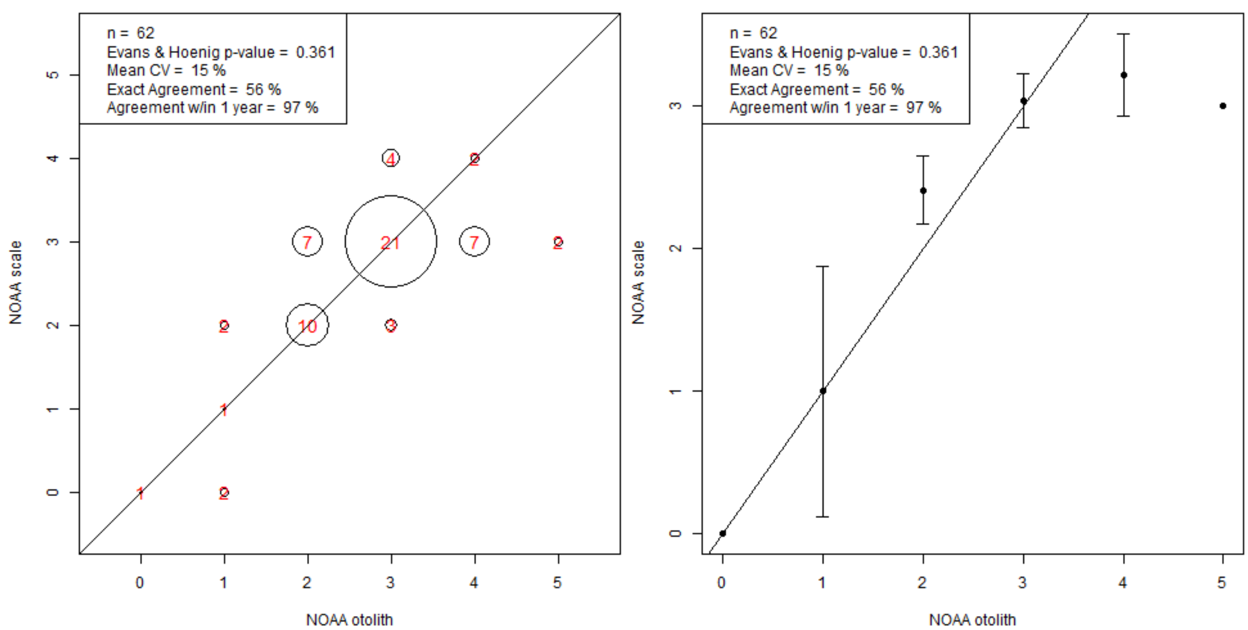

Figure 16. **Age frequency (left) and age bias (right) plots for NOAA Beaufort Atlantic menhaden scale and whole otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals.**

Appendix 1: Participating Ageing Lab Methods and Practices

Maine Department of Marine Resources (ME DMR)

ME DMR currently collects fish samples from the menhaden bait fishery. We collect 15 fish per sample and they are stored in labeled boxes and placed in the freezer until we can process them. We collect 15 in case there are damaged scales. It assures we can get 10 good fish. Samples are placed in luke warm water to thaw and process.

To process a sample; we document the length and the weight, remove a patch of scales and place them in a vial with water, remove the otolith and place in a tray. Otoliths are then put in a vial and stored in a labeled coin envelope.

Scales are dried on a paper towel and placed on glass slides and labeled with the sample information and stored in a coin envelope.

I have been ageing herring otoliths since 1996. I started ageing menhaden in 2021. Most of my experience is from the workshop as I am self taught. At this time I am the only menhaden ager. I digitize the otoliths and scales as a tiff file. I go back later and age them and annotate them. I have all ages saved with notations. Once everything is digitized, I send my samples to Amanda in Beaufort.

Massachusetts Division of Marine Fisheries (MA DMF)

MA DMF currently collects scale samples from menhaden captured primarily in the purse sein bait fishery. Scales are stored dry in coin envelopes. Scales are then cleaned with a pancreatin solution in an ultrasonic bath prior to being patted dry on a paper towel and sandwiched between two glass slides. Prepared samples are currently being sent to the NMFS lab in Beaufort for ageing. Beginning in 2023 otoliths were collected along with the scales. Otoliths are currently archived. Menhaden ageing experience within MA DMF is limited to the ASMFC QA/QC workshops and the menhaden ageing workshops. Scales aged for the exchanged were read through a microfiche reader. Otoliths were examined through a stereo microscope with reflected light on a black background.

Rhode Island Division of Marine Fisheries (RI DEM DMF)

Scale samples and otoliths are currently collected from fishery-dependent sampling of bait fisheries with supplementary fishery-independent samples collected from the RIDEM DMF Trawl Survey. The annual sample size target is 100 samples which is typically sufficient to satisfy the ASMFC requirement of one 10-fish sample per 300 metric tons landed.

After removal, scales are initially stored dry in coin envelopes until they can be processed in the laboratory. When processed, scales are lightly cleaned with a gentle cleanser and water, dried on paper towels, and pressed between two microscope slides. Fishery-dependent samples are currently shipped to the NOAA-Beaufort Lab where they are aged, and archived. Otoliths are stored in glass vials and not currently aged.

Menhaden ageing experience is limited to two ASMFC workshops in 2015 and 2023. For the 2023 workshop, states participated in a hard parts exchange. For that exchange, scales were read on a microfiche and otoliths were read on a stereo microscope with reflected light. Future ageing of scales and/or otoliths will depend on the findings of this workshop, and recommendations from the ASMFC technical committee and menhaden stock assessment scientists.

Connecticut Department of Energy & Environmental Protection

Prior to 2024, Menhaden scale samples were only collected from the Long Island Sound Trawl Survey (LISTS) if there were more than 10 menhaden caught in a particular tow. Scale samples were taken from a random 10 fish and the rest were measured. This procedure was done to emulate the sampling protocol from the fishery dependent sampling. Starting in 2024 scales were taken no matter the number of fish, from subsamples of menhaden, stratified by length group, to better represent the full range of sizes caught by LISTS. Individual weights were taken from menhaden being sampled starting in 2024. Scales were removed from about mid-body, below insertion of the dorsal fin. Before the sample was taken, slime was removed from the scales using a blunt knife, and the knife was rinsed between fish to prevent cross-contamination between samples. Scales were stored in coin envelopes until lab processing.

In the lab, scales were removed from the envelope and placed in a small beaker with water and a drop of dish soap. The beaker was placed in an ultrasonic cleaner and allowed to soak with the cleaner running for a few minutes. Scales are examined, looking for signs of regeneration. If the scale did not look regenerated, it was scrubbed using a toothbrush to remove and excess slime, or skin. The scale was then dipped in freshwater, blotted dry on a paper towel and placed on a slide for mounting. This process was repeated until there are 6-10 scales on the slide (depending on the size of the scales). A top slide was put on top on the scales and affixed together with two pieces of clear tape and then labeled with the sample number, date and fish ID number. The mounted scales were placed back in the envelope, along with the unused scales and cataloged by sample number and fish ID number.

Menhaden scales were aged using a microfiche, either a COM 200 or aperture card 6000). Annuli were defined as compression in the normal pattern of circuli that can be seen over the anterior field with continuous cutting over into the lateral fields. Annuli measurements are not currently taken on the scales. Previously, all scales were read blind by a single reader and assigned a confidence rating from 1-4 (1 being the best samples with little opinion on a different age estimate and 4 being unreadable). All samples with a confidence of 3 and a subsample of confidence of 2 samples would be re-read by the original reader. Starting in 2023 menhaden were blind read by either two separate readers or aged twice by a single reader. The two reads were compared for ACV, percent agreement and bias using statistical tests of symmetry (McNamar, Evans & Hoenig and Bowker's all using the FSA package in R). Any samples not in agreement were read blind again by the lead biologist for menhaden. The current lead biologist for ageing menhaden is Kelli Mosca, who has been ageing menhaden since 2022, and has had experience ageing shad since 2018.

New York Department of Environmental Conservation (NYSDEC)

NYSDEC currently collects Atlantic menhaden scale samples through our agency run fisheries dependent sampling program. Menhaden scales typically come from fish that have been sampled at local bait shops. These fish are caught primarily using cast nets or small bait seines. Additionally, and when available, menhaden scale samples are collected portside, directly from larger inshore seining operations. The Nearshore Trawl Survey, conducted by Stony Brook University, also collects scale and otolith samples that they provide to NYSDEC. Both scales and otoliths are stored in coin envelopes. Prior to 2024, scales were cleaned by soaking them for several hours in a diluted dish soap solution. After soaking, material on the scale was removed using a paper towel. The scales were then dried and pressed between two glass slides.   Feedback from the 2023 menhaden ageing workshop indicated that

NY's methodology for cleaning the scale samples should be modified as the samples were difficult to read. Scales are now cleaned with a pancreatin solution and run through an ultrasonic machine to better remove debris. Prepared samples are currently being sent to the NMFS lab in Beaufort for ageing. Menhaden ageing experience at NYSDEC is limited to menhaden ageing workshops. Scales aged for this recent exchange were read through a microfiche and otoliths were examined through a stereo microscope. There was only one reader for the scales and otoliths during this exchange.

New Jersey Division of Fish and Wildlife (NJ DFW)

Scale samples are currently collected from fishery-dependent sampling of commercial bait fisheries (purse seines and occasional paired trawls and gillnets). Samples are processed according to NOAA-Beaufort Atlantic Menhaden: Port Sampling and Scale Processing Protocols (Appendix A) before being sent to the NOAA Beaufort Lab for ageing. Age samples are not currently collected from fishery-independent sampling. Due to NOAA ageing NJ's samples, NJ agers have minimal experience with ageing menhaden outside of the 2024 workshop.

Delaware Division of Fish and Wildlife (DE DFW)

Fisheries staff pick up a 10-fish commercial Atlantic menhaden sample at the port and bring it back to the lab for processing. This usually occurs in the spring. Occasionally, a second set of 10 fish can be obtained in the fall. It all depends on availability of the samples. The fish arrive at the lab fresh, never frozen. Scales are taken from the side of the fish and just below the dorsal fin. Once removed from the fish, scales are placed in an envelope labeled with a fish identification number (beginning with a "C" to identify it as a commercial sample), date collected/processed (happens on the same day), location collected, gear type, fork length, and weight. Scales are then gently cleaned using warm water, Dawn dish soap, and a toothbrush. Scales are laid on a strip of paper labeled with their fish identification number to dry before being pressed between two slides. The slides are then secured together with Scotch tape and labeled with the species name and fish identification number (e.g. ATL MEN C-03001). Once pressed between slides, the scales are read on a microfiche. Scales are read blind (i.e. without the reader knowing any length or weight data and only using capture date once annuli are counted and a margin code is assigned) by two or more readers. The readers compare their ages, and a final age is agreed upon. Our first ager has been ageing menhaden scales since 2021. Our second ager just start ageing menhaden in November 2023.

Maryland Department of Natural Resources (MD DNR)

<u>Dependent Sampling (Pound nets)</u>

Each week, MD fisheries biologists collect 25 scale samples from Atlantic menhaden. The samples are taken with a clean, blunt knife from the fish; below the dorsal fin and above the lateral line. Ample scales are taken to ensure there are enough scales for processing. The fish are chosen at random; however, healthy, undamaged fish are selected. The scales are placed in a coin envelope with the location and fish data and identification written on it. These samples are then brought back to the office to be processed later that week.

<u>Independent Sampling (Gill nets)</u>

Each week, MD fisheries biologists collect 10 scale and otolith samples from Atlantic menhaden. The first 5 fish in each of the first 2 mesh sizes are chosen at random; however, healthy undamaged fish are selected. The menhaden are placed in a cooler and brought back to the lab for processing. Back at the lab, biological data and samples are collected. The scale samples are taken in the same process and location as the dependent samples. Then both the otoliths are removed by cutting open the head and pulling out the otoliths with forceps and placing them in a vial.

Scale Sample Process

Each week, the samples that were collected are processed. The scales are removed from the envelope, and the best 4 to 8 scales without regeneration are chosen to be cleaned and mounted. The scales are placed in a warm water and dawn soap bath for 1 min, then rinsed in clean warm water. Next, they are rubbed on a paper towel with the biologist's fingertip until clean. The scales are placed on a slide, all with the same orientation on the slide. Another slide is placed on top, and the two slides are secured with tape. During aging, the samples without 2 readable scales are remounted in this same process. If a sample has all regenerated scales, it is not used in aging. The scales are then aged by two MD biologists with a microfiche reader.

Otolith Sample Process

Atlantic menhaden otoliths are read whole, so they are removed from their vial and placed in a reading tray. Either water or light mineral oil is placed on the otolith to submerge it. After the otolith is aged with a compound microscope, it is cleaned and dried and placed back in its vial.

Virginia Marine Resources (VMRC)

VMRC published menhaden ageing methodologies in it's annual age and growth lab report (Appendix) available at: https://www.mrc.virginia.gov/ageing-lab/Reports/VMRC_2023_Ageing_and_Growth_Report.pdf

Virginia Institute of Marine Science (VIMS)

Sample collection

Atlantic Menhaden is a "Priority" species for its two major fisheries-independent trawl surveys, NEAMAP and ChesMMAP. Priority species means that length, weight, sex, maturity, stomach, and otoliths are collected for 5 individuals from each length bin on each tow. Paired otolith and scale samples were collected from the NEAMAP fisheries-independent trawl survey from 2016-2018. As otoliths are the preferred hard part for ageing, otoliths have been collected and aged since 2008 to present. Additionally, smaller grant funded projects and collaborations have paired scales and otoliths in 2018-2019 and again in 2022-2023. The total number of whole otoliths have been aged from the NEAMAP and ChesMMAP Trawl Surveys to date is 5,797 (NM 2,575, CM 3,222).

Scales

Paired Atlantic Menhaden scales and otolith samples will be removed from specimens at sea. Approximately 30-50 scales will be removed from each specimen. After collection, these scales are properly labeled and stored in capped vials in the freezer for later cleaning and processing in the laboratory. By freezing the scales rather than drying them, the scales will less likely be damaged. At the laboratory the scales will be thawed and lightly scrubbed in a soap and water solution to remove any debris and excess slime. Six of the best scales will be selected, thoroughly dried and pressed between two glass microscope slides with the sides of the slides taped closed on the ends. Due to variations in the scales, six samples are selected to provide the most accurate age for each specimen. Scale sample slides are preferably read using a microfiche reader. If no microfiche is available, then a stereo dissecting scope with transmitted light will suffice. Additionally, if the stereo microscope has imaging capabilities, larger images can be displayed to mimic the microfiche with sufficient lighting.

Scales and otoliths will be read independently of each other but will later be compared across both of these ageing structures.

Whole Otoliths

The whole head of each menhaden sample will be removed with a serrated knife behind the operculum, labeled and frozen in storage bags to later have their delicate otoliths removed back at the laboratory. Due to size and fragile nature of menhaden otoliths, careful extraction can more easily occur at the stable laboratory setting. After otolith extraction, samples are dried and stored in small vials. Otoliths are read whole in a petri dish full of water or ethanol (Ethanol dries quickly and samples can be sealed back in storage vials more quickly), under a stereo dissecting microscope with transmitted light for the best contrast. Depending on the clarity and size of the otolith 25x zoom should be used with the otolith viewed in a watch glass full of 70% EtOH. This will assure no “clearing” (loss of visible annuli) will occur if the otoliths are dried, then stored. Wet stored otoliths straight from the otic cavity, read in water or read in ethanol can cause clearing if sealed in a vial before drying. Sometimes a combination of reflected and transmitted light is necessary to distinguish annuli separation and boundaries.

The core of the otolith will appear as a circular, hollow shape in the center of the otolith. The core has a dark/opaque outline. This outline will be called the core boundary. The core boundary extends away from the core slightly up both the rostrum (long point) and antirostrum (“thumb”) of each otolith.

The first annulus is often a thicker, darker band. Establishing the first annulus is critical to proper age assignment. The first annulus will often not have a lot of separation from the core boundary. The clearest separation to identifying the first annulus will occur on the rostrum.

Similar to the first annulus, any of the additional annuli will be best identified on the rostrum of the otolith. Starting with the first annulus, the annuli will gradually get thinner and lighter as the fish grows older. The best and clearest annuli can be traced all the way around the otolith. More difficult annuli can be checked by observing annuli on both the rostrum and antirostrum. Annuli are often less visible on the antirostrum, however the annuli often morphometrically visible by raised bumps along the inner edge of the antirostrum. Additionally, these raised bumps can be seen as layers of the otolith (like a typographical map). These layers can be traced to individual annuli around the otolith and are usually most visible on fish exhibiting more than one annuli.

There are three readers at VIMS and the mode age for each sample (both scales and otoliths) is provided as the final age. If there is no mode from the initial read, the readers reread the sample and if there is still no mode, they examine the sample together and come to a consensus age. If a consensus age cannot be determined the sample is discarded. Very few samples are discarded. Precision tests are preformed within each reader (multiple reads of the same sample) and between readers. VIMS uses similar precision and symmetry tests to the NEFSC.

North Carolina Division of Marine Fisheries

NCDMF collects Atlantic menhaden samples from fishery-dependent sampling of the commercial bait fishery. This is comprised of a 10 fish representative sample from each catch. The side of the fish is wiped off with a rag or towel to get rid of any dirt or excess slime before taking a scale sample. Samples are collected with the dull edge of a scallop knife or similar instrument (collecting at least 20-30+ scales). The scales are placed in a coin envelope and dried to prevent mold growth. Depending on the size of the scales, 6-8 scales are selected and placed between two glass microscope slides (25x75x1 mm in size). The edges of the two glass slides are taped to secure the scales. The left side of the slide is labeled with the species name, date, fork length (mm), and sequence number and line number that NCDMF uses to link the sample back to its biological data. Age structures for the exchange were read on a microscope.

National Oceanic and Atmospheric Administration-Beaufort

Atlantic menhaden scale samples have been collected from fishery-dependent sampling (purse-seine nets) from reduction fisheries since 1955. Samples may be fresh or frozen and have been collected by one port sampler in Reedville, VA and are processed and mounted according to NOAA's sampling protocol before being sent to the Lab for ageing. Scales are removed from the lateral line below the insertion of the dorsal fin and soaked in soapy water. They are cleaned between two fingers before being mounted concave down (~10 scales) between microscope slides. In 2023 the reduction sampling protocol changed to collect 5 fish/collection instead of 10. In 2024, the port sampler retired with VMRC (Virginia Marine Resources Commission) to collect and mount reduction samples in the future. Scale samples from bait fisheries are collected by eleven state sampling programs with different protocols and are sent mounted to the Lab for ageing. The Lab currently ages an average of 1,000 scale samples from the reduction fishery and 1,800 samples from bait fisheries yearly. Otoliths are only received and aged for comparison studies.

An Olympus stereo microscope and monitor are used to view all samples for age determination using the Lab's ageing protocol. A digital image and annuli measurements are taken from the "best" scale using the imaging software cellSens. *Scales:* Mounted, 2x magnification, 0.5x objective, transmitted light, scope. *Otoliths:* Whole in watch glass with 70% alcohol, patted dry and dried overnight in vial with lid open, 6.3x magnification, 0.5x objective, transmitted light, scope or reflective light on dark background. Readability, annuli counts and edge codes are not recorded but a final age is entered in a database, taking edge growth and capture date into consideration. One ager has provided all ages until 2024, when an additional ager was added. Single reader ages are used in production but consensus ages were determined for this comparison study. The primary ager has been ageing scales for nine years, with limited otolith ageing. Ager 2 has extensive otolith ageing of other species and 1 year of menhaden scale ageing experience.

South Carolina Department of Natural Resources (SCDNR)

South Carolina is at the southern end of the menhaden range, and with no commercial landings there are no samples currently taken to meet any age reporting requirements. Therefore, menhaden ageing experience is limited to ASMFC ageing workshops. No samples were sent for the exchange set. Scales were read through a stereo microscope with transmitted light. The otoliths were read through the same microscope, but with reflected light. Ages were assigned by a single reader.

Appendix 2: Intra-Lab Comparisons

Ageing labs in Massachusetts, Delaware, and Maryland provided ages from multiple readers, so an additional analysis was run to make intra-lab comparisons between readers.

Massachusetts

Ages were provided for Kara Duprey (KD) and Scott Elzey (SE) from the MA DMF ageing lab. For the scales, the p-value for the test of symmetry was not significant (indicating no bias between readers) and the CV was relatively low at 8% (Figure A1). Agreement and agreement within one year were both relatively high at 71% and 97% respectively. For otoliths, the p-value for the test of symmetry was significant, indicating systematic bias, and the CV was 8% (Figure A2). Agreement and agreement within one year were 64% and 98% respectively. Agreement was higher on the scale samples for these readers and most disagreement on ages between the two readers occurred at older ages.

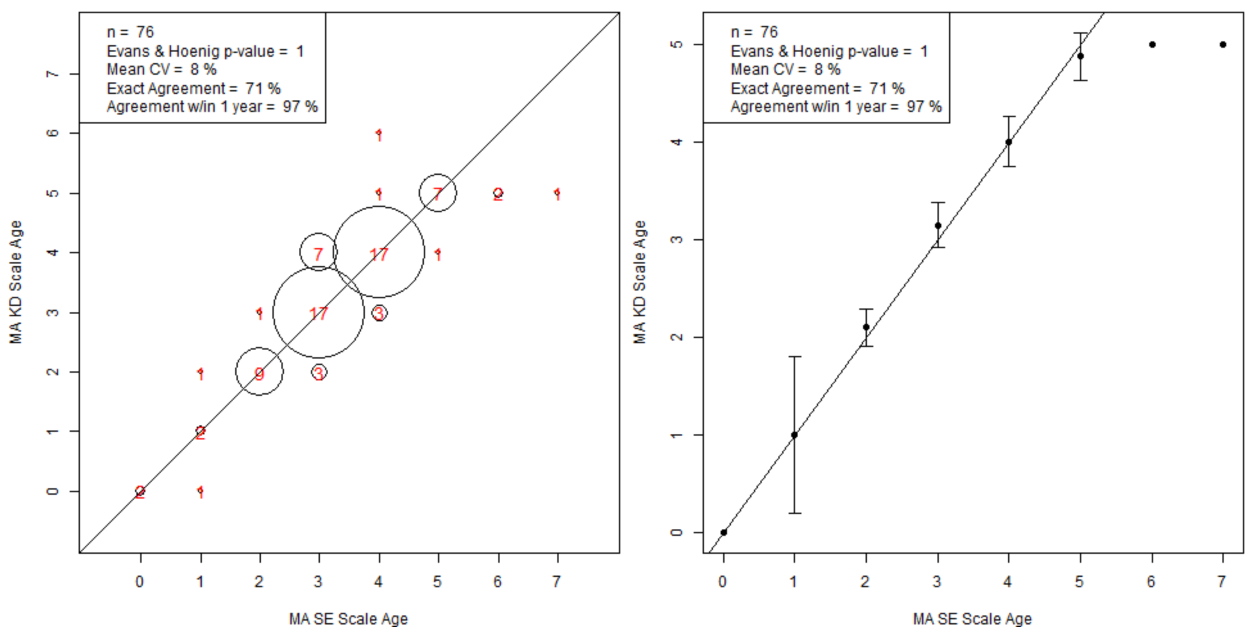

Figure A1. Age frequency (left) and age bias (right) plots for the two readers (KD and SE) in Massachusetts for the Atlantic menhaden scale age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

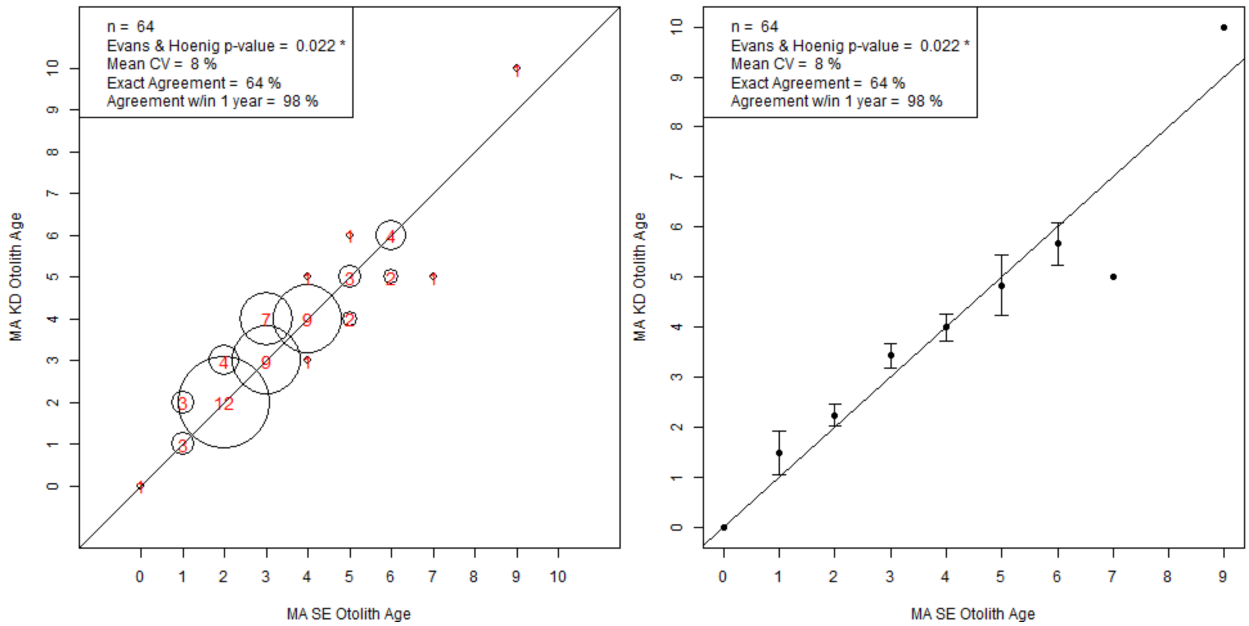

Figure A2. Age frequency (left) and age bias (right) plots for the two readers in Massachusetts for the Atlantic menhaden otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervalsError bars in the age bias plots are 95% confidence intervals.

Delaware

Ages were provided for Alexandria Hoffman (AH), Audrey Ostroski (AO), and Colt Williamson (CW) from the DE DFW ageing lab. For the scales, the p-value for the test of symmetry was not significant between AH and CW (indicating no bias between readers), but it was significant when comparing the ages between AH and AO and CW and AO (Figure A3). The CV was low at 3% when comparing CW and AH, but it was above 10% when comparing AO to AH and CW. Exact agreement ranged from 47-91%, with the highest agreement between AH and CW. Agreement within one year ranged from 89-100%. For otoliths, the p-value for the test of symmetry was not significant between AH and CW (indicating no bias between readers), but it was significant when comparing the ages between AH and AO and CW and AO, indicating systematic bias (Figure A4). The CV was low at 3% when comparing CW and AH, but it was above 10% when comparing AO to AH and CW. Exact agreement ranged from 34-91%, with the highest agreement between AH and CW. Agreement within one year ranged from 80-100%.

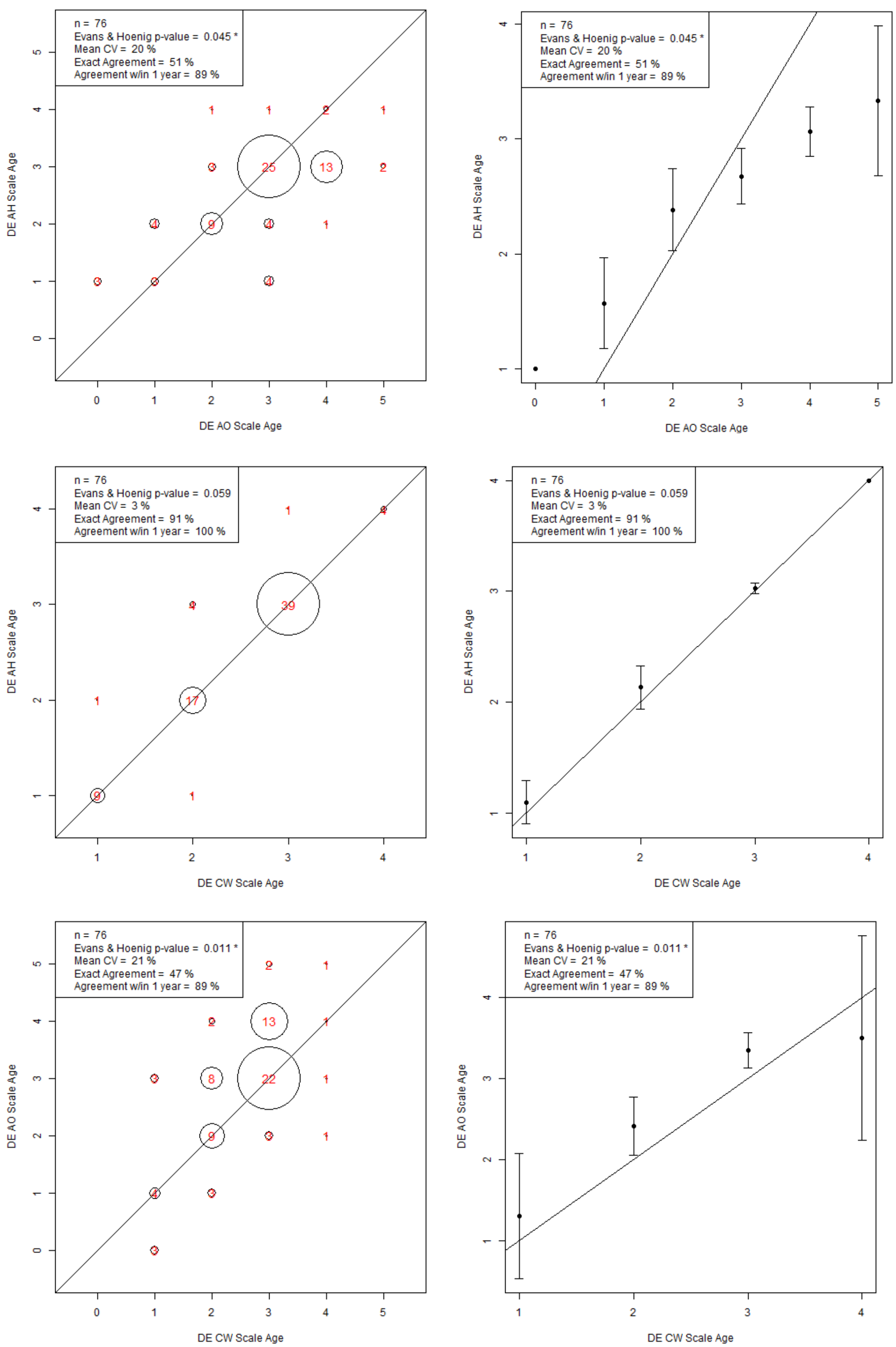

Figure A3. Age frequency (left) and age bias (right) plots for the readers (AO, AH, and CW) in Delaware for the Atlantic menhaden scale age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

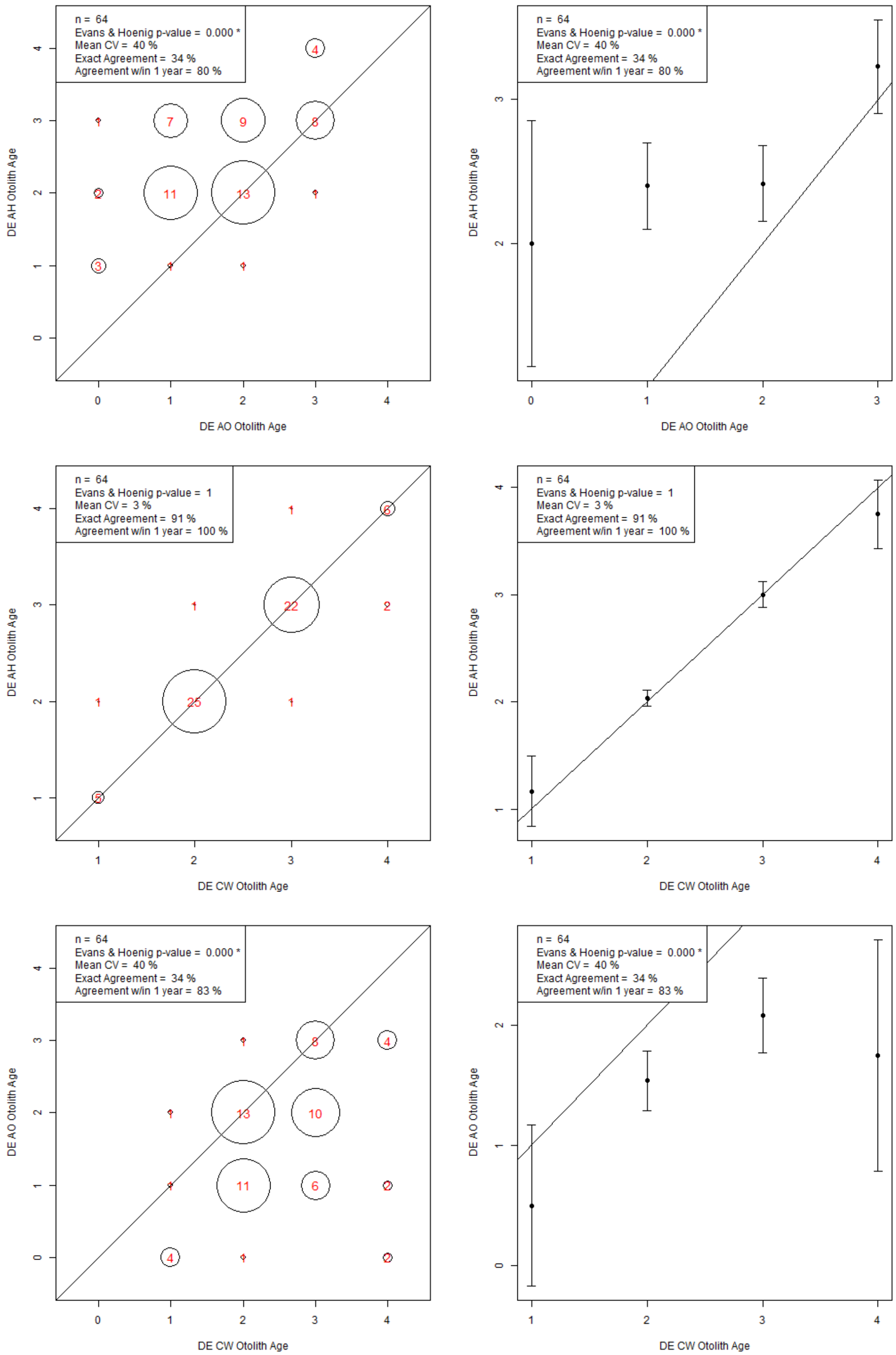

Figure A4. Age frequency (left) and age bias (right) plots for the readers (AO, AH, and CW) in Delaware for the Atlantic menhaden otolith age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

Maryland

Ages were provided for Katherine Messer (KM) and Harry Rickabaugh (HR) from the MD DNR ageing lab. For the scales, the p-value for the test of symmetry was not significant (indicating no bias between readers) and the CV was 12% indicating some imprecision (Figure A5). Agreement and agreement within one year were 64% and 93% respectively. Only KM provide ages for otoliths because HR did not have experience ageing that hard part, so only scales are compared for this analysis.

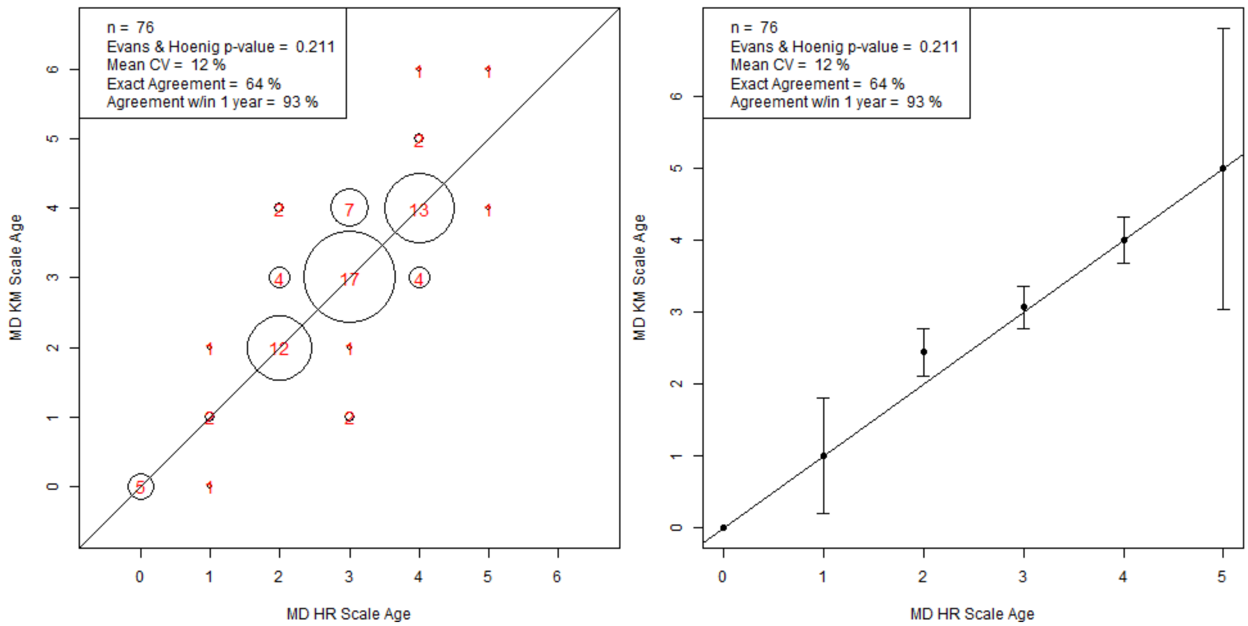

Figure A5. Age frequency (left) and age bias (right) plots for the two readers (KM and HR) in Maryland for the Atlantic menhaden scale age determinations. In the age bias plots, dots are the mean age and error bars are 95% confidence intervals

Appendix 3: Exchange Sample Details

Table A1. Ageing samples in the Atlantic menhaden exchange. Samples with a scale and otolith sample number (#) are paired samples. Table includes the state waters where the samples were collected, collection date, total and/or fork length (mm), and weight of the fish (g). Table continues on next two pages, followed by photos of the samples identified with the sample #.

Scale Sample #	Otolith Sample #	Collection State	Collection Date	Total Length (mm)	Fork Length (mm)	Weight (g)
3		RI	1/12/2021		302	660
5	161	MD	6/14/2017		208	147
6	162	NY	6/2/2021	322	277	300
7	163	DE	12/1/2016		90	
9		MA	6/15/2021		314	478
12	164	NY	5/3/2022	286	247	230
13	165	MD	6/22/2017		279	296
15		NC	1/16/2023	223	191	110
17	166	VA	5/22/1995		258	270
19	167	NY	4/30/2022	263	230	180
20	168	MD	6/28/2017		271	304
23	169	NY	5/12/2016		120	26
24	170	MD	6/28/2017		270	315
25	171	DE	12/2/2016		285	
26		MA	6/15/2021		295	398
28	172	MD	6/28/2017		258	254
29		NY	2/26/2022	110	98	6
35	173	MD	7/16/2018		274	307
38	174	NY	8/27/2021	305	263	180
40	175	MD	7/5/2017		298	404
41	176	NC	1/16/2023	241	218	140
42	177	NY	5/12/2016		90	120
44	178	MD	7/5/2017		247	280
46	179	MD	7/5/2017		236	235
50	180	MD	7/12/2017		243	230
53	181	VA	12/13/1995		111	20
55	182	NC	1/16/2023	191	166	80
59	183	MD	7/12/2017		255	269
60	184	VA	12/13/1995		194	115
64	185	DE	3/6/2017		125	
65		MA	6/21/2021		245	244
67	186	MD	7/19/2017		226	201

Table A1. *Continued*.

Scale Sample #	Otolith Sample #	Collection State	Collection Date	Total Length (mm)	Fork Length (mm)	Weight (g)
70	187	RI	4/27/2021		260	245
71	188	NJ	5/7/2016		275	372
73	189	NC	1/16/2023	237	201	140
76	190	VA	5/22/1995		257	268
80	191	DE	4/13/2017		245	
83	192	RI	4/27/2021		300	381
85	193	NY	5/12/2016		290	370
87	194	DE	5/17/2017		275	
89	195	NC	1/16/2023	215	185	100
90	196	RI	5/18/2021		251	262
92	197	NC	1/12/2023	270	231	250
93	198	NJ	10/30/2016		195	122
94	199	MD	6/14/2018		258	262
96		NY	5/31/2022		244	236
97	200	MD	6/18/2018		200	133
100	201	NC	12/20/2022	344	294	470
104	202	DE	6/12/2017		210	
106	203	MD	6/27/2018		259	278
114	204	NC	1/8/2023	140	196	233
115	205	VA	10/31/2016		285	350
118	206	RI	7/20/2021		292	290
123		NY	9/8/2022		256	227
125		MA	9/27/2021		302	438
126	207	MD	7/27/2018		291	386
128	208	NC	1/11/2023	207	180	120
129	209	NY	10/18/2016		290	396
130	210	RI	9/15/2021		210	119
132	211	NJ	2/15/2022		300	402
133	212	MD	7/30/2018		261	295
134	213	NC	1/11/2023	232	198	140
135	214	DE	10/6/2017		290	
138	215	RI	9/29/2021		228	159
141		MA	9/28/2021		250	291
142	216	MD	8/10/2018		290	398
144	217	NC	1/11/2023	286	244	230
145	218	RI	11/16/2021		266	258

Table A1. *Continued*.

Scale Sample #	Otolith Sample #	Collection State	Collection Date	Total Length (mm)	Fork Length (mm)	Weight (g)
146	219	MD	8/16/2018		222	166
147	220	NJ	3/1/2022		253	260
149	221	NC	12/20/2022	339	296	470
151	222	NJ	2/15/2022		280	328
155		MA	9/28/2021		285	343
156	223	DE	11/7/2017		265	
158	224	RI	11/16/2021		271	299
160	225	DE	10/30/2016		285	398